ON THE EXCRETION OF URINARY 17-HYDROXYCORTICOSTEROIDS (17-OHCS) IN ANTARCTIC REGION

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Abstract: In the study of the effect of chilliness and the tendency of cryodomestication, the urinary output of 17-Hydroxycorticosteroids (17-OHCS) was used, and its circadian rhythm was investigated.

Though urinary 17-OHCS increased during an extremely cold season, the change in relation to the cold weather has not been clarified. On the other hand, during the survey trip urinary 17-OHCS did not increase noticeably even when the test member met with an inexperienced chilliness, and it showed a rather lower level than the ordinary value.

Though the tendency of cryodomestication is difficult to clarify as there are many intermediary factors, some trend of domestication could be apparently observed with respect to all the known factors including urinary 17-OHCS.

The most interesting finding in this study was the circadian rhythm of urinary 17-OHCS whose pattern corresponded with the rhythm of the sun; while it showed normal pattern in the day that had clear daylight and night, in the polar rhythm of the sun circadian rhythm were disturbed. Namely, it seemed that our circadian rhythm depended on the solar rhythm, in other words, standard time of circadian rhythm was on movement of the sun.

1. Introduction

Cryodomestication is the theme of the medical study that was commenced by the 9th wintering party (1968–1969). The tendency of cryodomestication has been investigated mainly on the basis of the correlation between behavioral modality and metabolism (KAGEYAMA, 1963; OHKUBO, 1970). The 11th party (1970–1971) in which the author participated, investigated how the living body responds to divers conditions peculiar to the polar regions, especially the chilliness, using the amount of urinary 17-OHCS as an index.

The subjects of this investigation were as follows:

1. Effect of chilliness.
2. Tendency of cryodomestication.
3. Circadian rhythm.

It is known that the effect of chilliness causes various changes and responses in a living body. Nevertheless, reports on the mode of response in

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the hypophysis-adrenal cortex system against chilliness have been limited in number, and there seems to be no consensus of opinion (CHOWER *et al.*, 1970 ; SYMPSON, 1967 ; ITOH *et al.*, 1968). This is attributable not only to the difference in experimental procedures, but also, in the author's opinion, to the fact that the upper center of hypophysis-adrenal cortex system is so complicated that the amount of secretion of cortical hormones by a single stimulation may not always correspond to the stimulation, *i.e.*, any possible cause due to any other factor is not negligible.

The existence of the circadian rhythm by 17-OHCS has been revealed by many workers since PINCUS, but its mechanism is still debatable.

PERKOFF *et al.* (1954) reported that the rhythm of sleeping and awaking corresponds to the rhythm of 17-OHCS secretion. This is where the opinion is divided, some insist that the secretion rhythm of cortical hormones has a hereditary cycle while others think that the rhythm is formed through a habit of sleeping (ORTH *et al.*, 1967, 1969). In this connection, it seems that the modern society is largely disturbing the rhythm of sleep, body temperature, hypophysis-adrenal cortex system and other basic factors of life, thus it may not be too much to say that such disturbance is a cause of the so-called modern diseases. Moreover, the modern society is markedly complicating these experiments and researches, making their solution all the more difficult.

Taking advantage of an opportunity of the Antarctic wintering, the author investigated the effects of chilliness on living body and the effects of annual rhythm of the sun peculiar to the polar regions, *viz.*, the cycle of the nights with the midnight sun without sunset and the polar nights without the sun at all, on the rhythm of life of the team members under the circumstances where almost the whole period of wintering is spent in the cold, isolated from the modern society and the members are forced to live for a year in an entirely identical condition for sleeping, awaking, eating and surroundings, regardless of their different duties.

2. Study Subjects and Methods

2.1. Study subjects

The present investigation was made on the wintering members during the wintering as follows :

Long-term subjects : Only the author, investigated for consecutive eight months from February to October that roughly correspond to the essential period of wintering.

Seasonal subjects : Several persons selected from the team members in respect of age, occupation, character, with or without experience of wintering, etc., investigated twice, once in March during the former term of wintering and once again in July during the latter term, for two weeks each.

Inland survey trip subjects: The author and two persons out of the seasonal subjects, investigated for the whole period of the trip in principle.

2.2. Methods

Collection of urine: Urine was collected in three eight-hour periods, the 1st urine in 00:00-08:00, the 2nd urine in 08:00-16:00 and the 3rd urin in 16:00-24:00, and the total amount was dealt with.

Recording of behavior in life: Complying with the author's request, each subject member recorded his temporal behavior during the period of urine collection, and his physical condition in great detail. The author's own observations were added as objective findings.

Condition for investigation: No special restrictions for the investigation were imposed on the members so that they could lead an ordinary life as to works, amusements, drinks, etc.

Treatment of test specimens: Urine collection was made three times a day, and the quantity was measured. About 30 ml of each urine was preserved as test specimen in the freezing condition at -28°C, to be followed by successive measurements of the quantity of 17-OHCS. During the survey trip, urine was collected in a vinyl-film pouch and was left as it is, to be conveniently and naturally frozen. Some of the samples were lost in an unexpected trouble.

The total number of specimens amounted to 1,600.

Measurement of urinary 17-OHCS: Kit for measurement of urinary 17-OHCS was purchased from Iatron Co., Tokyo. Most of samples were measured at Syowa Station, and the rest were brought back to Japan in the frozen state, to be measured the same way.

The calculation and method of measurement are as follows:

$$\frac{E_U-E_R}{E_S-E_R} \times 0.06 \times \frac{\text{Volum of daily urine}}{6}$$

Here,

E_U: Extinction of urine blank,

E_S: Extinction of standard solution (10 μg of cortisol/ml) which was treated without β-glucronidase,

E_R: Extinction of reagent blank.

Procedure of modified method of SILBER and PORTER

A portion of 24 hour urine

Adjusted with 10% sulfic acid to pH 5.0–5.5

0.5 ml of acetate buffer

0.2 ml of β-glucronidase sol.

Left for 18 hours at 48°C to be hydrolyzed

30 ml of methylenchloride to hydrolyzed urine

Shaken for 30 sec (20 times)

Organic phase | Water phase (discarded)

3 ml of washing reagent A*

Shaken for 5 sec (20 times)

Organic phase | Water phase (discarded)

3 ml of washing reagent B**

Shaken for 5 sec (20 times)

Organic phase | Water phase (discarded)

Stoppered test tube containing 2 ml of 90% Ethanol

10 ml of extract | 10 ml of extract

Organic phase | Organic phase

Shaken for 30 sec (120 times) then left for aspirated organic (methylenchloride) phase

2 ml of color reagent*** | 2 ml of 68% sulfuric acid

Shaken for 30 sec (120 times) then left for aspirated organic phase. Cooled with tap water after heated for 30 min at 60°C.

Sample | Urine blank

Measurement at 410 mμ

*: 0.1 N-NaOH-10% Na_2SO_4

**: 10% Na_2SO_4

***: Phenylhydrazinhydrochloride

2.3. Summary of wintering life

Details of the condition for the wintering life are essential for discussion of the results of the present investigation. The life at Syowa Station is briefly described here.

Composition of members: 30 men, varying in age from 23 to 45, averaging 32 years old.

Duration of wintering: From February 20, 1970 to the latter half of February 1971. On November 25, 1969 the wintering party left Tokyo Port aboard the observation ship FUJI, and reached Syowa Station on January 4, 1970.

After transportation and construction were completed, preparations for wintering were made.

The wintering commenced on the February 20 and ended on February 16, 1971 when the party members were successively evacuated to FUJI, to be replaced by the 12th party.

The members left the frozen sea on March 17, 1971 and returned to Haneda on April 10, 1971 sixteen months after their departure from Japan.

Geographical conditions of Syowa Station: The station is located on East Ongul Island, 40°E and 69°S, facing the Indian Ocean. It is 5 km distant from the Antractic Continent beyond the Ongul Strait. There is a minus-six-hour difference in time between the station and Japan.

Meteorological condition: Because of the 5-km distance, the climate of the island is less cold than the main land, and the temperature recorded in 1970 was +7.7°C in maximum and −38.9°C in minimum (Fig. 1) (OHNO *et al.*, 1971), so the life at Syowa Station was not very severe. As a peculiar

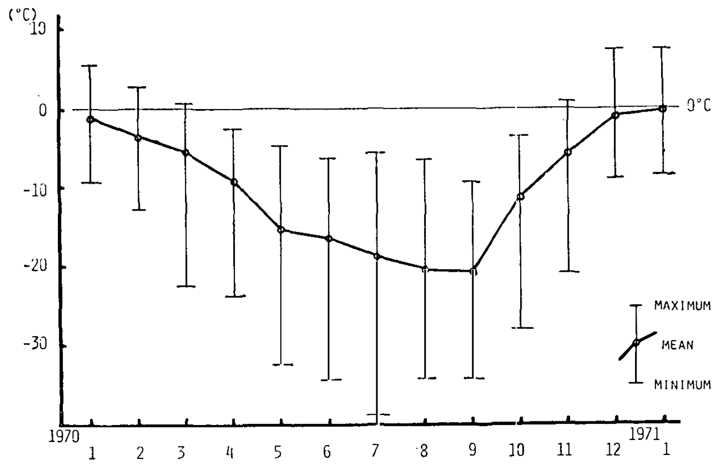

Fig. 1. Atmospheric temperature at Syowa Station (1970–1971).

condition, there is an annual change in the duration of sunshine, as will be described later.

Life environment: As seen in the records of observation by rocket that has been started by the 11th party (KAWAGUCHI *et al.*, 1970), the present Syowa Station has already cast off the character of an exploration base, and is now functioning as a modern scientific station where researches and observations in about twenty branches are being continued.

To support these scientific activities, construction and maintenance of the station are replenished yearly. The living quarters are kept as warm as around +18°C constantly.

Thus, there is no such heroic feeling as is frequently experienced when conducting research and observation in polar regions.

Daily schedule of the wintering members was as follows:

	Rising	Breakfast	Tea	Lunch	Supper
Weekday	07:00	07:30–08:00	10:00 15:00	12:00–13:00	18:00–19:00
Holiday	——	Brunch 10:00–12:00			18:00–19:00

Holiday comprises Sundays and national holidays and festival days just as in Japan.

In the survey trips the cold and the exploratory character were unavoidable in spite of the greatly improved vehicles and equipments.

3. Results and Discussion

3.1. Effect of chilliness and tendency of cryodomestication

3.1.1. Tendency of excretion throughout wintering term

Interrelationship between the author's daily total of excreted urinary 17-OHCS and the atmospheric temperature, both expressed by the average value for a period of every ten days, from March to October, is illustrated in Fig. 2. In the cold season from June to September the excretion of urinary 17-OHCS increased.

3.1.2. Effect of exposure to chilliness for a short time

In the cases of outdoor work or for survey for more than half a day, an increase in the amount of second urine was distinctly observed as seen in Fig. 3, while the daily total amount did not increase.

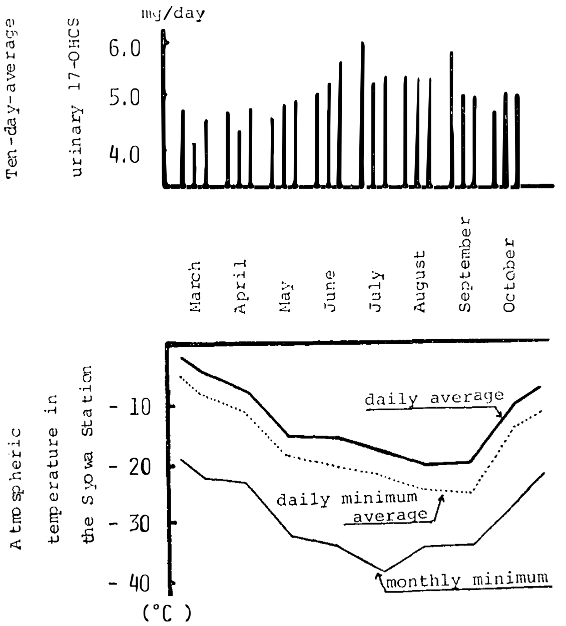

Fig. 2. Interrelationships between urinary 17-OHCS excretion and atmospheric temperature. In the cold season from June to September the excretion of urinary 17-OHCS increased.

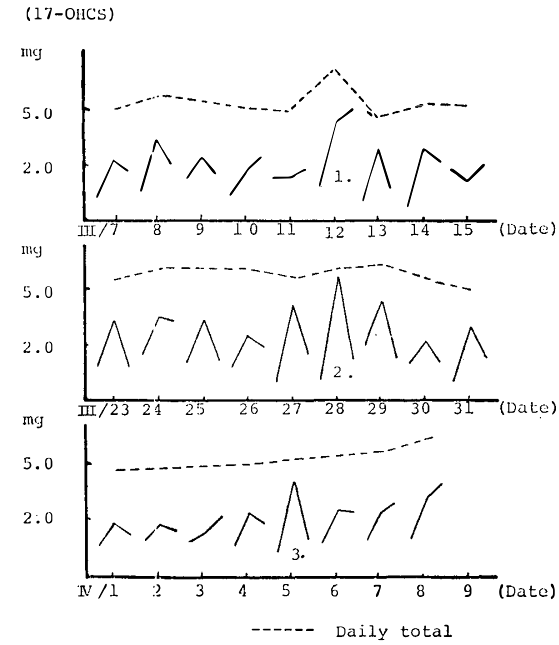

Fig. 3. Showing the author's urinary 17-OHCS level for each of the daily three intervals, and are arranged by date and are connected by line with one day as unit. Each graph is including a day of exposure to cold in term of routine indoor work; 1. Outdoor work, 2. Route to Tottuki Point, 3. West Ongul Is. survey. The amount of the 2nd urine in a day apparently increased, while the daily total amount did not so increse.

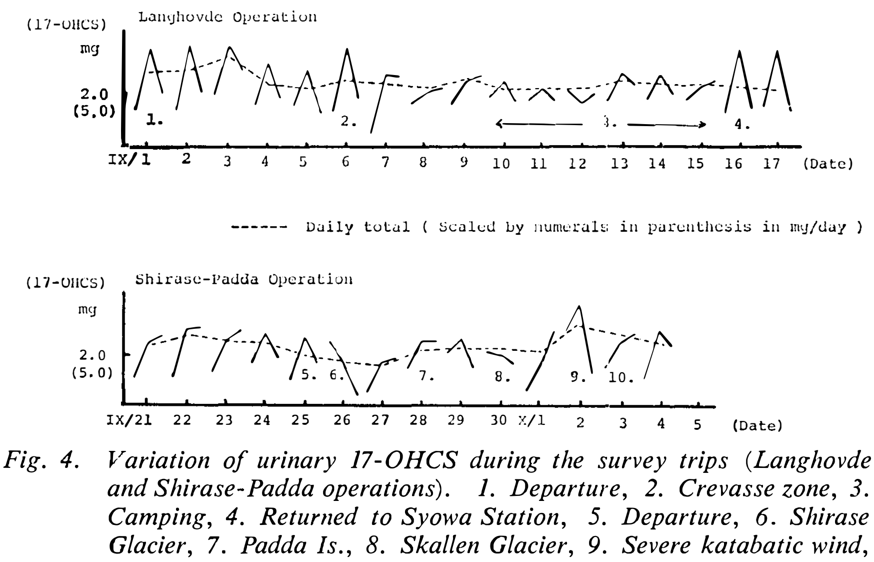

Fig. 4. Variation of urinary 17-OHCS during the survey trips (Langhovde and Shirase-Padda operations). 1. Departure, 2. Crevasse zone, 3. Camping, 4. Returned to Syowa Station, 5. Departure, 6. Shirase Glacier, 7. Padda Is., 8. Skallen Glacier, 9. Severe katabatic wind, 10. Syowa Station.

3.1.3. Tendency of urinary 17-OHCS excretion in survey trips (Figs. 4 and 5)

Langhovde operation: Urinary 17-OHCS increased for three days after the departure, but it gradually lowered to an ordinary level and afterwards did not change even in the worse condition such as camping. The atmospheric temperature during this term was −30.2°C in minimum and −17.3°C in maximum, averaging −24.0°C.

Shirase-Padda operation: This survey trip was attempted a week after the above-mentioned trip. Urinary 17-OHCS lowered immediately after the departure and recovered only after returning to station. During this period, the atmospheric temperature was −26.4°C in minimum and −12.9°C in

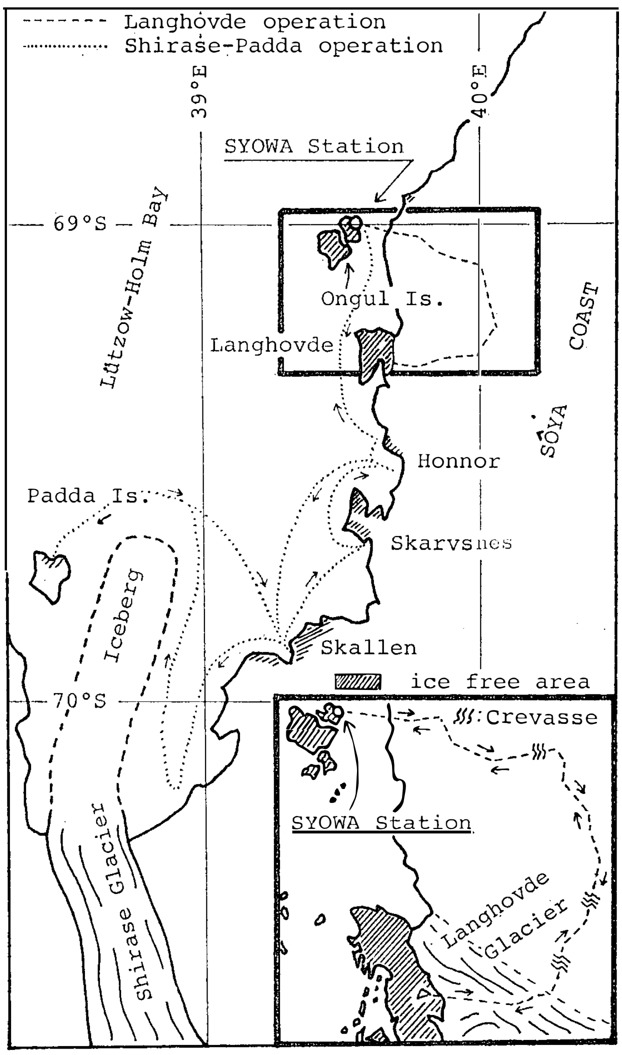

Fig. 5. Sôya Coast (east coast of Lützow-Holm Bay).

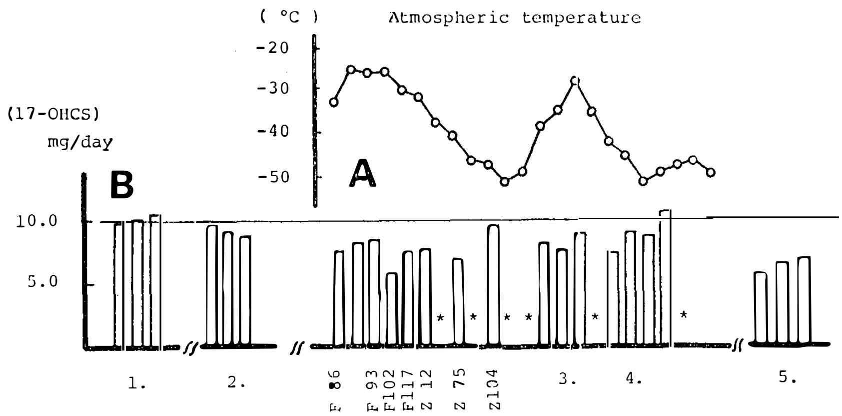

*Fig. 6. Showing the record of daily total urinary 17-OHCS in the winter travel (B) and of atmospheric temperature of the coldest term in this operation (A). 1. At the station, 2. Before departure (preparing for travel), 3. Arrival at Mizuho Camp, 4. Construction of Mizuho Camp, 5. After coming back, *: loss of the sample (Some of the samples were lost in an unexpected trouble), F and Z: Course flag.*

An inland trip and the establishment of an inland camp for scientific research work were carried out in the winter season, from June 23 to August 7, 1970, totaling 46 days, by an 11-man team with 4 snow vehicles. The Mizuho Camp was established, about 350 km distant from Syowa Station, at the terminal point of the trip (70°42′S, 49°17′E, and 2169 m above sea level by barometric altimetry) (SHIMIZU et al., 1972).

maximum, averaging −20.9°C.

Winter trip (Fig. 6): This was the worst trip as it was made during the sunless period when the lowest atmospheric temperature was recorded −54°C. Nevertheless, in this trip that took 46 days the values of urinary 17-OHCS were lower than the ordinary level at Syowa Station, and slightly increased on the days of temperature below −50°C.

3.2. Circadian rhythm of urinary 17-OHCS in relation to the rhythm of the sun

3.2.1. Circadian rhythm pattern of urinary 17-OHCS

Fig. 7 shows the author's urinary 17-OHCS level for each of the daily three intervals. The records are arranged by date and are connected by line with one day as unit. These lines are classified into four patterns as shown in Fig. 8.

The author calls them Type A (daytime-pattern), Type B (night-pattern), Type C (holiday-pattern) and Type D (others).

To discuss the patterns of circadian rhythm, however, the number of

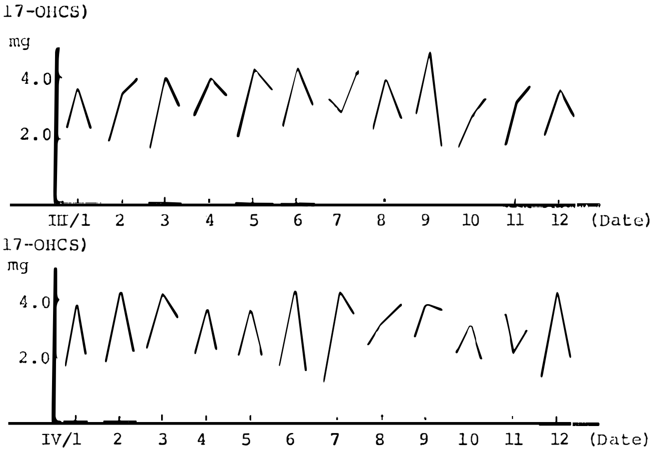

Fig. 7. Showing a part of the author's urinary 17-OHCS level for each of the daily three intervals. The records are arranged by date and are connected by line with one day as unit. These lines (circadian rhythm) transforms themselves into a variety of shapes day by day, and are classified into four pattern as Fig. 8.

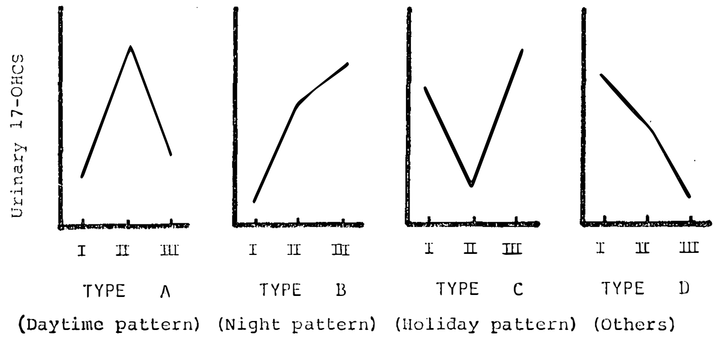

Fig. 8. Pattern and classification of circadian rhythm.
I: the 1st urine, II: the 2nd urine,
III: the 3rd urine.

times of sample collection must be taken into consideration.

In this connection, circadian rhythm of 17-OHCS in blood has been most frequently reported, especially the hourly measurement has been practiced (ORTH *et al.*, 1967). There is no specific reason for the author's collecting urine three times a day. In due consideration of experiments to be performed in some inconvenient place and collection of urine in the course of trip, it was decided to collect urine at the hours of 8, 16 and 24, which may little disturb the routine at the station and the observations in different divisions.

The reason Type A is expressed as daytime pattern is based on the fact that it occures in an ordinary life working in the daytime and resting in the nighttime. This pattern is most distinct in a more regular daytime-type life than recorded by the author. ORTH *et al.* (1967, 1969) reported that 17-OHCS in plasma varies with time, the maximum level appears around the hour of rising and the minimum level during sleeping hours. HONDA (1970) demonstrated that a normal man has the peak of urinary 17-OHCS in the forenoon and the slack in the evening or at night. DOE (1960) reported that there is a time lag of about three hours between 17-OHCS levels in plasma and in urine. Based on these observations the author defined a pattern in which the second urine attains the maximum as a daytime pattern or Type A.

On the other hand, Type B is characteristic to night workers in the author' investigations, and it appeared also when one was sitting up till late at night or doing midnight labor, thus the type was designated as a night pattern. HONDA reported that, for the subjects who show a nocturnal type behavior, the peak of circadian rhythm shift to afternoon.

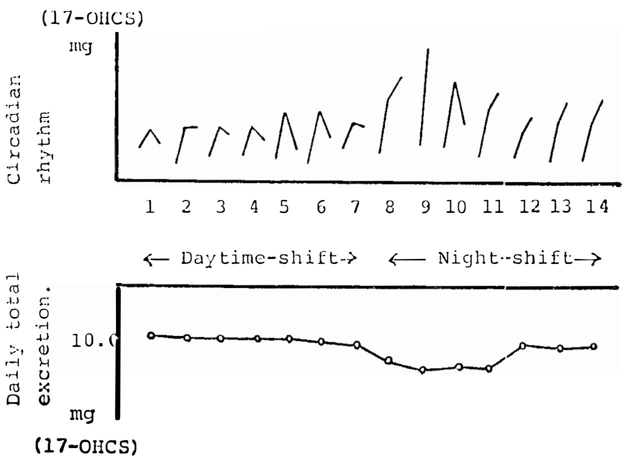

Fig. 9. Night worker's circadian rhythm and daily total of urinary 17-OHCS. Showing in a transitional stage from the daytime shift to the night shift (7th-8th day) appeared Type B rhythm (upper graph), and followed by an increase in daily total excretion (lower graph).

For instance, Fig. 9 is a record of circadian rhythm for a case of a meteorological member who was, at that time, in a transitional stage from the daytime shift to the night shift; Type B rhythm appeared immediately after he entered the night shift, followed by an increase in daily total excretion, but from about the third day of the night shift it gradually returned to the ordinary level. This is very interesting as it implies domestication of the subject to the night shift schedule.

Type C appeared frequently on holidays. This is probably because brunch on holidays was taken between 10:00 and 12:00 o'clock, and the subject used to sit up till late on the previous night, consequently late in rising, which accounts for the minimum level of the second urine on the holiday. Hence, the author called it a holiday pattern.

Type D has not been analyzed in detail, but like Type C it may ordinally belong to Type B, and appears only when a rhythm of the daytime-type life is disturbed.

3.2.2. Frequency of each pattern

Type A	Type B	Type C	Type D
53%	31%	11%	5%

3.2.3. Variation of the average duration of sunshin by months

As seen in the graph of Fig. 10A, the rhythm of the sun in the polar region starts from January with the midnight sun, to be succeeded by the months when the sunset is observed and the duration of sunshine becomes shorter and shorter until June, after which the sun does not appear for 45 days.

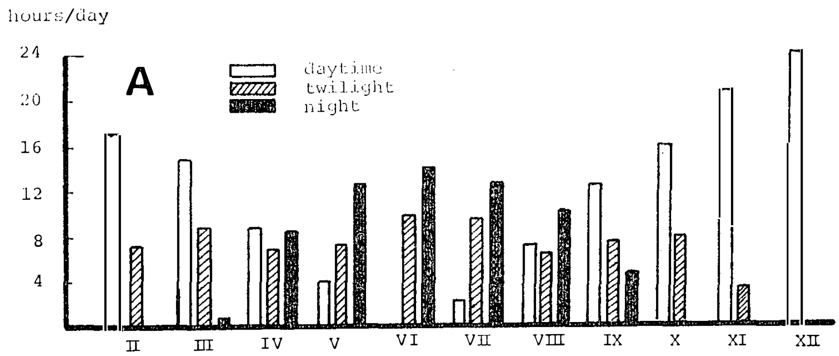

Fig. 10A. Bar chart showing the average hours of daylight, twilight and dark night for each month as the rhythm of the sun in polar region.

Then, with the return of sunrise, the duration of sunshine grows longer, and in October dark nights disappear, to be followed by the season of the midnight sun without sunset which begins in December, the sun travels over our heads for 24 hours a day. This is the rhythm of the sun in this polar region.

3.2.4. Frequency of each pattern of circadian rhythm by months

As seen in the graph of Fig. 10 B, Type A has the peak in March-

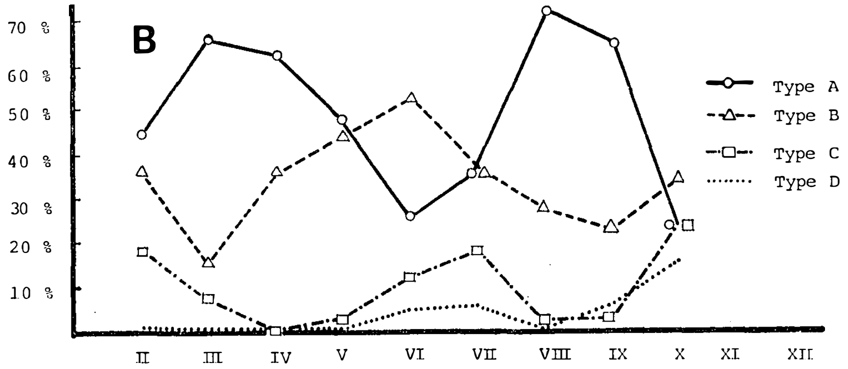

Fig. 10B. Line graph showing the frequency of each circadian rhythm pattern of urinary 17-OHCS for each month. Type A has the peak in March-April and August-September, and the slack in June. Type B slacks in March-April and August-September, and culminates in June.

April and August-September, and the slack in June, showing descent after October and ascent before February. On the other hand, Type B slacks in March-April and August-September, and culminates in June, showing ascent after October and descent before February in contrast with Type A. Types C and D show the same profile as Type B.

3.3. Discussion

The atmosperic temperature was as low as below −20°C approximately when we worked outdoors at the station and during our travel, so we experienced much difficulty.

On the other hand, analyzing the results of short-term exposure to cold or travel (Figs. 3 and 4), it is not assumed that adrenocortical hormones increased due to cold.

If the rise of the level for three days after the departure as seen in the Langhovde operation (Fig. 4) is attributable to the effect of chilliness, it must be that the cryodomestication was performed only for three days, but how can it be?

If the effect of chilliness should be detected, the slight increase in the level when the temperature was below −50°C as seen in the winter trip (Fig. 6) may be attributed to cold, because the tested member said that he had experienced physical suffering more serious than could be expected at the time.

In other words, the cryodomestication must be taken into consideration more than half a year after the commencement of wintering. However, it is likely that a living body does not take such cold as −30° to −40°C as an external invasive attack, *i.e.*, one does not feel cold on those days.

In addition, OHKUBO's report (1970) indicates that in the season of severe cold an apparently increase in the basal metabolism is observed, and that 3181 kcal of consumption and 3407 kcal of intake, namely, about 200 kcal of positive balance of energy, were observed during the winter season, with simultaneous finding of increased thickness of the abdominal subcutaneous fat followed by increased body weight.

These phenomena may be thought as the means of protection of living body against the cold, and further, one acquires an art of escape from feeling cold empirically and unconsciously, getting more patient and more resistant to the cold, *i.e.*, the threshold temperature of the cold that the living body feels an invasive attack, is lowered. This is probably the reason there was no observable change in urinary 17-OHCS.

From the above viewpoint, it may be concluded that the cryodomestication was clearly observed in this study.

On the other hand, the increased urinary 17-OHCS during the intensely cold season, from June to September (Fig. 2), must be undoubtedly related to the cold, but also sufficiently to the physical and mental effects since the wintering members were subjected to the most harassing conditions physically during the dark season when the sun did not appear.

For examples, the level rose higher when the members were in a strained situation like passing over crevasses and cracks on the frozen sea (Figs. 3 and 4) or engaged in a tournament of "go" games and billiards held at the station.

Accordingly, the increased level observed after the departure for the trip in the Langhovde operation is supposed to be the strain caused by a change of circumstances, and it also assumed that the decreased strain before undertaking the next trip as seen in the Shirase-Padda operation (Fig. 4), shows a sign of cryodomestication.

In this connection, the levels of 17-OHCS lower than ordinary values during the trip must be discussed further, although the lower levels may be partly explained by the regularity of activities in the field.

Correlation of the two annual variations of the circadian rhythm of urinary 17-OHCS and the rhythm of the sun (Fig. 10) reveals the following facts: the periods from March to April and August to September in which Type A forms the peak, have distinct sunrise, sufficient duration of sunshine, distinct sunset and nights, thus corresponding to the ordinary rhythm of the sun under which we have been living for a long time.

On the contrary, June and the period from October to February are either without the sun or with the sun all the time over the head, resulting in the decrease of Type A.

In contrast with this, Type B decreases during the season provided with distinct sunrise, and cycles of broad daylight and night, and increases during the season of the nights with midnight sun and the polar nights.

Types C and D show a similar tendency to Type B. Almost identical results were obtained by the investigation made on other subjects.

From the results described above, it may be concluded that our life rhythm depends largely on the movements of the sun, and the circadian rhythm of the sun consisting of distinct cycles of sunshine, sunset and night is essential for us to spend a life in an ordinary rhythm featured by daytime activity. Actually, during the nights with midnight sun and the polar nights, our thought of time was so completely disturbed that we could recognize the time only by watch and sirens for meals.

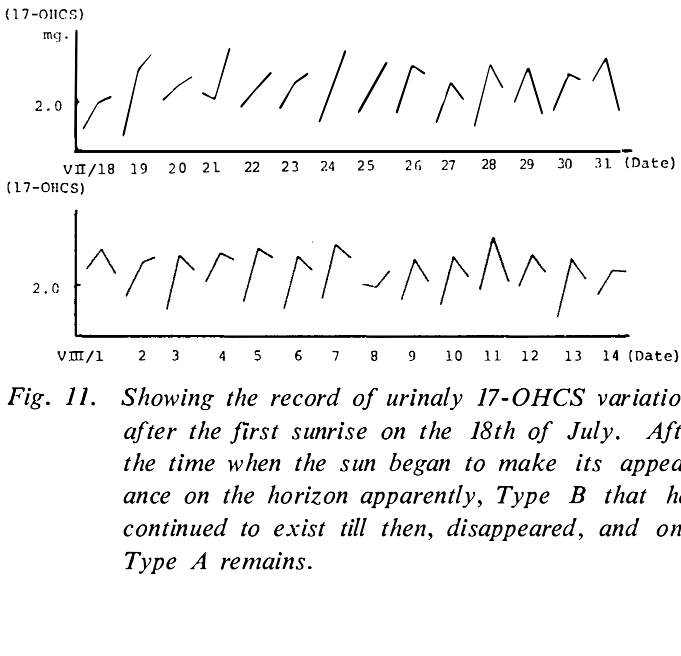

Fig. 11. Showing the record of urinaly 17-OHCS variations after the first sunrise on the 18th of July. After the time when the sun began to make its appearance on the horizon apparently, Type B that had continued to exist till then, disappeared, and only Type A remains.

Fig. 11 is the record of urinary 17-OHCS variations after the first sunrise on the 18th of July. It indicates that after the time when the sun began to make its appearance on the horizon, Type B (night pattern) that had continued to exist till then, disappeared, and only Type A (daytime pattern) remained. In other words, the normal rhythm in the body, that had been about to be lost, was recovered. And our actual feeling of the emerging sun out of the darkness was really impressive, and the lengthening duration of sunshine drove us out of doors, swimming in bliss of extricating ourselves from the most harassing dark 45 days.

This is probably why Type C (holiday pattern) decreases during the periods of March to April and August to September.

What has been mentioned so far can be summarized as follows: The distinct rhythm of the sun (of non-polar region) is necessary for us to tick the rhythm featured by daytime activity, and the rhythm of the life featured by daytime activity is likely to get disturbed under the rhythm of the sun in polar regions.

4. Summary

Urinary 17-OHCS excretion in the Antarctic region was investigated and the following results were obtained:

1) The urinary 17-OHCS level increased during the period from June to September that corresponds to the coldest season, but its relation to cold has not been clarified.

2) Urinary 17-OHCS during the survey trip did not show any noticeable increase even when the subject met with an inexperienced chilliness, and the level was rather lower than ordinary value.

3) The tendency of cryodomestication is not easily clarified as many intermediary factors are involved, some trend of domestication could be observed with respect to all the factors as well as 17-OHCS.

4) An interesting correlation was found between the circadian rhythm of excreted urinary 17-OHCS and the rhythm of the sun.

Acknowledgments

It was fortunate for the author that he was able to make this investigation in the Antarctic region. His sincere thanks are extended to Professor Hisato YOSHIMURA, Hyogo Medical College, Professor Kazuo ASAHINA, Faculty of Health and Physical Education, Chukyo University, and Professor Sigeru MORITA, the Jikei University School of Medicine, for providing the opportunity and kind guidance. The author is also greateful to every member of the 11th wintering party as well as to Leader Tatsuro MATSUDA, for their kind understanding and cooperation for this investigation.

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(*Received April 22, 1975*)