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THE URINARY EXCRETION OF 17-KETOSTEROIDS BY MEN UNDER FIELD CONDITIONS IN EXTREME COLD*

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Fort Knox, Kentucky
27 October 1949

*Sub-project under Study of Physiological Effects of Cold. Approved 24 September 1942. MDFRL Project No. 6-64-12-02-(11).

Project No. 6-64-12-02
Sub-project MDFRL 02-(11)
MEDEA

27 October 1949

ABSTRACT

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OBJECT

To determine the excretion of 17-ketosteroids on a group of seven subjects participating in two bivouac exercises, first under conditions of a temperate climate, and later under arctic conditions, which were so designed as to be similar except for the factor of extreme cold.

RESULTS

When the excretion of 17-ketosteroids by seven healthy soldiers under field conditions in a temperate climate was compared with their excretion of these substances under field conditions in extreme cold, no significant changes were noted.

CONCLUSIONS

The determination of the excretion of 17-ketosteroids in the urine did not indicate a stress due to cold under the circumstances described in this report.

RECOMMENDATIONS

Since one cannot suppose that urinary excretion of these steroids reflects conditions in the circulating blood, studies should be directed toward finding a convenient and reliable method for the determination of 17-ketosteroids or their precursors in blood.

There are numerous factors which affect the level of urinary excretion other than the actual amount of active principle secreted into the blood. Attempts should be made to determine what factors modify the pathway of metabolism of the active compounds and what affect variation in kidney and liver function has in determining the level of excretion.

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I. INTRODUCTION

Many observations have been recorded showing a relationship of the adrenal cortex to the process of adaptation to stress (1, 2, 3, 4). The determination of the excretion of 17-ketosteroids has been employed as a means of studying certain aspects of adrenal cortical functions.

A study of the excretion of the 17-ketosteroids was, therefore, included in the observations carried out on a group of seven subjects participating in a bivouac exercise under arctic conditions at Ft. Churchill, Canada. It was anticipated that changes in the excretion of the 17-ketosteroids would provide an objective measure of the response to the stress (cold and exercise) to which the men were subjected. As a basis for comparison with the Ft. Churchill conditions, the excretion of these steroids was determined (previous to the Ft. Churchill bivouac) for the same group of subjects during a bivouac at Ft. Knox, Kentucky under conditions which were so designed as to be similar in every way except for the factor of extreme cold.

II. EXPERIMENTAL

A. The Ft. Knox and Ft. Churchill Bivouacs

Complete details of the two bivouacs and the major investigation will be described in another report (5); for the present purpose the conditions of the study may be summarized briefly. The subjects were seven healthy male enlisted volunteers. After a month of physical conditioning (daily hikes), the 14-day control bivouac was carried out at Ft. Knox during the month of November, 1947. Throughout this test period, the men marched 13-15 miles a day except that no marches were scheduled on the two Sundays that were included in the bivouac period. The diet consisted of the Army "C-2" ration, and the clothing worn during this period was the Army fatigue uniform.

The subjects were taken to Ft. Churchill, Canada early in January, 1948. After a period of conditioning which included the 2-week Canadian arctic indoctrination course, a second 13-day bivouac exercise was carried out during February, 1948. The men hiked (snow-shoes) 4 to 6 hours a day, and the average exposure to the outdoor weather was 6.1 hours per day for the entire period. Their food was again the "C-2" ration. Regulation arctic uniforms were worn outdoors.

The two bivouac exercises were carried out under as nearly identical conditions as possible (personnel, food, activity, procedure) except for the difference of environmental temperature and the clothing worn. Thus two realistic field situations were attained which could be compared directly with respect to physiologic measures under temperate and arctic temperatures, respectively. The temperature and wind conditions during the two exercises are summarized in Table 1.

TABLE 1

SUMMARY OF CONDITIONS OF TEMPERATURE AND WIND
DURING THE TWO BIVOUAC EXERCISES

Bivouac	Mean Temp. (deg. F)	Temp. Range (deg. F)	Mean Wind Vel. (m.p.h.)	Wind Vel. Range (m.p.h.)	Mean Windchill
Ft. Knox	41.3	27.5 to 62.8	4.8	0 to 37	590
Ft. Churchill	-19.7	-37.0 to 12.0	16.5	0 to 44	1790

B. Methods

1. Collection and preservation of urine.

Complete urine collections were made during both bivouac periods. "Waking" urines were collected from 0600 through the last voiding before retiring; "sleeping" urines were collected through the first morning urine at 0600. An exact record of the time elapsed during each collection was made in order that the hourly output for each period might be calculated.

The urine was voided directly into tinned metal cans and brought to the laboratory within 24 hours after the beginning of any collection. At Ft. Churchill, the samples became frozen in the collecting cans within a few minutes after voiding and were allowed to thaw at room temperature after arriving at the laboratory.

When the urines were received at the laboratory, the volumes were measured and a sample (usually 250 ml.) was set aside for the steroid determinations. Since some of the specimens were alkaline in reaction during the bivouac at Ft. Knox, the pH of such samples set aside for steroid analyses was adjusted to 5 with hydrochloric acid (nitrazine paper). During the Ft. Churchill bivouac, all specimens were acid in reaction and consequently the pH was not adjusted. The samples were then frozen rapidly and were maintained in the frozen state until the time of analysis.

2. The 17-ketosteroid analytical procedure.

Hydrolysis and extraction of the urines for separation of the ketonic neutral fraction were carried out according to the procedure outlined by Pincus (6). This method includes the micro-Girard separation of ketonic from nonketonic substances using Girard's reagent T. The colorimetric determination of the 17-ketosteroid content of the ketonic fraction was carried out according to the method of Callow, Callow and Emmons (7). This is the Zimmerman reaction employing an alcoholic potassium hydroxide solution. All results are reported in terms of dehydroisoandrosterone which was used as a standard. The ether used for extraction was checked daily for substances oxidizing potassium iodide and none was employed which showed a positive test.

The accuracy and reproducibility of the method as carried out in this laboratory are indicated by the following observations:

Twenty pairs of duplicate determinations were carried out at the beginning of the study; after that only a single analysis was done on each sample. Of these twenty pairs, thirteen showed a difference (between duplicates) of 0-5% of the mean for the two values, and eighteen agreed within 10%.

At intervals during the course of the analytical work the whole procedure was checked by processing 100 or 200 micrograms of dehydroisoandrosterone added to 100 ml. of distilled water. The average error in recovery for nine such tests was 13%. However, individual errors in recovery ranged from -21% to +15%.

C. Results

The mean 24-hour excretion of 17-ketosteroids for each subject during the Ft. Knox and Ft. Churchill bivouacs is listed in Table 2. Inspection of these data will show that there is no significant difference between the means for the two bivouacs in any one of the seven subjects. Neither was there any definite trend in the level of 17-ketosteroid excretion during either bivouac exercise for the group as a whole. The values for the 17-ketosteroid excretion for the most part fell within the normal ranges reported by others (8).

TABLE 2

MEAN DAILY 17-KETOSTEROID EXCRETION;
RATIO OF MEAN HOURLY WAKING TO SLEEPING OUTPUT

Subject	Bivouac	Number of Determin.	Mean 17 K.S. (mg./24 hrs.)	Mean Deviation	Waking/Sleeping Output
1	Ft. Knox	14	9.5	± 1.4	1.3
	Ft. Churchill	13	8.9	0.6	1.4
2	Ft. Knox	12	13.8	1.9	1.4
	Ft. Churchill	9	14.3	1.2	1.2
3	Ft. Knox	13	14.6	1.7	1.6
	Ft. Churchill	13	11.9	2.5	1.2
4	Ft. Knox	12	11.4	1.8	1.3
	Ft. Churchill	10	10.7	1.6	1.4
5	Ft. Knox	13	7.3	1.3	1.3
	Ft. Churchill	12	7.6	2.2	1.2
6	Ft. Knox	12	9.1	1.2	1.1
	Ft. Churchill	10	8.0	0.9	1.2
7	Ft. Knox	11	8.5	1.3	1.0
	Ft. Churchill	13	8.3	1.3	1.6

The diurnal variations in 17-ketosteroid output which has been studied by Pincus (9) was noted in these subjects as shown by the ratio of the mean hourly outputs during the waking and sleeping hours (Table 2). The ratio of waking to sleeping hourly output was greater than 1.0 in 79% of the individual values recorded. However, there was no consistent change in the ratios for the group as a whole during the Ft. Churchill bivouac as compared with the ratios at Ft. Knox.

III. DISCUSSION

No change was found in the excretion of 17-ketosteroids which might have been expected as the result of the cold stress to which these men were subjected during the Ft. Churchill bivouac. Neither the 24-hour totals, nor the ratio of the excretion during the day (when the exposure was greatest) to that at night (when it was least) showed any consistent change. The findings seem to indicate that the subjects may have been adapted to the stress (cold and exercise) during the bivouac period at Ft. Churchill prior to the first urine collections. No 17-ketosteroid determinations were carried out during the period immediately after arrival at Ft. Churchill. It is quite possible that a change in 17-ketosteroid excretion might have occurred during that time. However, the main object of this investigation was to compare the 17-ketosteroid excretion during two bivouac exercises, first under conditions of a temperate climate and later under arctic conditions which were so designed as to be similar except for the factor of extreme cold. The results reported on the 17-ketosteroid excretion are in accord with the findings of Stein et al., who found no significant change in 17-ketosteroid excretion following repeated exposure of subjects to a temperature of -20°F. for 5 hours in a cold chamber (10).

IV. CONCLUSIONS

The 17-ketosteroid excretion of seven healthy male subjects was not significantly changed during a field exercise at Ft. Churchill, Canada under conditions of extreme cold from the levels of excretion recorded for the same men during a similar exercise under temperate climatic conditions at Ft. Knox. A diurnal variation in 17-ketosteroids was noted in these subjects, the hourly excretion during the waking hours being consistently greater than that during sleep. The measurement of the excretion of 17-ketosteroids during the two bivouac periods seems to indicate that the subjects may have already been adapted to the stress (cold and exercise) during the period at Ft. Churchill prior to the first urine collections.

V. RECOMMENDATIONS

Since one cannot suppose that urinary excretion of these steroids reflects conditions in the circulatory blood, studies should be directed toward finding a convenient and reliable method for the determination of 17-ketosteroids or their precursors in blood.

There are numerous factors which affect the level of urinary excretion other than the actual amount of active principle secreted into the

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