BIOLOGICAL RHYTHMS AND TEMPERATURE REGULATION IN RHESUS MONKEYS DURING SPACEFLIGHT

FINAL REPORT

NASA NAG2-587

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Biological Rhythms and Temperature Regulation

INTRODUCTION

Mammals have developed the ability to maintain homeostasis, i.e. a relatively constant internal environment, despite most variations encountered in their everyday environment. For example, as homeotherms mammals maintain a relatively constant body temperature. The most prominent variation encountered in the terrestrial environment is the daily alternation of light and dark. The evolution of the circadian timing system (CTS) has allowed animals to coordinate their internal time with the external environment. This physiological system enables an organism to anticipate and prepare for daily (and yearly) alterations in the environment.

Living organisms have evolved under the unvarying level of earth's gravity. Physiological and behavioral responses to changes in gravity are not completely understood. Exposure to altered gravitational environments (i.e., via hyperdynamic fields or spaceflight) has pronounced effects on physiological and behavioral systems, including body temperature regulation and circadian rhythms.

The physiological changes that occur during exposure of animals to increased acceleration fields produced via centrifugation are usually triphasic (cf. Smith, 1975, for review; Oyama, 1971). Typically, the immediate response of a system is followed by a recovery period and then a new steady state is established. One aspect of the response of primates (Fuller, 1984a), rats (Fuller, et al., 1977b; Oyama, et al., 1971; Schertel et al., 1980) and dogs (Oyama, 1975) to increased acceleration fields (hyperdynamic environments) is a substantial decline in colonic temperature. In rats, this initial decrease occurs immediately, reaching a minimum within 2 hours. The recovery period, however, can take up to several

days. During this recovery period the circadian rhythm in body temperature remains markedly depressed (Oyama, 1975).

The initial decrease in colonic temperature observed in rats is accompanied by an increased heat loss (Fuller et al., 1977b). Decreases in oxygen consumption (heat production) have also been observed in rats (Oyama & Chan, 1973). These changes in heat production and loss were determined not to be thermoregulatory responses elicited by changes in temperature at the sites of temperature reception. This was determined by comparison of the skin (tail), hypothalamic and spinal cord temperatures. These anatomic locations are the site of receptors which, in the rat, serve as inputs for the neural control of core temperature (Fuller et al., 1975; 1977a). All sites showed depression similar to that seen in core temperature (Fuller et al., 1977b). The increased heat loss found to occur during the fall in body temperature would not have been predicted on the basis of thermoreceptor inputs to a normally functioning controller.

During the recovery phase of the temperature response, there is a marked deficit in the ability of rats to maintain body temperature during acute cold exposures (Fuller et al., 1977b; Giachino et al., 1979; Horowitz et al., 1979). The cause of this thermoregulatory impairment is not clear. It may be the result of changes in any of several factors including: 1) central and/or peripheral thermosensitivity, 2) the ability to gain or lose heat, or 3) integrative ability of the central neural controller for thermoregulation.

Recently, a time of day component to the response of the thermoregulatory system to exposure to hyperdynamic fields has been identified (Fuller et al., 1992). In both rats and squirrel monkeys, acute exposure to 2G during the inactive or rest phase causes a significantly greater fall in body temperature than exposure during the active phase. The larger decreases in body temperature are seen when exposure occurs during the day in nocturnal rats and during the night in the diurnal squirrel monkey. Interestingly, in squirrel monkeys, the recovery phase is also affected by the time of day of onset of centrifugation.

Body temperature amplitude is significantly decreased when onset of centrifugation is begun at the midpoint of the light portion of the cycle.

The Bioflights in the late 1950's provided the first temperature data from a primate in microgravity (Graybiel et al., 1959). Although many problems existed in interpretation of data (i.e., axillary instead of core temperature, short flight duration, highly stressed animals, etc.), there was a suggestion of a general decline in body temperature during the flights.

The earliest record of primate temperature data during spaceflight is that of Biosatellite III (Hahn et al., 1971). Although this animal was compromised, three applicable observations are apparent from the data: 1) there was a depression in body temperature during the flight, 2) the circadian rhythm of body temperature persisted but was "free-running" and not entrained to the ambient light-dark cycle and 3) there were substantial changes in the sleep-wake behavior, including shifts in the phase-angle relationship to the LD cycle (with synchrony to 24-hr maintained) and fragmentation of the sleep-wake cycle (Hanley & Adey, 1971; Hoshizaki et al., 1971). The loss of synchronization of the temperature rhythm, while the sleep rhythm remained synchronized, suggests that the altered gravitational field had a selective effect on the circadian timekeeping system. These data are consistent with our current understanding of the mammalian circadian timekeeping system. The circadian clock regulating the temperature rhythm is different from the clock regulating behaviors such as drinking and rest-activity (Fuller et al., 1981). Some of the recent Soviet COSMOS monkeys also showed a decrease in body temperature of 0.50-0.75°C, with a suggestion of altered phase-control of the temperature rhythms.

This program examined the influence of microgravity on temperature regulation and circadian timekeeping systems in Rhesus monkeys. Animals flown on the Soviet Biosatellite COSMOS 2229 were exposed to 11 2/3 days of microgravity. The circadian patterns temperature regulation, heart rate and activity were monitored constantly. This experiment has extended our previous observations from COSMOS 1514 (Sulzman et al., 1986) and

2044 (Fuller et al., 1993a), as well as provided insights into the physiological mechanisms that produce these changes.

METHODS

Two male Rhesus monkeys (M151 and M906), weighing 3.5 to 4.0 kg, were used in three experimental paradigms to study the effects of microgravity on circadian rhythms and temperature regulation. The animals were studied in a 3-5 day baseline control experiment verifying all procedures and collecting baseline data prior to the flight of the Biosatellite. The animals were flown for 11 2/3 days on the Biosatellite and subsequently studied in a 3 day postflight experiment which began 13 days after the flight. Six weeks after recovery, a second, longer control study was performed, but the data are not yet available for analysis. In all conditions, the animals were housed in a 24 hour light-dark cycle (LD 16:8; lights on 08:00-24:00). The primates were fed 250 gram meals twice each day (08:00-10:00 and 18:00-20:00) and performed behavioral tests four times per day (08:00, 13:00, 16:00 and 21:00). The atmosphere inflight was maintained at sea level partial pressures and barometric pressure. The animals were extensively trained to sit in a chair-like restraint device for the duration of the experiments.

Measured parameters for each animal included: brain temperature, axillary temperature, head skin temperature, thigh skin temperature, heart rate, motor activity, and ambient temperature at the upper portion of the chair. Except for brain temperature (which was collected at one minute intervals), data were collected at 10 minute intervals. All data were stored on a battery operated data logger (Vitalog, modified by L & M Electronics).

The heart rate was derived from the Soviet EKG signal by a US supplied R-wave detector (L & M Electronics). The axillary temperature measurements were taken from the output signals of a German biotelemetry transmitter (BTS BT) implanted in the axilla of each animal. The activity measurements were derived from a piezoelectric transducer (L & M Electronics) attached to a harness over the animals chest. Brain temperature was recorded by means of an electrode implanted superior to the caudate nucleus (A15; L10, V-25mm;

Snider & Lee, 1961) using a microbead thermistor encased in 25 gauge stainless steel tubing. The rest of the temperatures were measured via thermistors (YSI series 400 probes). The head skin temperature was attached to the temple of the animals. The thigh and ankle skin temperature sensors were super-glued to the animals' skin in those locations. To provide strain relief, the leg sensors were then taped in place. The ambient temperatures were measured at the top of the restraint system.

After each experiment the data were transferred to a microcomputer for analysis and storage. The analysis of the data included: examination and plotting of the raw data files, phase, waveform, period and statistical analysis. The statistical analyses yielded daily 24 hour means, light means, dark means and standard errors. Phase analysis was performed by fitting a sine wave to 24 hours of data using the least squares technique to compute circadian phase and amplitude. Waveform analysis was performed by repetitive waveform reduction. Period analysis was performed by the periodogram technique. Data were subjected to analysis of variance for repeated measures and the individual values compared using the Tukey test.

RESULTS

Circadian rhythms persisted in both subjects in all three conditions (preflight, flight, postflight). There was an increase in ambient temperature during the last three days of the flight. This increase was such that several of the temperature sensors (skin and ambient) reached the upper maximum of their range. This fact will bias the data from these sensors at this time such that maxima and means will be higher than actually reported. In addition, some alteration of the waveform of the data also occurred, which may slightly bias the circadian analysis of the data. To avoid any confounding influence this increase may have had, to examine variables across comparable lengths of time of collection, and to examine the response of the thermoregulatory system to the increased ambient temperature, the following data were examined: preflight days 2-3; flight days 2-3 (early), 7-8 (mid), 11-12 (late) and postflight day 2-3. Analysis of the data at intervals over the course of flight also

allowed a preliminary examination of the acute vs. chronic effects of the spaceflight environment. Average data for each measured variable are given in Table 1. The summary of the responses of each animal is described individually by variable below. All values given are 2-day means with standard errors.

Brain Temperature. This is the first of the COSMOS bioflights to measure brain temperature in a non-human primate. As mentioned previously, this variable was recorded at one minute intervals, allowing a very precise delineation of the daily rhythm in brain temperature. Averaged data from each animal is presented as a histogram in Figure 2. The average phase of the brain temperature was 15.1 $\pm$ 0.12 hours preflight. The is advanced from the position of 16.6 $\pm$ 0.23 hours which was seen on days 2-3 of flight. There was a slight delay to 17.0 $\pm$ 0.38 hours by days 7-8 of flight and a return back to an earlier time during days 11-12 (16.6 $\pm$ 0.85 hours). Postflight the average phase of the brain temperature rhythm was 14.4 $\pm$ 0.39 hours. The phase of brain temperature was significantly earlier postflight than during any other interval ($p<0.05$).

The amplitude of the brain temperature rhythm was 0.85 $\pm$ 0.01 °C preflight. This was slightly larger than the 0.76 $\pm$ 0.03 °C exhibited early in the flight. There was a slight decrease to 0.70 $\pm$ 0.05 °C by midflight with an increase to 0.86 $\pm$ 0.11 °C by the end of the flight. The postflight brain temperature amplitude was 0.74 $\pm$ 0.08 °C.

Mean brain temperature was 38.73 $\pm$ 0.20 °C preflight, comparable to the 38.80 $\pm$ 0.15 °C seen at the start of flight. Average brain temperature decreased slightly (to 38.56 $\pm$ 0.3 °C) by midflight and returned back to preflight levels (38.82 $\pm$ 0.11 °C) by the end of flight. The pattern was generated by the response of animal M151. While the average brain temperature of M906 remained between 38.95 and 39.01, M151's average brain temperature fell from 38.63 °C at the start of flight to 38.1 °C midflight. It returned back to 38.3 °C during the last two days of flight. Postflight mean brain temperature averaged 39.02 $\pm$ 0.06 °C.

Axillary Temperature. Axillary temperature data is reported as a frequency output of the sensor, and not as absolute body temperature. The frequency output of the sensor

decreased as body temperature increased. The raw plots of axillary temperature thus show high body temperatures as occurring during the dark (the reverse of the actual body temperature rhythm for this diurnal species). This is also the reason that the phases (which are the time of the acrophase of a sine wave fitted to 24 h of data) of axillary temperature occur during the dark period. Further, axillary temperature was not recorded in animal M151 during the preflight experiment, nor was it recorded from either subject postflight. The following data are shown graphically in Figure 3.

Animal M906 had an average phase of the axillary temperature rhythm of 3.45 $\pm$ 0.03 hours preflight. The average axillary temperature phase inflight for M906 and M151 began at 4.62 $\pm$ 0.18 hours. This delayed slightly by midflight to 5.07 $\pm$ 0.39 hours. A further delay to 6.10 hours was seen on days 11 of flight, the data from day 12 of flight was not complete enough to perform a curve fitting.

The amplitude of the axillary temperature rhythm for M906 preflight was 16.41 $\pm$ 0.14 . For both animals the average axillary amplitude was 14.0 $\pm$ 1.54 on days 2-3 of flight, 14.7 $\pm$ 0.87 on days 7-8 and 15.0 $\pm$ 3.11 on days 11-12.

Mean axillary temperature showed a slight decrease at midflight, compared early and late flight. This is shown by the slightly higher average frequency recorded at this time.

Head Skin Temperature. This variable also showed a later phase inflight than preflight. Preflight phase was 14.9 h $\pm$ 0.74 hours; inflight phase began at 16.4 $\pm$ 0.79 hours, delayed to 18.0 $\pm$ 0.16 hours by midflight and slightly advanced to 17.8 $\pm$ 1.65 hours by late flight. Postflight the average phase of the head temperature rhythm was 14.63 $\pm$ 1.27 hours. This response was seen in both animals. The phase of this variable became very unstable during the high ambient temperatures seen during the last three days of flight. Results of data analysis are plotted in Figure 4.

The amplitude of the head skin temperature rhythm decreased from preflight to early flight (o.59 $\pm$ 0.13 °C to 0.35 $\pm$ 0.09°C). As flight advanced the amplitude increased to 0.56 $\pm$ 0.06 °C. During the late flight, there was a further increase in this amplitude (1.01 $\pm$ 0.18 °C)

as the animals were increasing blood flow to the skin in order to thermoregulate in the face of the thermal load of the increased ambient temperature. The postflight amplitude was 0.82 ± 0.17 °C.

The use of vasomotion was also seen in the average head skin temperature, which rose from a relatively constant level (33.53 ± 1.69 °C pre; 33.86 ± 0.85 °C d2-3; 33.59 ± 0.78 °C d 7-8) to 36.59 ± 0.4 °C on d 11-12. Postflight the mean head skin temperature was 34.17 ± 1.29 °C.

Thigh Skin Temperature. This variable was not measured during the preflight or postflight conditions. The following data are presented as a histogram in Figure 5. During flight the average phase of the upper leg skin temperature was fairly stable (18.5 ± 1.63 hours d2-3; 18.1 ± 1.22 hours d7-8; 18.2 ± 1.71 hours d11-12).

The amplitude of the thigh skin temperature rhythm showed an initial decrease (from 1.09 ± 0.15 °C d2-3 to 0.73 ± 0.07 °C d7-8), but, as with head skin temperature increased during d11-12 (1.20 ± 0.23 °C).

This pattern was also seen in the mean thigh skin temperatures; averages were 32.64 ± 0.63 °C for d2-3. 33.69 ± 0.36 °C for d7-8; and 36.59 ± 0.40 °C for d11-12.

Ankle Skin Temperature. These data are presented graphically in Figure 6. The phase of the ankle skin temperature rhythm was very different during spaceflight. Preflight the average phase was 15.0 ± 2.55 hours; while postflight it was 21.41 ± 2.95 hours. However, during spaceflight the average ankle skin temperature phase was 6.9 ± 3.53 hours on d2-3; 6.1 ± 3.73 hours on d 7-8 and 18.7 ± 1.84 hours during the high ambient temperature of d 11-12.

The amplitude of the rhythm was also initially increased in spaceflight (1.0 ± 0.63 °C preflight; 3.16 ± 1.17 °C d2-3). The amplitude decreased to 1.04 ± 0.45 °C on d7-8. There was a slight increase to 1.34 ± 0.46 °C on days 11-12. Postflight ankle skin temperature amplitude averaged 1.64 ± 0.79 °C.

Mean ankle skin temperature again reflected the vasomotor response to the ambient temperature. Mean skin temperature at this site was 26.8 ± 1.88 °C preflight, 27.35 ± 0.84 °C d2-3 of flight, 26.83 ± 0.4 °C d7-8 of flight. It rose to 35.5 ± 0.55 °C during d11-12 of flight. Postflight mean ankle skin temperature averaged 32.06 ± 1.21 °C. Mean ankle skin temperature was significantly higher during late flight than during either preflight or mid flight ($p<0.05$).

Heart Rate. Figure 7 is a plot of the average heart rate circadian phase, amplitude and 24 hour mean for each animal in the five experimental data sets. Preflight, the animals had an average phase of approximately 14.9 ± 0.94 hours. At the start of flight, the average phase was 12.7 ± 0.28 hours. This delayed to 17.9 ± 2.43 hours at midflight and advanced back to 15.1 ± 1.35 hours at the end of flight (during the high ambient temperature). Postflight, the average phase of the heart rate rhythm was 13.0 ± 0.52 hours.

The average amplitude of the heart rate rhythm was larger preflight (30.1 ± 4.29 bpm) than at the start of flight (10.6 ± 2.51 bpm). This increased slightly midflight (12.1 ± 3.44 bpm) and late in the flight (12.5 ± 2.22 bpm). The postflight amplitude averaged 11.2 ± 1.10 bpm. A statistical difference was revealed by ANOVA ($p<0.025$). A Tukey test revealed that the preflight amplitude differed from all flight amplitudes as well as from the postflight amplitude.

Mean heart rate during the preflight experiment was 144.8 ± 17.41 bpm. This was higher than the average heart rate during days 2-3 of flight (128.7 ±3.46 bpm), days 7-8 (117.2 ± 20.1 bpm) and days 11-12 (108.0 ± 17.0 bpm). Postflight, the daily mean heart rate was 141.4 ± 7.5 bpm. It should be noted that while the average heart rate for both animals was smaller inflight than preflight, the drop was noticeably larger in M151. This animal also exhibited a decrease in food consumption and activity level over the flight.

Activity. The activity measurement is a relative one, depending on the placement and sensitivity of the sensor used. As such, activity levels and amplitudes can only directly be compared within the course of a single experiment. The average phase of the activity rhythm of the two animals was 15.6 ± 0.23 hours preflight, as shown in Figure 8. This is close to the

value of 16.1 ±.35 hours seen during days 2-3 of flight. However, by days 7-8 of flight the average phase of the activity rhythm had delayed to 19.6 ±.51 hours. As was the case with the phase of the heart rate rhythm, this advanced (to 17.2 ± .42 hours) during the last 2 days of flight. Postflight the average activity phase was 14.7 ± 0.37 hours. Activity phase showed a statistically significant difference (p,0.005). The phase at midflight was significantly later than preflight, early in the flight, during late flight and during the postflight experiment.

During flight the amplitude of the activity rhythm rose from 120.4 ± 27.43 counts/10 minutes during days 2-3 to 173.9 ± 27.41 counts/10 minutes on days 7-8. The mean activity amplitude remained high at the end of the flight (172.1 ± 30.62 counts/10 minutes).

Mean activity levels rose slightly from 662.5 ± 26.7 counts/10 minutes on days 2-3 to 687.0 ±131.2 counts/10 minutes on days 7-8. Mean activity levels then decreased to 582.1 ±160.5 counts/10 minutes during the last two days of flight. This pattern of average activity was driven by animal M906 who exhibited an increase followed by a slight decrease in activity. M151 exhibited a continued steady decline in activity level over the course of the flight.

Ambient Temperature. Mean ambient temperature was 25.2 ± 0.24 °C preflight. From early to midflight it remained relatively stable (24.9 ± 0.31 °C; 25.6 ± 0.78 °C), but rose significantly during the late flight (31.5 ± 0.24 °C; $p<0.005$). Postflight the mean ambient temperature was 27.24 + 1.19 °C. These data are presented in Figure 9.

DISCUSSION

Temperature Regulation. Previous experiments have shown that skin temperatures are reduced in spaceflight. This, in concert with a reduced heart rate and activity level, combined with reduced food intake of the animals during the flight suggest a decrease in metabolism; analysis of metabolic rate using the double-labeled water method revealed a decrease of up to 40% in these two subjects during spaceflight (Fuller et al, 1993b). In this experiment, both head and ankle skin temperatures were lower inflight (early and mid) than during the postflight experiment, despite the fact that ambient temperature was higher in the

postflight experiment. However, during the preflight experiment, head skin temperature was lower than during flight. Ankle skin temperature was higher preflight than at the start of flight, but decreased at midflight to the preflight level. These data continue to suggest that the thermoregulatory system is operating at a lowered level while the organism is in a microgravity environment. However, there were variations between the animals and analysis of the second postflight data will be required to more completely understand these responses.

Circadian Timing System. Several previous observations of different organisms suggest that the circadian timing system is composed of two or more central pacemakers. The limited data sets that are available from spaceflight experiments suggest that these pacemakers show differential responses to the microgravity environment.

The pacemaker involved in regulating the timing of variables such as heart rate and motor activity consistently evidence appropriate 24 hour rhythms in the presence of a 24 hour light-dark cycle. Various reports have suggested the possibility of a phase angle shift but not a change in period. In contrast, the pacemaker that is involved with the circadian regulation of parameters such as body temperature does not show such stability. Frequent reports have indicated that temperature rhythms. in the microgravity environment, even in the presence of a light-dark cycle have non-24 hour or significantly altered 24 hour rhythms. While such robust changes were not observed in this flight, there were deviations from the 24 hour patterns in some of the temperature variables, most notably in ankle skin temperature.

Conclusion. The data that were collected during this experiment further confirm that temperature regulation, metabolism and circadian timing are altered in the microgravity environment of spaceflight. Increased knowledge of these alterations will assume increasing importance as our forays into this environment increase in frequency and duration.

REFERENCES

Fuller, C. A., 1984a. Aviat. Space. Environ. Med. 55:226-330.

Fuller, C. A., Horowitz, J. M., Horwitz, B. A., 1977a. J. Appl. Physiol. 42:154-158.

Fuller, C. A., Horwitz, B. A., and Horowitz, J. M., 1977b. J. Appl. Physiol. 42:74-79.

Fuller, C. A., Horwitz, B. A., and Horowitz, J. M., 1975. Am. J. Physiol. 228:1519-1524.

Fuller, C. A., Tremor, J., Connolly, J. P. and Williams, B. A., 1981. Physiologist 24:S111-112.

Fuller, C. A., T. M. Hoban-Higgins, D. W. Griffin and D. M. Murakami. 1992. COSPAR, in press.

Fuller, C. A., T. M. Hoban-Higgins, A. Alpatov, and V. Klimovitsky. 1993a. In: COSMOS 2044 Biosatellite Mission Description and U. S. Final Reports of Monkey, Rat and Radiation Experiments. NASA, in press.

Fuller, C. A., T. P. Stein, D. W. Griffin, M. Dotsenko, A. Truchenekov and V. Koroldov. 1993b. In: COSMOS 2229 Final Results Symposium, Moscow, 1993.

Giacchino, J., Horowitz, J. M., and Horwitz, B. A., 1979. J. Appl. Physiol. 46:1049-1053.

Graybiel, A., Holmes, R. H., Beischer, D. E., Champlin, G. E., Pedigo, G. P., Hixson, C., Davis, T. R. A., Barr, N. L., Kistler, W. G., Niven, J. E., Wilbarger, E., Stullken, D. E., Augerson, W. D., Clarke, R., Berrian, J. H., 1959. Aerospace Med. 30:871-931.

Hahn, P. M., Hoshizaki, T., and Adey, W. R., 1971. Aerospace Med. 42:295-304.

Hanley, J., and Adey, W. R., 1971. Aerospace. Med. 42:204-313.

Horowitz, J. M., Schertel, E. R., and Horwitz, B. A., 1979. The Physiologist 22:S57-S58.

Hoshizaki, T., Durham, R., and Adey, W. R., 1971. Aerosp. Med. 42:288-295.

Oyama, J., 1971. In: *Regulatory Biology: Depressed Metabolic States.* NASA TM-X-69354, pp. 27-51.

Oyama, J., 1975. COSPAR Life Scis. Space Res. XIII. P. H. A. Sneath, eds. Akademik-Verlag (Berlin) p. 2-17.

Oyama, J., and Chan, L., 1973. Fed. Proc. 32:392.

Schertel, E. R., Horowitz, J. M., and Horwitz, B. A., 1980. J. Appl. Physiol. 49:663-668.

Smith, A. H., 1975. In: *Foundations of Space Biology and Medicine.* M. Calvin and O. Gazenko, eds. NASA (Washington, , DC) p. 125-172.

Sulzman, F. M., Fuller, C. A. and Moore-Ede, M. C., Klimovitsky, V., Magedov, V., and Alpatov, A.M., 1986. In: *Final Reports of U.S. Monkeys and Rat Experiments Flown on the Soviet Satellite COSMOS 1514*, eds. R.C. Mains and W.W. Gomersall, NASA [Moffett Field] , p. 71.

Weir, J.B. deB., 1949. J. Physiol. (London) 109:1-9.

FIGURE LEGENDS

Table 1: Circadian phase and amplitude and 24 hour means for each variable. The data are averaged and given with standard errors for: preflight, early flight (d2-3), midflight (d7-8), late flight (d11-12) and postflight. All phases are in hours. Activity amplitude and mean are in counts per 10 minute interval. Heart rate amplitude and mean are in beats per minute (bpm). Amplitude and mean values for all temperatures other than axillary are in °C; axillary temperature is reported as the frequency output of the sensor. A higher frequency is indicative of a lower temperature.

Figure 1: The complete data set for one animal (M906) for recorded during the flight of COSMOS 2229. All variables are plotted vs. time of day. The light-dark cycle is indicated by the light and dark bars at the top of the graph. Data shown include: A - brain temperature (in °C), B - axillary temperature (in frequency of the sensor), C - head skin, D - thigh skin, and E - ankle skin temperatures (in °C), F - heart rate (in beats per minute), G - activity (in counts per 10 minute interval) and H - ambient temperatures (in °C). Brain temperature was recorded at 1 minute intervals, all other data were recorded at 10 minute intervals. The frequency of the axillary temperature sensor increases as temperature decreases, thus higher values, indicating a decreased temperature, are seen at night. Data records begin when the animals were placed in the BIOS chamber. Launch which occurred on 12-29-92 at 1630 hours. Landing and recovery occurred on 1-10-93 at 0716.

Figure 2: Brain temperature data. These data are two day means from: days 2 and 3 of preflight, days 2 and 3 of flight (early), days 7 and 8 of flight (mid), days 11 and 12 of flight (late) and days 2 and 3 of the short postflight experiment that was conducted 10 days after flight (spgrd).

Panel A: Average circadian phase (in hours).

Panel B: Average amplitude of the circadian rhythm of heart rate (in °C).

Panel C: Average mean heart rate (in °C).

Figure 3: Axillary temperature data is presented as described in Figure 2. The axillary temperature is reported as the frequency of the output of the sensor, and which has an inverse relationship to actual temperature. Therefore, the highest values of frequency occur during the night, the time of the minimum body temperature.

Figure 4. Head skin temperature data is presented as described in Figure 2.

Figure 5. Thigh skin temperature data is presented as described in Figure 2.

Figure 6: Ankle skin temperature data is presented as described in Figure 2.

Figure 7 Heart rate data are shown as described in Figure 2. Heart rates are in beats per minute.

Figure 8. Activity data is presented as described in Figure 2. Activity levels are in counts per 10 minute interval.

Figure 9: Mean ambient temperature data for each condition (°C).

II

K-7-35 (II)

Metabolism

INTRODUCTION

Few measurements of metabolism have been made in altered gravitational fields. The work of Oyama and Chan (1973) suggests that a reduction in metabolism occurs during an acute exposure to a hyperdynamic environment. The observed fall in body temperature during spaceflight, such as discussed in Section I, could in part be explained by an alteration in metabolism. Further, the depression of the skin temperatures suggests a heat conservation mechanism was activated for the duration of the flight. Whether these two factors (reduced body and skin temperatures) indicates an alteration in metabolism or a decrease in the thermoregulatory set point cannot as yet be determined.

A priori, one would expect an organism's energy requirements at 0 G to be less in space because of the absence of the work necessary to oppose gravity (Rambaut et al., 1977). However, data collected during the Skylab missions suggest that human energy expenditure may be increased during space flight (Leonard, 1983; Rambaut et al., 1977). The astronauts lost weight throughout the flight with most of the weight being lost early in the flight.

Energy expenditure can be regarded as the sum of two components; the basal metabolic rate and the energy costs of activity. Weight loss is usually associated with an energy deficit. A negative energy balance exists when energy intake is less that energy utilization. The deficit is made up by tissue catabolism (principally fat, but also some protein). By analyzing food and water intake, urine and fecal output, and changes in body weight, the Skylab investigators reached the unexpected conclusion that energy expenditure during

spaceflight was about 5% greater than at 1 G (Leonard, 1983; Rambaut et al., 1977).

Conversely, two recent COSMOS flights (1514 and 1667) examined the daily food intake of rhesus monkeys during spaceflight. All four monkeys exhibited a decrease in food intake during spaceflight (5 to 7 days) relative to the amount consumed before flight (Sulzman et al., 1986; Gazenko & Ilyin, 1987). Similarly, two squirrel monkeys flown on Spacelab 3 showed decreased food intake (Fuller, 1985). It is not certain, however, what role space adaptation syndrome played in depression of appetite on these flights.

Possible explanations for the human metabolic responses are an increased workload during spaceflight (Leonard, 1983), or as Rambaut and coworkers (1977) suggested, a progressive decrease in metabolic efficiency. It is likely to be very difficult to distinguish between these two possibilities in man because the activity component may be different during spaceflight than it is on the ground. The problem is to measure energy expenditure with efficient precision during spaceflight in a non-invasive manner which will not interfere with other investigations or take any time. The doubly labeled water (DLW) method meets these criteria.

The doubly labeled water ($^2H_2{}^{18}O$) method for measuring energy expenditure is simple, non-invasive and highly accurate. The method was originally described by Lifson in 1955 and applied to man by Schoeller in 1982 (Lifson & McClintock, 1966; Schoeller & Webb, 1982). If $^2H_2{}^{18}O$ is given orally, it mixes with the body water in about 3 hours (Schoeller & van Santen, 1982; Schoeller, 1983). The two isotopes then leave the body at different rates. ^2H leaves as water, mainly in the urine, whereas ^{18}O leaves both as water and exhaled CO_2. Thus the turnover rate of isotopic hydrogen and oxygen labeled water differ, and that difference is proportional to the rate of CO_2 production.

The method has been validated in man, normal animals, and in animals in metabolically perturbed states and animals in the grossly non-steady state such as a complete fast (Coward & Prentice, 1985; Klein et al., 1984; Schoeller & van Santen, 1982; Schoeller & Webb, 1982; Stein et al., 1987). It has been used to measure the energy cost of the flight of birds as well as of free ranging wild animals (for review, see Nagy, 1982). Thus, the method has been shown to be widely applicable. The principal sources of error with the method are analytical errors and non-steady state sampling of body fluids (Jones et al., 1988; Nagy, 1982; Schoeller & Taylor, 1988; Stein et al., 1987).

The DLW method is the only method available for continuously measuring energy expenditure during spaceflight given the severely restricted conditions in the spaceflight environment. Therefore, this study focuses on the development and use of this procedure on nonhuman primates during spaceflight.

Energy expenditure and total body water was determined in two Rhesus monkeys by the doubly labeled water ($^2H_2{}^{18}0$) method. Three determinations were made. Monkey B (#2483) was studied twice, during the flight of COSMOS 2044 and during a follow-up ground control study a month later. A second monkey was studied on the ground only (Monkey D, #782).

METHODS

A. Procedure

Two monkeys were studied, with one monkey (#2483) being studied twice, once during the flight of COSMOS 2044 and once postflight. The second monkey (#782) was a ground control.

The urine collection protocol is outlined in Figure 12. The same protocol was used for the inflight and two ground control studies. The first sample is the preflight baseline specimen. Samples 2, 3, and 4 (3,6,9 hr post DLW) are

the post-dosing urines for determination of TBW by extrapolation. Samples 5 and 6 (Preflight) are dual purpose samples. They should be about 3 hours apart and are for: i) completion of the TBW extrapolation and ii) the first of the two points for calculation of the ^{18}O and ^{2}H loss rates. They are essentially duplicates. Samples 7 and 8 (Postflight) are also dual function points. They are the second point of the two points for the two pair determinations of the ^{18}O and ^{2}H loss rates and secondly, the baseline for the second dosing with ^{18}O to determine body water postflight. They are essentially duplicates. Samples 9, 10 and 11 (3,6,9 hr post DLW) are the post-second dose of isotope urines to be used for the poststudy TBW determination by extrapolation back to t = 0 (isotope dosing time).

17.0 g of 99.1% $^{18}0$ labeled water (Isotec Inc., Miamisburg, OH, batch #NS0894) was added to 4.32 g of 99.9% Deuterium oxide (ICONS Inc., Summit, NJ, batch #2084-2919) to give a mixture with the final composition listed in Table 1. In the subsequent paragraphs this is referred to the stock DLW or water.

TABLE 1

ISOTOPIC ENRICHMENT OF LABELED WATER MIXTURE

$^{18}0$ enrichment	77.71%
^{2}H enrichment	21.74%

A baseline urine was collected (sample #1) and then the animals dosed with $^{2}H_2{}^{18}0$ as indicated in Table 2 (1.25 g/kg of the stock DLW). The DLW was mixed with apple juice and administered orally.

TABLE 2.

ISOTOPE DOSES

Monkey #	Study	Dose of DLW (g)
B' (2483)	Preflight	5.142
B' (2483)	Postflight	0.757
B" (2483)	Preground control	4.704
B" (2483)	Postground control	0.724
D' (782)	Preground control	5.151
D' (782)	Postground control	0.596

Serial urines were then collected at three hourly intervals for the next 10-15 hours. Approximately 17 days later, at the end of the study period, two postflight/poststudy urines were collected. The animals were then redosed with DLW (0.2 g/kg) and three serial urines collected at three hourly intervals later.

B. Calculation

(1) Isotope distribution spaces and the total body water (TBW) _. The ^{18}O and ^{2}H isotope distribution spaces (IDS, in grams) were calculated from equation (1):

$$IDS = (d*APE\Delta_D*18.02*f)/(MW_D*(100*0.00208*\Delta_D)) \quad (1)$$

d = dose (grams)

$APE\Delta_D$ = Atom Percent Excess = enrichments of the dose relative to the predose enrichment (Δ units)

f = fractionation effect (1.047 for CO_2)

MW_D = the molecular weight of the dose water

Δ_D = enrichments of the urine relative to the predose enrichment (Δ units)

For this study we assumed that the isotope distribution space is equal to the TBW. In man, the total body water (TBW) is assumed to be equal to the ^{18}O isotope distribution space divided by 1.01.

(2) Energy expenditure _. The rate of isotope loss from the body was calculated from equation 2:

$$k_O \text{ or } k_H = (\ln \Delta pre - \ln \Delta post)/t \quad (2)$$

Δpre and $\Delta post$ = differences in isotopic enrichments of the sample and the pre-dosing background for ^{18}O or ^{2}H on pre- and postflight respectively

k_O = fractional $H_2{}^{18}O$ turnover rates

k_H = fractional $^{2}H_2O$ turnover rates

The rate of CO_2 production was calculated from the equation of Schoeller, equation 3 (Schoeller et al, 1983):

$$rCO_2 = 0.481\ N(1.01k_O - 1.04k_H) - 0.0258N(k_O - k_H) \quad (3)$$

rCO_2 = rate of CO_2 production (mol/day)

N = total body water (moles).

The Weir equation (equation 4) was used to convert the rate of CO_2 production into energy expenditure values.

$$EE = 3.941\ VO_2 + 1.106\ VCO_2 - 2.17U \quad (4)$$

$$rCO_2 = VCO_2/22.4 \quad (5)$$

$$RQ = VCO_2/VO_2 \quad (6)$$

RESULTS

The actual isotopic enrichments found are tabulated in Tables 3, 4 and 5. Note that all delta values are relative to standard mean ocean water (SMOW).

TABLE 3.

ISOTOPIC ENRICHMENTS FOR FLIGHT STUDY
MONKEY B' (#2483)

Sample #	$\Delta\ ^{18}O$	APE ^{18}O	$\Delta\ ^{2}H$	APE ^{2}H
B'1	42.20	0.0148	150.34	0.0030
B'2	536.56	0.1165	1636.03	0.0253
B'3	592.42	0.1280	1792.98	0.0276
B'4	614.61	0.1325	1871.94	0.0288
B'5	605.40	0.1306	1861.86	0.0287
B'6	588.57	0.1272	1809.16	0.0279
B'7	24.01	0.0111	121.24	0.0026
B'9	-0.47	0.0061	-49.74	0.0000
B'10	192.78	0.0458	614.50	0.0100
B'11	175.55	0.0423	567.23	0.0093

TABLE 4.

ISOTOPIC ENRICHMENTS FOR POSTFLIGHT GROUND CONTROL STUDY
MONKEY B" (#2483)

Sample #	$\Delta\ ^{18}O$	APE ^{18}O	$\Delta\ ^{2}H$	APE ^{2}H
B"1	-4.15	0.0053	-48.01	0.0000
B"2	778.92	0.1662	2189.52	0.0336
B"3	726.91	0.1556	2068.98	0.0318
B"4	722.10	0.1546	2005.82	0.0308
B"5	524.55	0.1140	1558.95	0.0241
B"7	142.07	0.0354	422.09	0.0071
B"9	213.15	0.0500	693.32	0.0112
B"10	192.29	0.0453	616.81	0.0100
B"11	201.35	0.0476	659.31	0.0106

TABLE 5.

ISOTOPIC ENRICHMENTS FOR GROUND CONTROL STUDY
MONKEY D' (#782)

Sample #	$\Delta\ ^{18}O$	APE ^{18}O	$\Delta\ ^{2}H$	APE ^{2}H
D'1	-3.97	0.0054	-40.68	0.0002
D'2	631.89	0.1360	1831.97	0.0275
D'3	648.97	0.396	1858.06	0.0286
D'4	664.35	0.1427	1914.85	0.0295
D'5	494.01	0.1077	1467.96	0.0228
D'7	202.91	0.0479	469.00	0.0078
D'9	222.62	0.0520	749.89	0.0120
D'10	224.69	0.0524	575.27	0.0094
D'11	220.77	0.0516	754.00	0.0121

DISCUSSION

A. Monkey B' (#2483) flight study 9/12/89 to 9/29/89

The data base in this study demonstrates the viability of this technique as an inflight measure of metabolism. Sample B'1 shows that the monkey had received a previous dose of isotopes. Day one (9/12/89, samples B'2-B'7) shows the expected enrichment pattern, a pre-equilibrium period and then by about 6 hours after body water has equilibrated with isotope, a steady decline in isotopic enrichment. Extrapolation of these points back to t = 0 gives ^{18}O = 646Δ.

The TBW value of 2.986 liters is also reasonable. It corresponds to 77.76% of body weight (4.20 kg). It is possible that this animal was in a dehydrated state when the measurements were made since a subsequent study on this animal was done October 31, 1989 (B") and the animal weighed 3.84 kg. Correlation with other TBW studies or clinical parameters (PCV, TP, BUN, Osm) would be useful in clarifying this observation.

Energy Expenditure Calculation. As predicted by the protocol, any one of points B'3, B'4, B'5 and B'6 can be used as the "first point" in a two pair calculation for calculating the isotope loss rates. The second data point is given by sample B'7. B'7 is a reasonable value, however, the duplicate B'8 sample for verification was not available. Again, the energy expenditure value of 266 kcal/monkey/d or 69.3 kcal/kg/d appears physiological.

Postflight TBW Analysis. Sample B'9 seems problematical. It appears to be unenriched, i.e., the sample is the same as the background. Accordingly, this sample was not considered in the data analysis. Further, not enough data points were available to obtain a reliable estimate of the postflight TBW. In future studies we need to increase the postflight DLW doses to enhance the reliability of this analysis.

Summary. The data demonstrate that the inflight protocol works, but the accuracy of the data could be greatly enhanced by collecting more urine samples. Specifically, two more urine samples collected at 15 and 18 hours after the initial dose and two more on return before redosing with ^{18}O would be recommended. Because the re-determination of body water postflight is of a lower priority, consideration should be given to dropping these urines in favor of collecting two more urine samples postflight approximately 3 hours apart.

B. Monkey B" (#2483) postflight study 10/31/89 to 11/19/89.

First TBW Study. Data points B"2 to B"4 are reasonable, with B"3 and B"4 suggesting that an equilibrium has been reached. Another point would have confirmed the extrapolation back to t = 0. In theory this should have been B"5. However, B"5 (^{18}O = 524.6) is so different from B"3 (^{18}O = 726.9) and B"4 (^{18}O = 722.1) that it cannot be correct. Since the ^{2}H values show the same anomalous

pattern (B"3 ^{2}H = 2069; B"4 ^{2}H = 2006; B"5 ^{2}H = 1559) it must be concluded that there is a problem with the actual sample. Thus, the TBW (2.246 liters) value is best regarded as an estimate.

Energy Expenditure. Again, of the two "post" data points, only one (B"7) was available. However, this sample seems anomalous as the ^{2}H value is too high compared to the ^{18}O value. It may have been collected just after giving the second ^{18}O dose, rather than before. This would explain the anomalously high enrichment. Again, sample B"8, had it been available, might have helped resolve this problem.

Post TBW Determination. As described above, the crucial baseline points B"7 (questionable) and B"8 (missing) are not available. Samples B"9, B"10, B"11 looks fine, but with an uncertain baseline little can be done with them.

C. Monkey D' (#782) ground control 10/31/89 to 10/19/89

First TBW Study. As with data point B"5, data point D'5 seems to be much too dilute. Thus, the enrichments of D'3 (^{18}O = 649) and D'4 (^{18}O = 664), which are about 3 hours apart are reasonable. However, 3 hours later the D'5 enrichment had dropped to ^{18}O = 494. Since the ^{2}H values show the same trend, it suggests a sample problem and not an analytical problem.

Energy Expenditure & Post TBW Determination. As with B"7, D'7 looks questionable and there is no D'8 (back-up) available According to the protocol, following the collection of D'7, the animals were redosed with ^{18}O, (0.2 g/kg) and samples collected post-dose (D'9, D'10, D'11). D'9 to D'11 looks right, and D'7 (Table 6), is so similar that it is suggestive of a post-second ^{18}O dose sample. This may be possible for D'7 since the absence of a D'8 sample precludes both the calculation of energy expenditure rate and the second TBW determination.

CONCLUSIONS AND RECOMMENDATIONS

The successful inflight data from Monkey #2483 (B' samples) show that the DLW protocol is very workable. Furthermore, the results suggest that four changes be made on future flights to ensure the accuracy of the data set.

1. Following isotope dosing preflight, urine samples should be collected every 3 hours for the first 15 hours.

2. If the flight is delayed for a few days two additional urine samples should be collected as close to flight as feasible. Large volumes of fluid intake should be avoided at this time. If fluid intake records are available, they should become part of the data set.

3. Postflight serial urine samples should be collected at 3 hour intervals for 12 hours.

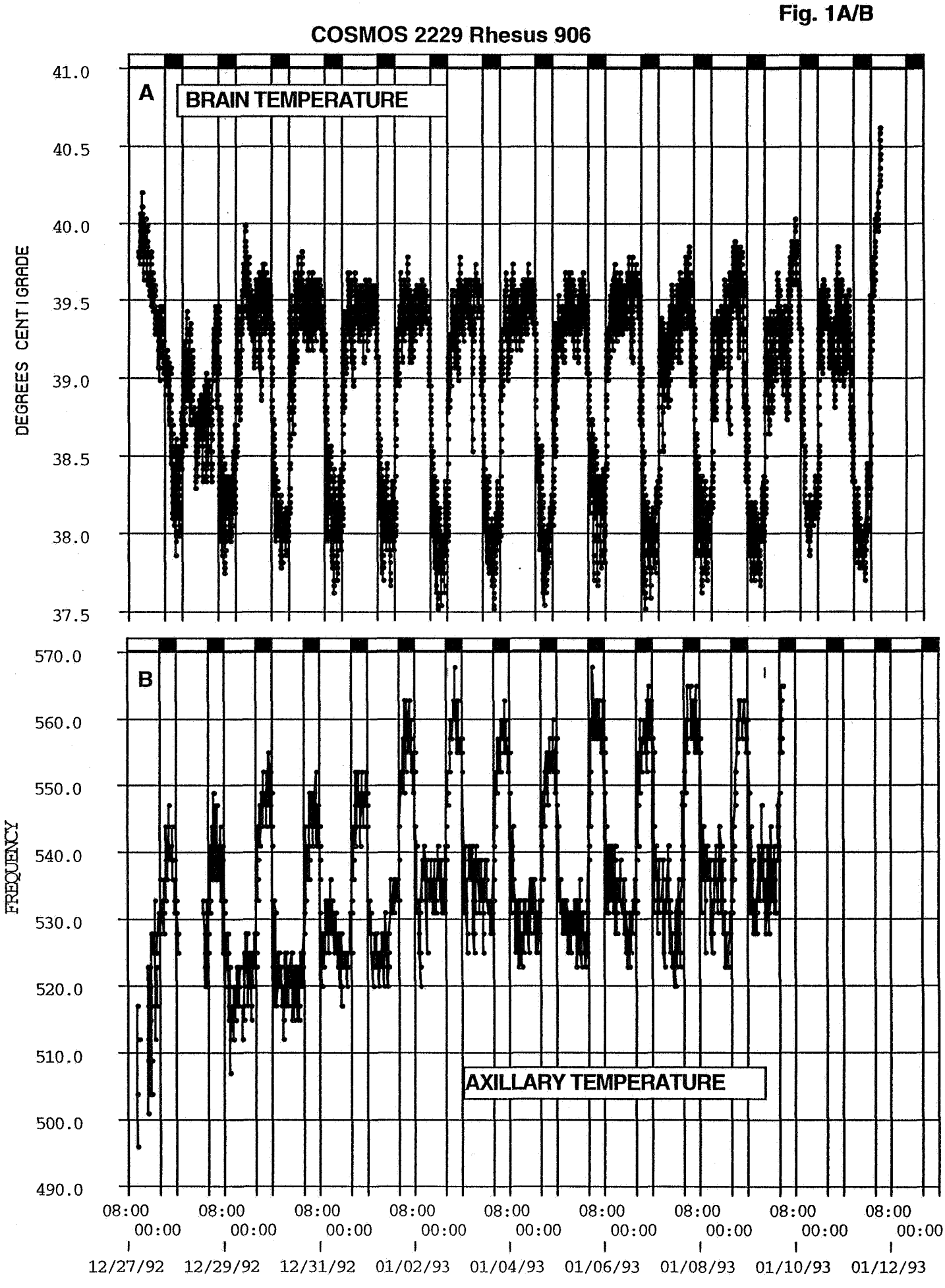
4. The postflight ^{18}O dosing should be discontinued or increased and delayed several hours.

Table 1

	ACTIVITY			HEART RATE			AXILLARY TMP			BRAIN TMP		
PREFLIGHT	PHASE	AMP	MEAN	PHASE	AMP	MEAN	PHASE	AMP	MEAN	PHASE	AMP	MEAN
AVERAGE	15.59	179.78	435.37	14.87	30.11	144.80	3.45	16.41	532.13	15.05	0.85	38.73
SEM	0.23	110.74	281.09	0.94	4.29	17.41	0.03	0.14	3.05	0.12	0.01	0.20
FLIGHT-EARLY												
AVERAGE	16.06	120.43	662.53	12.69	10.59	128.73	4.61	13.97	533.08	16.58	0.76	38.80
SEM	0.35	27.42	26.66	0.28	2.51	3.46	0.18	1.54	2.45	0.23	0.03	0.15
FLIGHT-MID												
AVERAGE	19.62	173.93	686.98	17.89	12.14	117.22	5.07	14.67	543.13	16.95	0.70	38.56
SEM	0.51	27.41	131.17	2.43	3.44	20.12	0.39	0.87	3.11	0.38	0.05	0.30
FLIGHT-LATE												
AVERAGE	17.23	172.06	582.13	15.07	12.45	107.96	6.10	14.98	535.50	16.64	0.86	38.82
SEM	0.42	30.62	160.48	1.35	2.22	16.99	0.39	3.11	3.11	0.85	0.11	0.11
POSTFLIGHT												
AVERAGE	14.70	201.20	419.19	12.99	11.21	141.37				14.39	0.74	39.02
SEM	0.37	29.27	93.94	0.52	1.10	7.46				0.59	0.08	0.06

	HEAD SKIN TMP			THIGH SKIN TMP			ANKLE SKIN TMP			AMBIENT TMP		
PREFLIGHT	PHASE	AMP	MEAN	PHASE	AMP	MEAN	PHASE	AMP	MEAN	PHASE	AMP	MEAN
AVERAGE	14.88	0.59	33.53				14.98	0.99	26.80	11.99	0.56	25.18
SEM	0.74	0.13	1.69				2.55	0.63	1.88	3.74	0.10	0.24
FLIGHT-EARLY												
AVERAGE	16.35	0.35	33.86	18.48	1.09	32.64	6.88	3.16	27.35	14.12	0.32	24.86
SEM	0.79	0.09	0.85	1.63	0.15	0.63	3.53	1.17	0.84	5.43	0.09	0.31
FLIGHT-MID												
AVERAGE	17.97	0.56	33.59	18.12	0.73	32.31	6.12	1.04	26.83	20.03	0.28	25.57
SEM	0.16	0.06	0.78	1.22	0.07	0.36	3.73	0.45	0.40	1.84	0.09	0.78
FLIGHT-LATE												
AVERAGE	17.80	1.01	36.59	18.15	1.20	36.11	18.69	1.34	35.53	18.07	0.87	31.51
SEM	1.65	0.18	0.40	1.71	0.23	0.36	1.84	0.46	0.55	1.57	0.49	0.24
POSTFLIGHT												
AVERAGE	14.63	0.82	34.17				21.41	1.64	32.06	18.03	0.52	27.24
SEM	1.27	0.17	1.29				2.95	0.79	1.21	2.34	0.21	1.19

Fig. 1A/B


COSMOS 2229 Rhesus 906
A
BRAIN TEMPERATURE
DEGREES CENTIGRADE
41.0
40.5
40.0
39.5
39.0
38.5
38.0
37.5
B
AXILLARY TEMPERATURE
FREQUENCY
570.0
560.0
550.0
540.0
530.0
520.0
510.0
500.0
490.0
08:00
00:00
12/27/92
12/29/92
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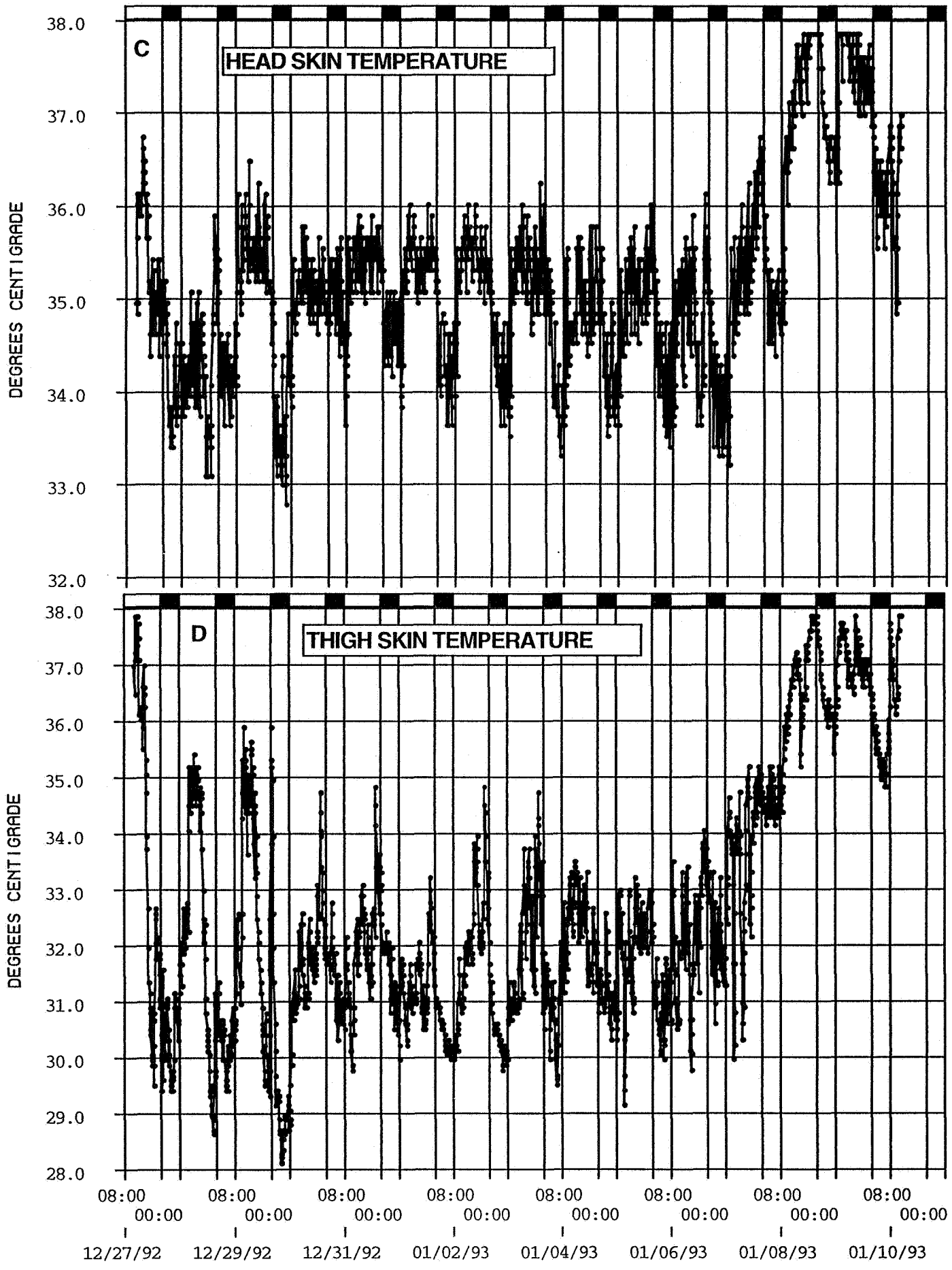
01/08/93
01/10/93
01/12/93

Fig. 1 C/D


COSMOS 2229 Rhesus 906
C
HEAD SKIN TEMPERATURE
D
THIGH SKIN TEMPERATURE
DEGREES CENTIGRADE
DEGREES CENTIGRADE
38.0
37.0
36.0
35.0
34.0
33.0
32.0
31.0
30.0
29.0
28.0
08:00
00:00
12/27/92
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01/06/93
01/08/93
01/10/93

FIG. 1 E/F

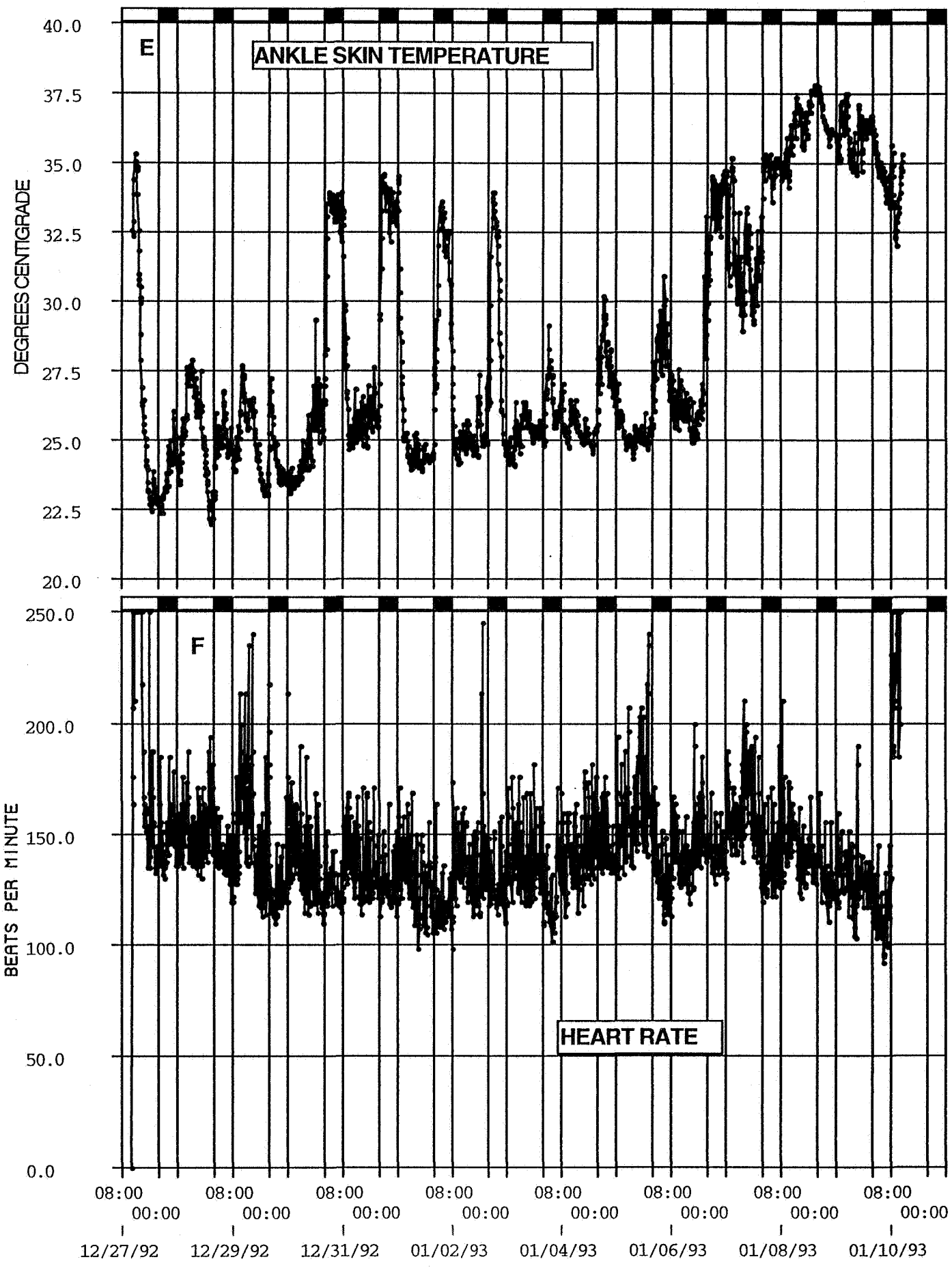
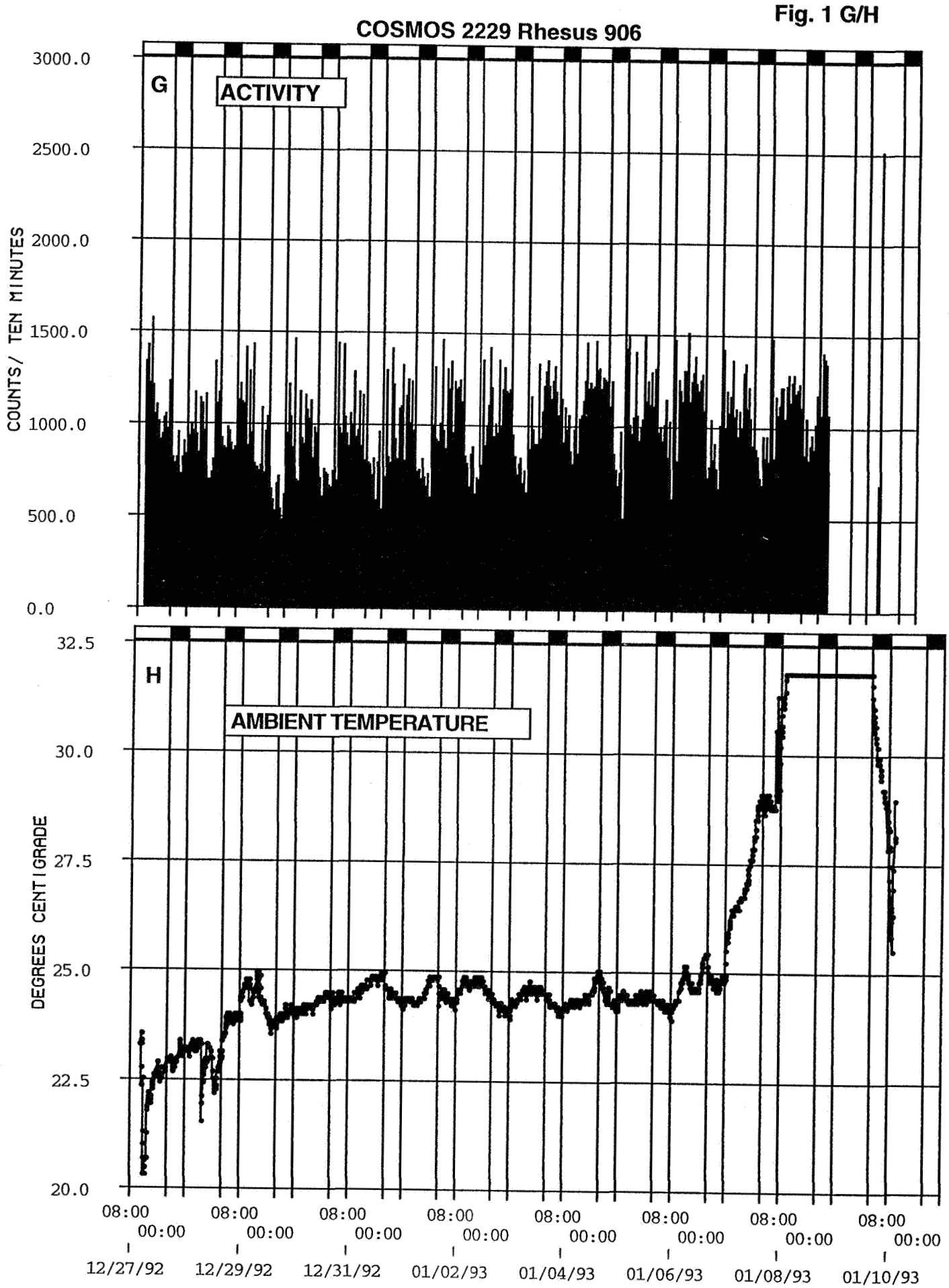

COSMOS 2229 Rhesus 906
E
ANKLE SKIN TEMPERATURE
DEGREES CENTIGRADE
40.0
37.5
35.0
32.5
30.0
27.5
25.0
22.5
20.0
F
HEART RATE
BEATS PER MINUTE
250.0
200.0
150.0
100.0
50.0
0.0
08:00
00:00
12/27/92
12/29/92
12/31/92
01/02/93
01/04/93
01/06/93
01/08/93
01/10/93

Fig. 1 G/H


COSMOS 2229 Rhesus 906
G
ACTIVITY
COUNTS/ TEN MINUTES
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
H
AMBIENT TEMPERATURE
DEGREES CENTIGRADE
32.5
30.0
27.5
25.0
22.5
20.0
08:00
00:00
12/27/92
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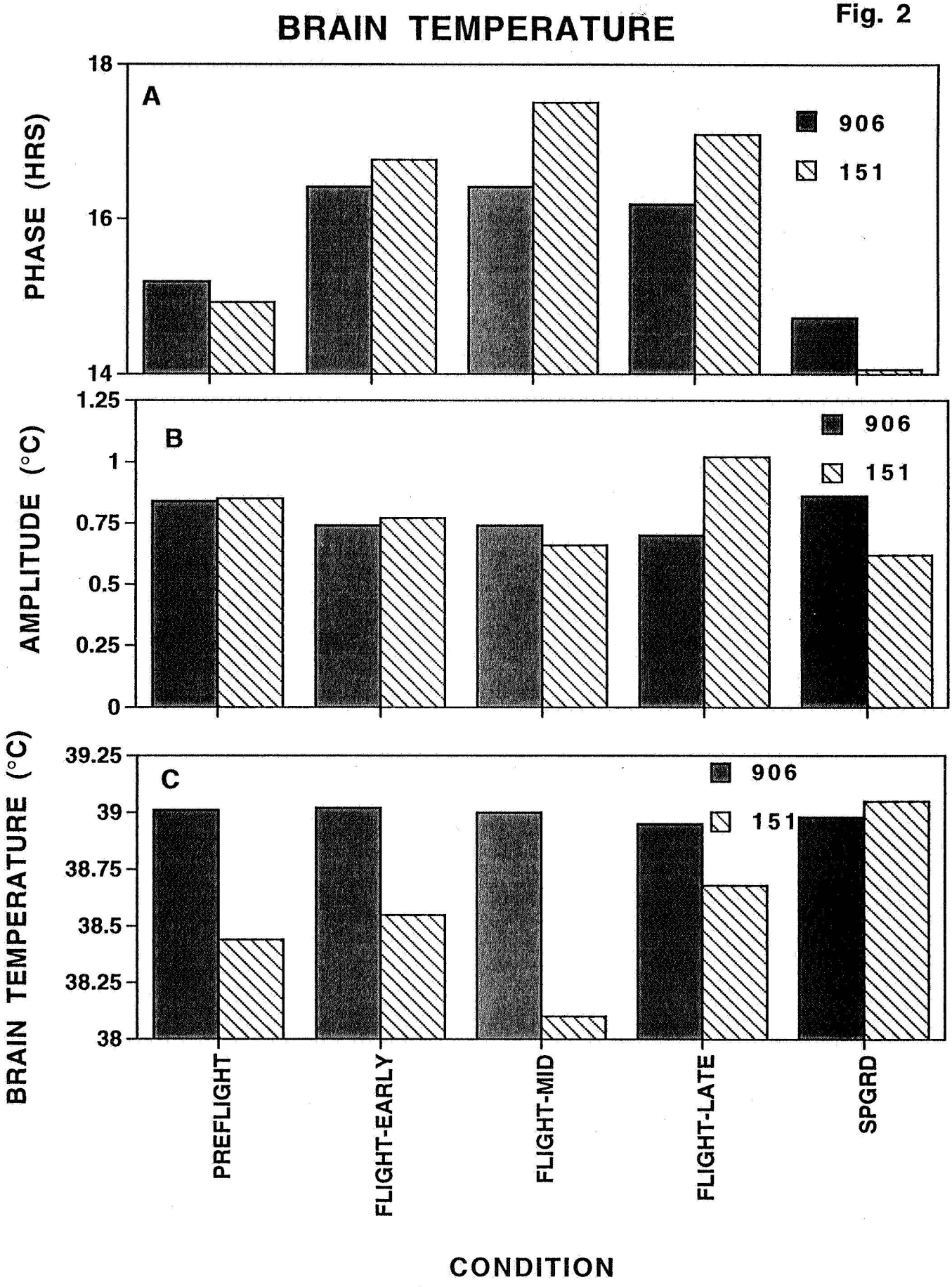
01/06/93
01/08/93
01/10/93

Fig. 2


BRAIN TEMPERATURE
A
B
C
906
151
PHASE (HRS)
18
16
14
AMPLITUDE (°C)
1.25
1
0.75
0.5
0.25
0
BRAIN TEMPERATURE (°C)
39.25
39
38.75
38.5
38.25
38
PREFLIGHT
FLIGHT-EARLY
FLIGHT-MID
FLIGHT-LATE
SPGRD
CONDITION

AXILLARY TEMPERATURE

Fig. 3

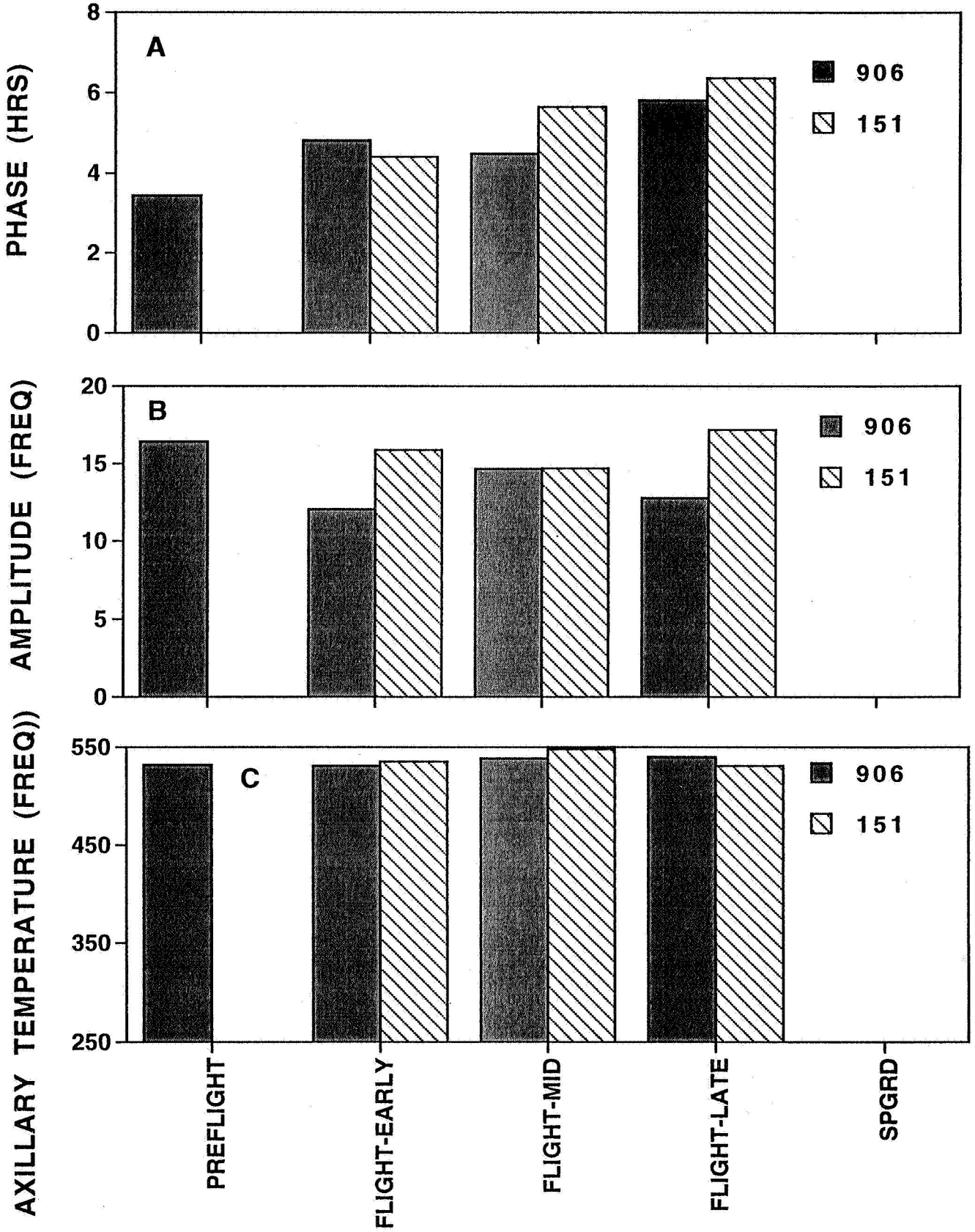
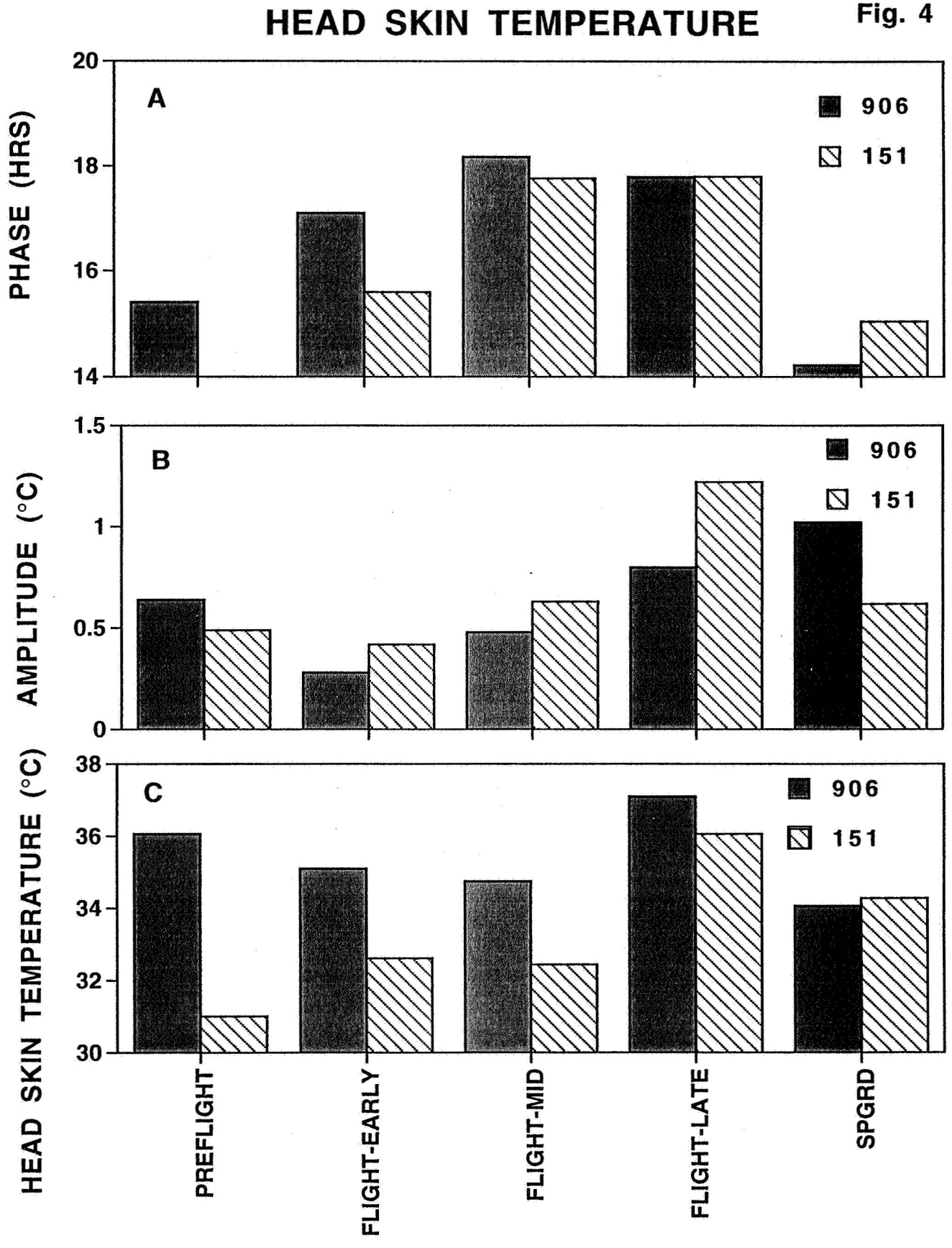


Fig. 4


HEAD SKIN TEMPERATURE
A
B
C
906
151
PHASE (HRS)
AMPLITUDE (°C)
HEAD SKIN TEMPERATURE (°C)
20
18
16
14
1.5
1
0.5
0
38
36
34
32
30
PREFLIGHT
FLIGHT-EARLY
FLIGHT-MID
FLIGHT-LATE
SPGRD
CONDITION

THIGH SKIN TEMPERATURE

Fig. 5

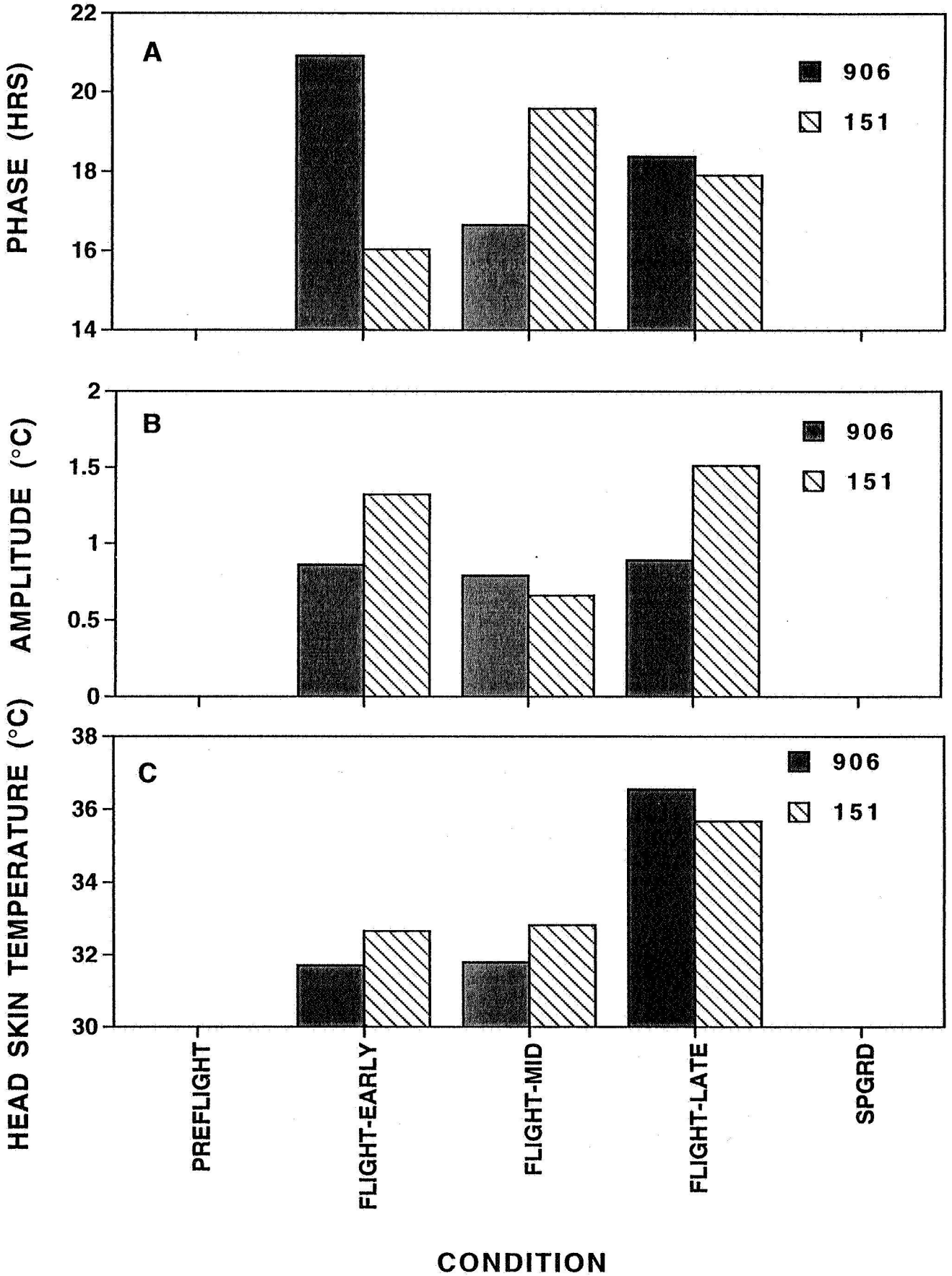
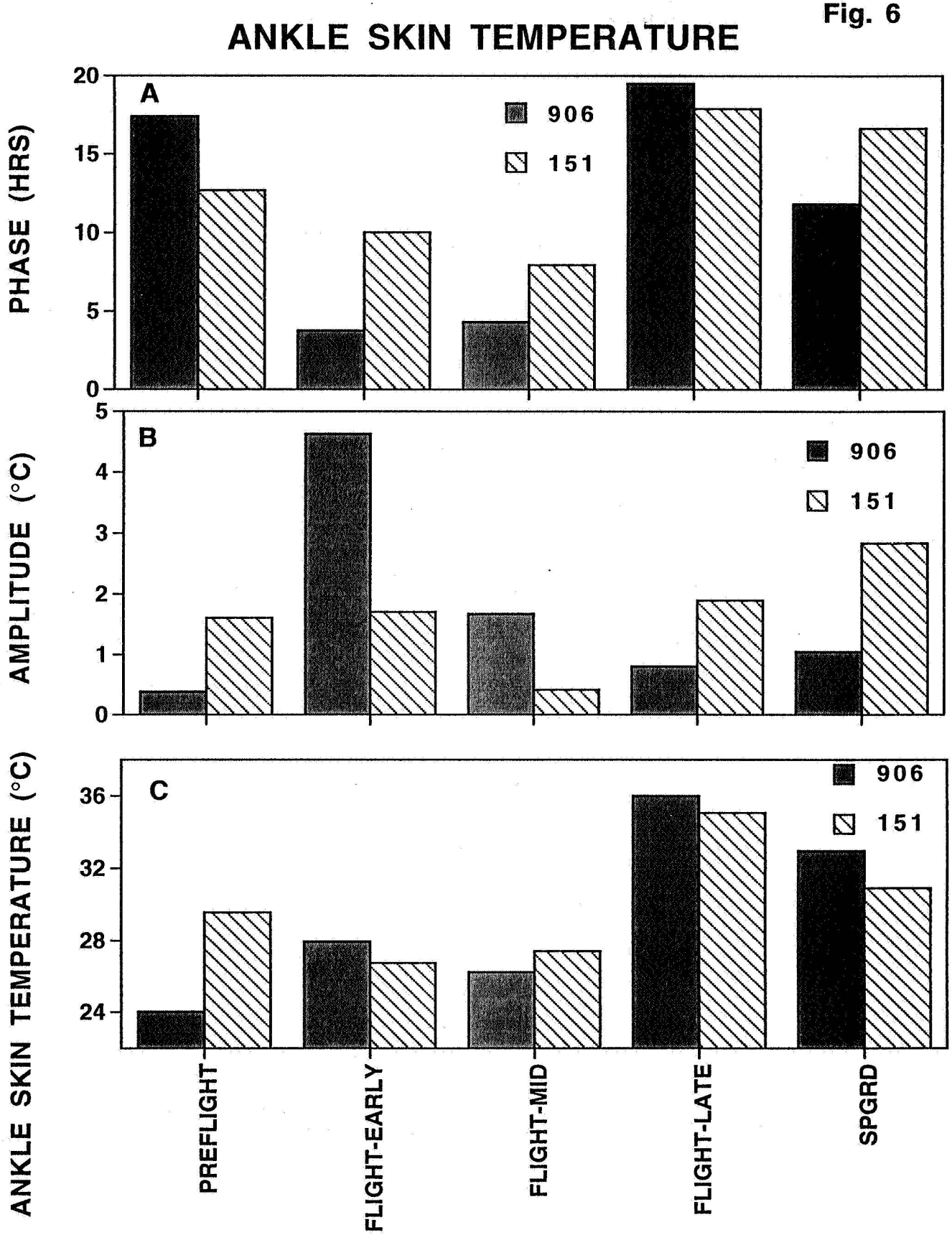
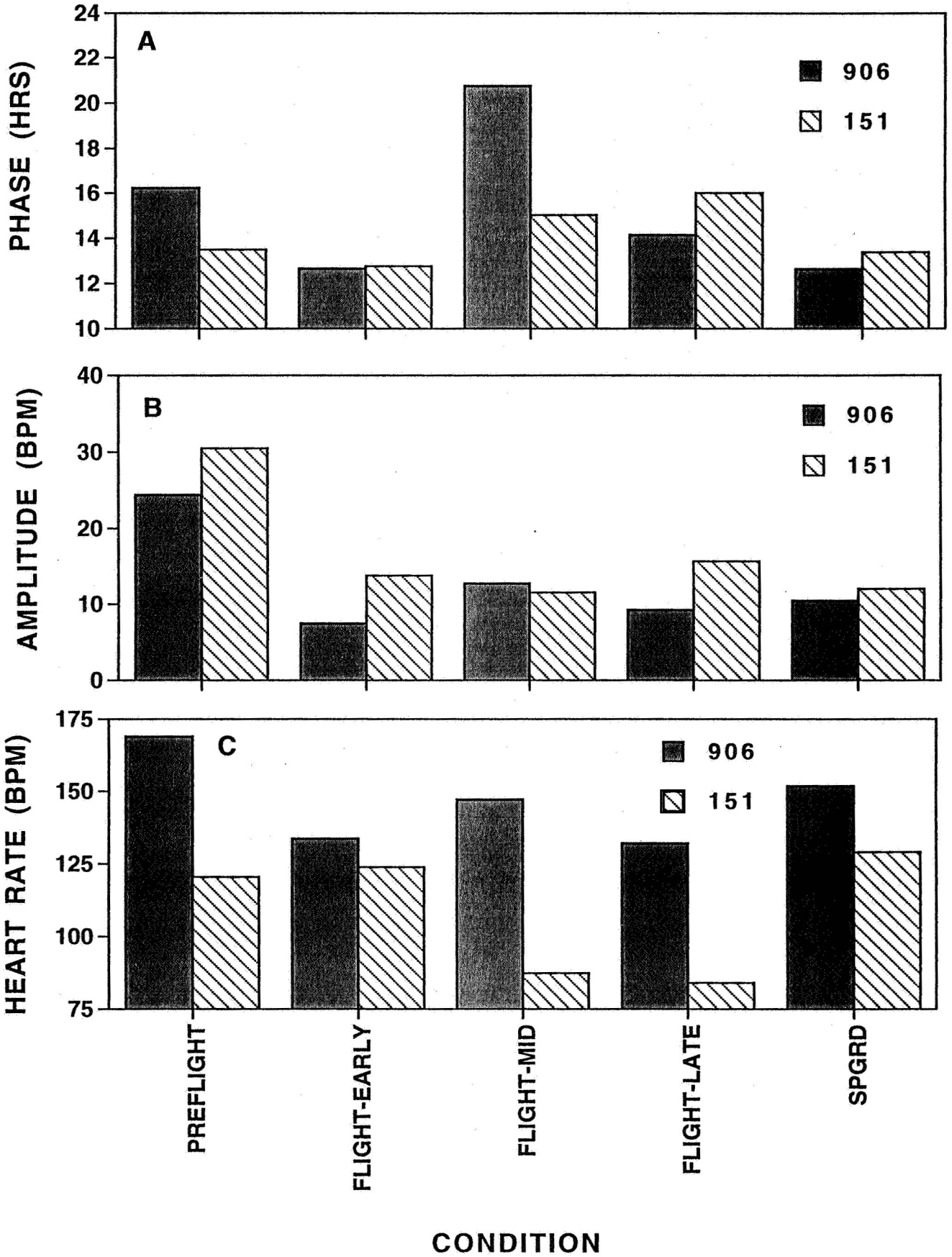


Fig. 6


ANKLE SKIN TEMPERATURE
A
B
C
906
151
PHASE (HRS)
AMPLITUDE (°C)
ANKLE SKIN TEMPERATURE (°C)
20
15
10
5
0
4
3
2
1
36
32
28
24
PREFLIGHT
FLIGHT-EARLY
FLIGHT-MID
FLIGHT-LATE
SPGRD
CONDITION

HEART RATE

Fig. 7



ACTIVITY

Fig. 8

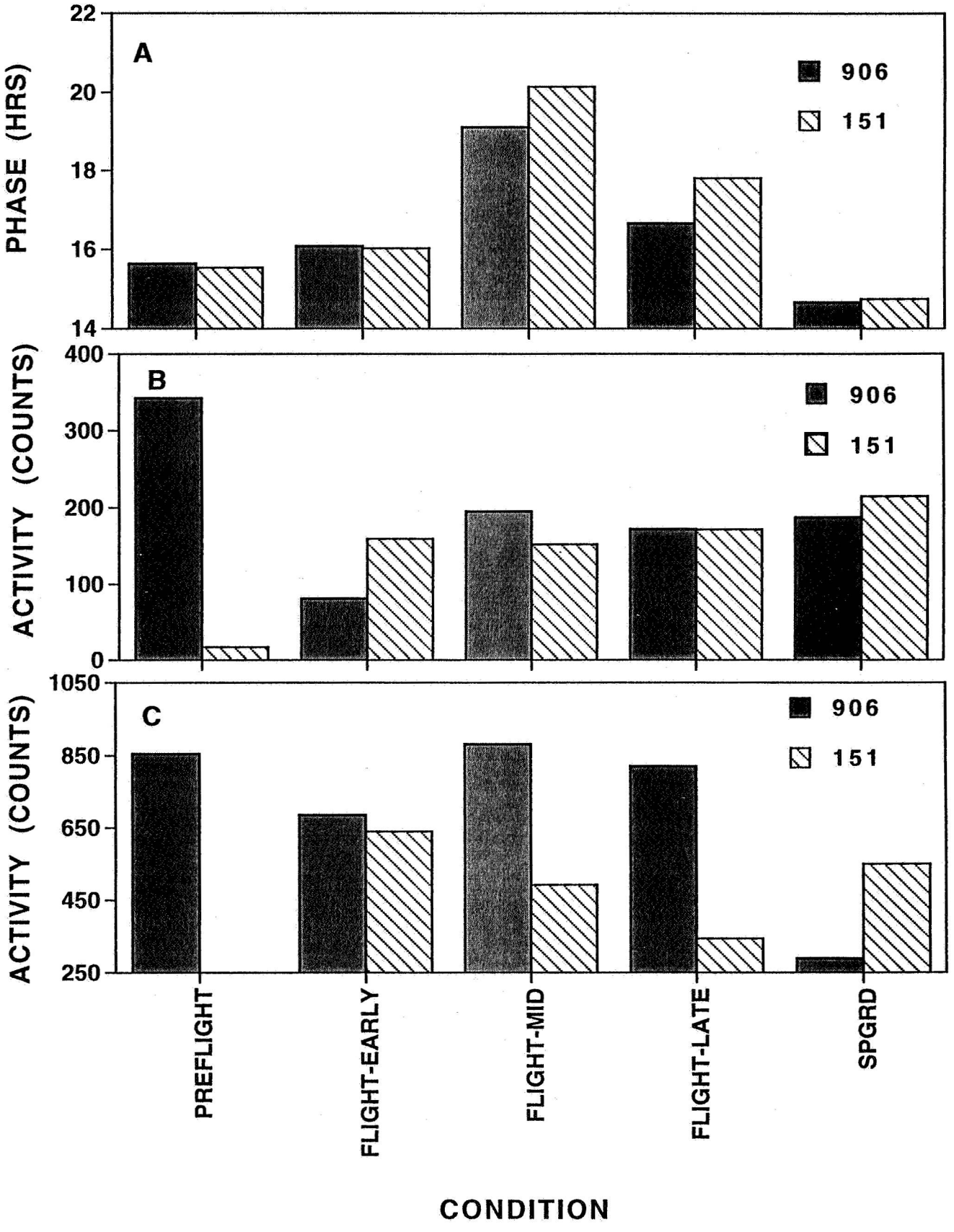
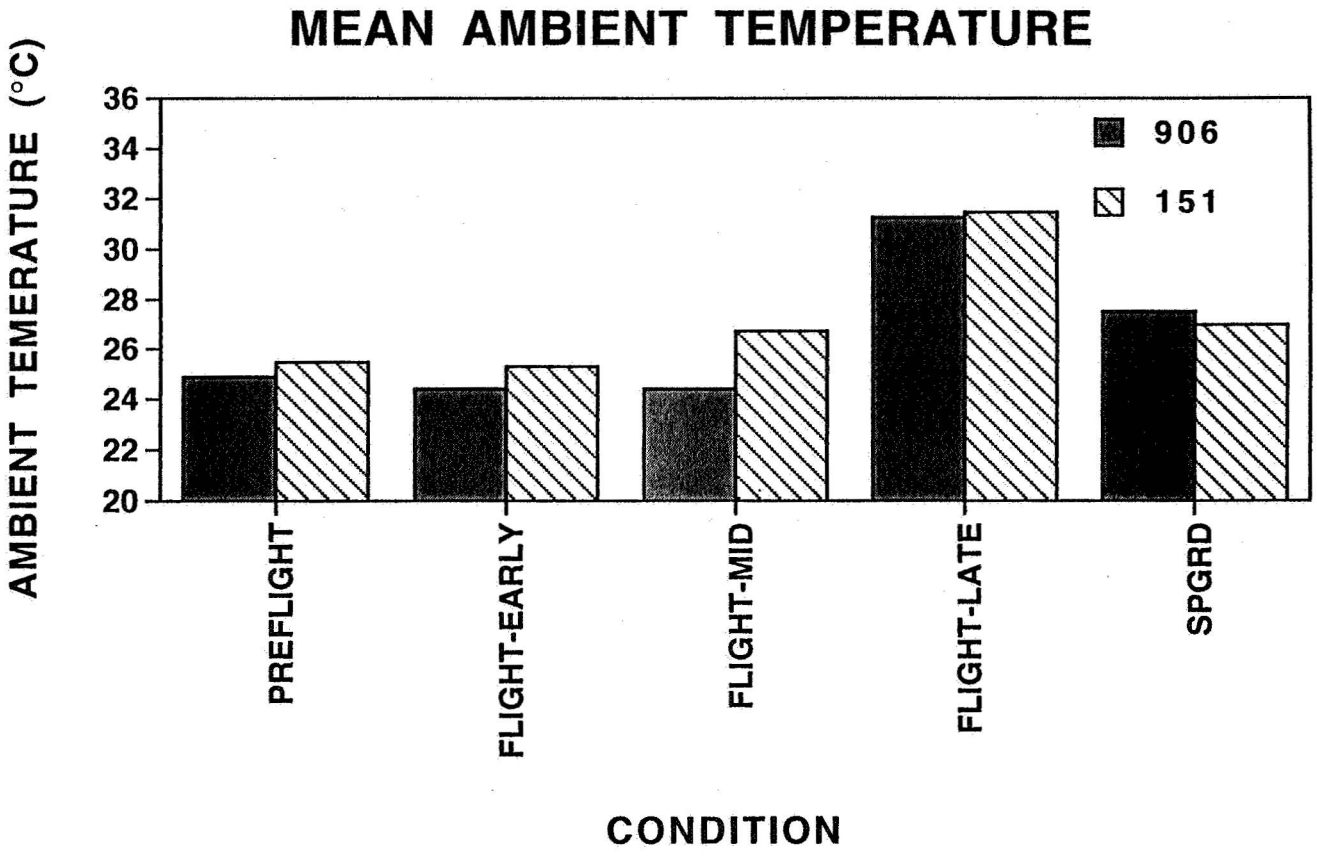


Fig. 9


MEAN AMBIENT TEMPERATURE
AMBIENT TEMERATURE (°C)
36
34
32
30
28
26
24
22
20
906
151
PREFLIGHT
FLIGHT-EARLY
FLIGHT-MID
FLIGHT-LATE
SPGRD
CONDITION