WHITE MOUNTAIN RESEARCH STATION

UNIVERSITY OF CALIFORNIA, BERKELEY

SEMI-ANNUAL STATUS REPORT

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OBJECTIVE

To establish physiological base line data, and to develop physiological procedures and instrumentation necessary for the automatic measurement of hemodynamic and metabolic parameters during prolonged periods of weightlessness.

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I. BIOCHEMISTRY

A. *Elemental analysis of biological materials.*

Eight elements, namely: nitrogen, phosphorus, chloride, calcium, magnesium, sodium, potassium and iron, are presently analyzed with reasonable confidence according to the procedures outlined in Status Reports No. 20 and 21. Whenever feasible, a given element is analyzed by two different methods to check the accuracy. Eventually the Kjeldahl procedure will be used for the analysis of nitrogen and potassium only, and the alkaline ashing for the remaining elements. This would of course save both time and effort.

During the present report period, substantial effort has been devoted to the development of a new method for the quantitative analysis of total sulfur content in biological materials. The method is based on the principle of reducing the sulfur to hydrogen sulfide, and subsequently determining it electrometrically. The method has worked well for simple compounds such as ammonium sulfate, sodium sulfate, sodium sulfite and cystine. However, when the ratio of nitrogen to carbon in the material to be analyzed increases, there apparently is interference due to the formation of cyanide, which results in falsely high sulfur values. In case of methionine the "recovery" is 110 per cent, and in dry powdered milk it is almost 200 per cent.

It appears now that the problem can be solved by preliminary ashing of the biological sample at 550°C and in presence of potassium carbonate to remove nitrogen and carbon. The remaining ash can then be analyzed for sulfur reductometrically as outlined above. The technical details of sample preparation and analytical procedure for sulfur content determination will be described in the next status report.

B. *Radioimmunoassay procedures.*

It has been established recently that the omnipresent cyclic AMP has a very important role in regulating a remarkable array of biochemical and physiological processes. Cyclic GMP is perhaps not less ubiquitous, but its exact function is not as defined as that of cAMP. Since radioimmunoassay (RIA) kits became available which permit the determination of picogram quantities of cAMP and cGMP in the microliter range of urine, we included the quantitative determination of these parameters in any urinalysis.

The kits for cAMP and cGMP are available from only one or two manufacturers in the USA. In principle, the procedures using RIA for the above constituents appears simple and straightforward. In reality, it is at present far from an analytical chemist's dream of simplicity. Some of the contributing difficulties are: (1) The binding protein is not a true solution. (2) The "sticky" reagent adheres to the capillary pipets and sometimes clogs the orifices, thus failing in quantitative transfers. (3) The proteins denature readily at temperatures above 0°C. (4) Small changes in pH of buffers also adversely affect the binding protein. (5) The concentration range relative to standard is very limited. (6) Sufficient data are not available on the limits of incubation periods. (7) Generally the procedure supplied with the kit lacks in important details, such as: (a) The kinds of pipets to use; (b) What size test tubes to use (and whether glass or plastic); (c) How to wash the "insoluble" (invisible) complex efficiently while on the filtration membrane.

Once it is realized what the inherent difficulties are, it is possible to compensate for most of them. In the next report we should be able to outline a procedure for cAMP and cGMP which avoids most of the difficulties listed above.

The data for cAMP and cGMP of urines from bed-rest subjects obtained in this laboratory are reported elsewhere.

C. *Rat blood electrophoretograms in hyperoxia.*

In the last semi-annual report it was stated that rats exposed to pure oxygen at 760 torr show increasing levels of haptoglobin up to 3 days. On the 7th day of continuous exposure, the haptoglobin level is drastically decreased relative to control animals.

To ascertain further the individual response of rats to hyperoxia at the 3-day exposure, 8 animals were subjected to pure oxygen breathing for 3 days. Another 8 rats breathing normal air were used as controls.

The results of this experiment are detailed in Table 1. The haptoglobin binding capacity appears relatively constant in the control group, whereas the experimental group shows a wide variation. This essentially agrees with our previous observation in which a rise in haptoglobin occurred for the first 3 days and then fell sharply below the control group. The 8 animals in this experiment reflect their individual responses in which in some animals the haptoglobin is still climbing, while in others it has passed the peak and is on the decline. To trace this transition, that is from normal to high and then low haptoglobin, it is obvious that more points along the curve are required.

The other constituents in the blood of these rats yield a much more consistent picture. It is seen that the plasma protein content, albumin beta-1, and especially gamma globulin, have decreased in the experimental rats. The hemoglobin and fibrinogen on the other hand show a very consistent and considerable increase.

It is interesting to note here, that in 1959 Nyman (Scand. J. Clin. Lab. Invest. 11, Suppl. 39) suggested that generally variations in the

concentration of fibrinogen in some pathological conditions correlate with changes in haptoglobin. In the nephrotic syndrome, however, no correlation was seen. This is because the haptoglobin with a molecular weight of 85,000 is filtered across the glomerulus more easily (and hence is eliminated by the way of the urine) than fibrinogen with a molecular weight of 400,000. Further investigation of the kidney pathology of the hyperoxic rats is needed and may be done elsewhere.

The statistical treatment of the data in Table 1 will be done in collaboration with Dr. H. Leon of NASA-Ames Research Center, who is co-investigator in this particular experiment.

II. BIOINSTRUMENTATION

A. *Monkey pod feeder and waterer system.*

A description of this system and the results of a caged monkey interface trial were presented in Status Report No. 22. The results of tests of the feeder and waterer as part of the monkey pod system are given in the Physiology Section of the present report. During the current report period, both cage and pod tests were conducted with the feeder and the waterer, and a number of mechanical problem areas were identified. Corrective action was initiated and solutions found to most of the problems, as follows.

1. Feeder.

a) If the food lever was left in a forward position, either by action of the monkey or the failure of the spring to return the movable plate to a neutral position, the stirrer in the food reservoir continued its action and tended to disintegrate the food tablets over a period of time. By electronically limiting the stirring to 10 seconds with each lever action, a flow of food tablets reach the delivery tube in good condition. In addition, even if the spring is broken, food can be obtained by the

monkey subject through his actuation of the lever in the full 90° arc and return.

b) When the food lever was actuated and food was not delivered to the monkey by reason of an obstructed delivery tube or empty food tablet reservoir, the electronic signal produced could not be distinguished between this circumstance and an actual food delivery. The total intake of food over a given time period is accurately determined, however, by counting the number of tablets put in the reservoir, and subtracting the number that remained or were discarded by the subject during the test period.

With the provision of a light-sensing transistor in the path of food flow immediately before it is presented to the animal, only tablets dropped into the food cup are counted, and the differentiation from lever actuation without tablet delivery is discernible.

c) The size and consistency of the PMC 5040 food tablets was another area of concern. The range of tablet size has varied between batches of food. However, by increasing the internal diameter of the delivery tube, this aspect as a source of occlusion can be eliminated. Broken tablets or those which do not meet a minimum degree of hardness contribute to delivery difficulties. A hand selection process for each individual tablet was used as an aid. In addition, correspondence with the manufacturer has been conducted in an effort to improve the quality of this food item.

2. Waterer.

The main problem in the original design proved to be the triggering of the waterer by normal variation in ambient barometric pressure, which can exceed the 10 torr differential pressure exerted by the monkeys through sucking action to activate the waterer. Accordingly, the device has been modified by the addition of a compensatory bellows to cancel variation in barometric pressure. This modification is currently under test.

B. *Pod restraint and lower body negative pressure system.*

During this report period a simplified version of the monkey pod fiberglass shell has been designed and fabricated. This shell has two parts instead of four, and its upper and lower sections join at a level about 12 cm cephalad to the iliac crest of a monkey. The junction of the two parts is made via two circular flanges, one of which includes a rubber "O" ring. A closure device compresses the flanges together against the "O" ring and makes a gas-tight seal between the inside and outside of the pod.

The new pod will be internally divided into upper and lower sections at the level of the iliac crest of a monkey by the LBNP seal and rubber divider seal, as is the present model. The new pod includes a circular rim of 35 cm diameter built into the lower section which will support a LBNP seal and a rubber divider seal. The increased area provided by the large circular opening between the upper and lower sections of the pod should simplify the design and fabrication of the LBNP seal, the rubber divider seal and the pod restraint jacket. This circular opening between upper and lower pod should also allow a gas and fluid "O" ring seal to be made at the perimeter of the LBNP seal. This feature should minimize the potential problem of urine leakage from the lower to the upper section when the pod must be placed in the horizontal position during LBNP tests.

Design modifications for the LBNP seal, the rubber divider seal, and the pod restraint jacket are currently being made and it is planned that these items will be fabricated in the near future for testing in the new pod. It is anticipated that the monkey pod restraint, feeder, and waterer subsystems will be similar in the new pod to those in the present version.

C. *Blood pressure and temperature telemetry system.*

Although both the blood pressure telemetry system and the temperature system work well separately, when they are combined and switched from one to the other, the pressure unit develops an unacceptable drift. In addition, the transmitter develops a temperature error which affects the pressure data. Arrangements were made with the College of Engineering, University of California, Berkeley, to have a graduate student work with us on this problem. The effort is continuing.

D. *Fabrication of a biobattery power supply.*

Since the life of mercury batteries is limited, an effort is being made to design a power source which uses in part some of the chemical constituents of living tissue. Bench experiments were performed with $Zn-O_2$, $Al-O_2$, $Mg-O_2$ and $Mg(alloy)-O_2$ cells. Only the latter system, using magnesium alloys, was satisfactory from the point of view of voltage level and durability. The best results were obtained from a welding rod composed of 90% Mg, 9% Al and 1% Zn. A one-inch section of the rod and a small square of platinized platinum mesh was implanted in the abdomen of a rat for one month. A 27 thousand ohm resistor was used as the load so as to draw approximately 50 microamperes. Leads were externalized and daily measurements were made. Output ranged between 1.3 and 1.5 volts. Electrodes were removed and examined. Both electrodes were partly encapsulated with tissue. The Mg alloy rod was only slightly depleted.

An implantable transmitter powered with the system has been inserted in the abdomen of a monkey. The transmitter is designed so that the signal data is proportional to the voltage of the power supply.

E. *Biocompatible ion electrodes.*

Effort is being made to fabricate a small pH electrode which could

be used in an implantable blood pCO_2 electrode. Good pH electrodes have been made with a diameter of 2 mm. The pH sensitive glass is sealed across the end of a lead glass cylinder. pCO_2 electrodes will be made by incorporating a silver sleeve, and captive electrolyte behind a membrane stretched across the end of the pH electrode.

F. *Unitized vascular catheter and thermocouple device.*

A polyvinyl chloride vascular catheter was spirally wound with copper-constantan thermocouple wires and inserted into a large silicone tube. The device can be used for simultaneous blood pressure and temperature, or for withdrawal of blood samples for analyses. This device was implanted in the aorta of a pig-tailed monkey on 23 May 1973, and two weeks later continuous temperature of the couched animal was recorded.

G. *Biological testing of potentially useful plastic materials.*

Heat-shrinkable plastic tubing would be useful for covering implanted instruments, provided it was compatible with tissue. Two such materials have been inserted subcutaneously in an animal for 68 days without apparent reaction. These are heat-shrinkable silastic and heat-shrinkable viton, both produced by Raychem Corporation. Continuing tests with different materials are planned.

III. PHYSIOLOGY

A. *Monkey pod respiratory gas exchange measurements.*

A series of fasting, 24-hr trials of respiratory gas exchange determinations in pig-tailed monkeys was described in Status Report No. 22. During the present reporting period, the development of automated feeding and watering devices for the monkey pod system permitted a substantial extension of the measurement period.

Respiratory gas exchange was monitored continuously during several 4-day metabolic balance trials, and the gas metabolism results of one such experiment are shown in Table 2. Hourly O_2 consumption, CO_2 production, and respiratory quotients were tabulated for the 96 hours of recorded data. Oxygen consumption rates fluctuated between a high of 6.64 liters/hour and a low of 2.71 liters/hour, and is presumably associated with feeding and activity patterns. Carbon dioxide production rates similarly showed a variability of 6.34 to 2.31 liters/hour. On the other hand, as can be seen in Table 3, the O_2 consumption for each 24-hr period ranged from 100.6 liters to 115.4 liters with a mean value of 108.5 liters, indicating a generally stable condition of the animal. CO_2 production for the 24-hr periods ranged from 91.2 to 102.0 liters with a mean of 98.5 liters. It may be noted that the respiratory quotient (RQ) displayed 24-hr mean values ranging from 0.88 to 0.92, with an overall mean value of 0.91 for the entire 4.1 days.

The total caloric value of the food ingested by the animal during the experiment, 2,183 kcal, may be compared with the metabolic energy expenditure during the same period as computed from O_2 consumption rate, using the standard conversion factor of 4.85 kcal/liter O_2 consumped. During the 4.1-day period the animal consumed 445 liters of O_2, which corresponds to an expenditure of 2,158 kcal. Thus, the animal was in nearly perfect energy balance. This conclusion is supported by the observation that the body weight of the animal was 12.35 kg when he was put into the pod, and 12.34 kg when he was removed 4 days later.

As further verification, according to Kleiber the metabolic rate for mammals may be computed from the relationship $M = 72\ W^{0.75}$ where M is the resting metabolic rate in kcal/24 hr and W is the body weight in kg. In the present instance, M is computed to be 490 kcal/24 hr, or 2,009 kcal

in 4.1 days. This agrees within 7 per cent with the energy expenditure of 2,158 kcal computed from the O_2 consumption measurements.

B. *Monkey pod metabolic balance measurements.*

In Semi-Annual Reports 21 and 22, procedures were outlined for the quantitative analysis of a number of elements with the aim that it be used in metabolic balance studies of monkeys subjected to enclosed and isolated environments such as the pod. During this reporting period the last of the intended elements, namely sulfur, was added to the list making a total of 9 elements. The total analytical procedure was carried out twice. Once, the monkey spent 4.10 days in the pod, and the pod contents, including the feces, urine and the supporting blotting paper, were analyzed. The metabolic balance was calculated from previously analyzed Purina monkey chow pellets. The second time, the same animal spent 3.98 days in the pod. At this time the pod contents were analyzed simultaneously with a representative sample of the monkey chow. Thus, for the first time a metabolic balance study was made where the "input" and "output" were known precisely as they were measured, using identical procedures and run concurrently.

A summary of the results of the two experiments is shown in Tables 4 through 7.

The redundancies in certain analyses, using both the Kjeldahl method and the alkaline ashing sample preparation for analysis, is only a temporary measure. It is important to ascertain which method yields the most accurate and consistent results.

Other measurements, besides the 9 elements, include the fat and ash content of both the monkey pellets and monkey excreta and the moisture content for the pellets only.

C. *Integrative testing of potential pod monkeys.*

Various biological aspects of the pod experiment system have been reported upon in recent status reports. The restraint system has been tested with several selected subjects and, as physical and mechanical changes were made, the same subjects were retested in order to evaluate the impact of the actual modifications on their physiological status. While this longitudinal approach has benefited the implementation of bioinstrumentation, a wider cross section of individual monkeys has been sought to increase the potential pod pool.

The monkey candidates have been selected with the following criteria.

a) Freedom from pathological entities which would defer their admittance to the stock colony. Monkeys that have had surgery or were subjects on prior environmental physiology experiments, were not excluded for consideration.

b) At least a twofold range in weight of available subjects. Thus, the minimum weight was 5.50 kg representing a young adult male pig-tailed monkey in the adolescent spurt or the early part of the late growth phase of maturation, while, on the other hand, the heaviest candidate was a full-grown adult weighing almost 14 kg who displayed a rather high level of obesity.

c) Key anthropoidimetric measurements made on each monkey were utilized to fabricate restraint garments which, on the basis of past experience, would most likely accommodate the particular monkey in a comfortable position.

d) Previous training in a chair, couch, or similar device, although noted for later evaluation, was not considered for the initial selection.

A 24-hr experience in the lower and middle pod sections provided a pool

of 12 male pig-tailed monkeys. All monkeys were carefully examined prior to and following this one-day sojourn, and were judged ready to participate in further phases when appropriate bioinstrumentation was available. Ten of the original 12 monkeys (83%) completed 4 continuous days in the lower plus middle pod configuration, and an indication of their tolerance is shown in Table 8 by the body weights recorded immediately before and after 4 days of pod restraint. Termination of the trial period prior to the full 4-day schedule was deemed clinically necessary for 2 monkeys and they were returned to a cage situation. Both of them are presently in good condition and may be recycled in future trials.

It would appear, as a result of these trials, that a high percentage of male pig-tailed monkeys are amenable to the pod configuration regardless of their past histories in restraint. Fasting 6-hr and 24-hr gas metabolism trials with the pod hood in place have been conducted on these monkeys and the results are reported elsewhere in this document.

Following extensive mechanical testing of the feeder-waterer and food acceptability trials, 8 of 11 monkeys were able to operate this device when accessible in the cage situation. No inducement was made to increase the candidates' operative ability. The monkey's own curiosity, dexterity, and ingenuity were the deciding factors. If food and water were not obtained within 2 days' exposure, the test was considered negative and the monkey was returned to his normal cage environment. As a final step for a monkey interface with this device, the pod hood was modified to allow the placement of the feeder-waterer. The monkey is able to receive food and water within the hood and replenishment of the nutrient reservoirs can be made from outside the pod without disturbing the experimental subject. All 3 of the monkeys tried have completed acceptable trials with the feeder-waterer in

the pod mode. As a result, an accurate measurement of food and water intake can be made during the same time period as the total excreta collection.

D. *Monkey biorhythms.*

Development of a scheme for determining the number of days required for a body temperature to shift following a shift in an environmental cycle, has been ongoing for the past few months.

In this scheme, 2 parameters of the body temperature rhythm are considered: the maximum Spearman cross-correlation coefficient occurring in a body temperature cycle, and the time of that maximum, when a string of 15-min temperature values is cross-correlated with a 24-hr data segment containing average temperature values at each 15-min increment of the day from a control set of entrained data.

The maximum value of the Spearman cross-correlation coefficient (R_S) is taken as a measurement of degree to which the temperature waveform being tested matches the control waveform. The time at which the maximum occurs is taken to be the phase angle of the rhythm (Φ_{R_S}).

The sequential values of $R_{S_{max}}$ and Φ_{R_S} can be tested for day-to-day consistency by regression analysis. A regression line of $R_{S_{max}}$ or Φ_{R_S} on sequential days is fitted, and various statistics of the regression line are calculated. If the confidence interval of the slope of the regression of Φ_{R_S} on sequential days includes the value zero, the rhythm may be thought of as having a 24-hr period. If the value of the confidence intervals of the regression of $R_{S_{max}}$ on sequential days includes zero, the waveform may be thought of as stable.

The following procedure is used to determine the number of days necessary to re-establish the rhythm in terms of its phase angle and fit with the control waveforms. A series of regression lines are determined by moving

the regression analysis with fixed length over a series of Φ_{R_S} and $R_{S_{max}}$. The values of the regression parameters and statistics are compared with a regression line through the control data.

The residual variances of the control and each of the test lines are tested by an F test. Both the slopes and the means of the lines are tested against a T distribution.

When the comparison of $R_{S_{max}}$ regression lines shows them to be identical following a shift, the first day which is in the regression line can be taken to be the point at which the waveform was re-established.

When the comparison of Φ_{R_S} regression lines shows them to have the same variance and same slope, the first day which is in the regression line can be taken to be the point at which the new phase angle was reached.

This analysis is still in its developmental stages. Fortran programs have been written to perform the analysis, but it remains to be decided what is the best line length, what confidence intervals should be used, and what is the resolution of the technique.

E. *Monkey temperature and humidity tolerance.*

In the period covered by this report, experiments have continued investigating temperature tolerance in the pig-tailed monkey (see p. 18, Status Report No. 21). Three monkeys have been exposed to a series of ambient temperatures from 25-40°C at 80% relative humidity. An identical series of temperature tests conducted at 20% relative humidity is presently near completion. When the results of the high and low humidity tests are available, they will be added to the earlier completed series of temperature tests at 50% relative humidity and the effect of humidity on temperature tolerance can be determined.

A modification of the environmental test chamber has been recently made. The sensor for the temperature amplifier was replaced with a thermistor with a faster response time and was relocated to an area of high air flow near the exhaust duct of the chamber. This change has significantly reduced the range of cycling about the temperature set-point. Formerly this range was as large as $\pm$ 3°C. This range has now been reduced to $\pm$ 1°C or less. This change has simplified the task of programming the chamber for making step-changes in ambient temperature.

A 24-hr thermal stress protocol was evaluated during this report period using a pig-tailed monkey as the test subject in the environmental chamber. This protocol was designed to determine the effects of thermal stress on the concentration of several urinary metabolites. A monkey was fitted with a rectal temperature probe harness (see page 24, Status Report No. 19), a silicone urine collection tube (see page 14, Status Report No. 21), and placed in an EPL fiberglass couch restraint system and then into an EPL isolation box located inside the environmental chamber.

The environmental chamber was programmed for a control temperature of 24°C and either a heat stress of 33°C or a cold stress of 15°C. Each experiment was of 4 days duration, with days 1, 3 and 4 at the control temperature and day 2 at either the high or low temperature. Urine was collected continuously and removed for analysis at 24-hr intervals. Rectal temperature was monitored daily from 0900-1000 hours by attaching the temperature probe to the special harness device and then inserting it into the subject. The subject had an adequate ration of Purina Monkey Chow available daily from 1400-1700 hours and water *ad lib*.

The monkey tolerated the 24-hr heat and cold stresses well and showed only moderate changes in rectal temperature. Water and food consumption

were normal throughout both stress tests.

Monkey #341 was the subject for the 3 separate thermal stress tests that were conducted. Urine was collected from this monkey via a silicone rubber tube, without known leakage for a total of 15 test days over a period of about 5 weeks, with 7 days as the longest continuous collection period.

At the end of each test trial when the urine collection tube was removed there was always about 2-4 ml of coagulated seminal fluid inside the tube. This coagulate did not block urine flow to a significant degree but it did add a considerable amount of weight to the tube, which in turn increased the force acting on the monkey at the glans penis where the tube was attached. At the end of the 7-day test a small excoriation was found on the glans penis and compression lines were evident on the shaft of the penis under the rubber ring. It is possible that this was due to the increased weight of the urine collection tube during this test. This problem might be minimized by daily manually expressing any coagulated semen out of the tube.

It appears that this 24-hr thermal stress protocol is well-tolerated by a pig-tailed monkey. Whether or not the urine collection system and procedure adequately meet the experiment goals must await further analysis of the collected urine samples.

A newly developed (see Bioinstrumentation section, this report) aortic vascular catheter-thermocouple combination was implanted in monkey #392, Grey on 23 May 1973. On 5 June this monkey was placed in the environmental chamber in the same restraint system used for the 24-hr thermal stress tests. Chamber temperature was kept at 24°C and 50% relative humidity, and the thermocouple output was monitored continuously on a Grass strip-chart recorder for 48 hr. The chamber was set for a cycle of 12 hr light and 12 hr dark beginning at 0600 hours. Excellent recordings of aortic temperature

were obtained which showed a typical circadian rhythm with rather sharp rises and drops in temperature following the lights on and off cycles, respectively. This technique of monitoring core temperatures would be most useful during thermal stress studies such as the type described above. This thermocouple remained functional for 61 days post-surgery. The reason for failure has yet to be determined.

F. *Monkey blood oxygen transport.*

Studies on the pig-tailed monkey erythrocyte level of 2,3-diphosphoglycerate were continued. The values of 2.3-DPG were found to be in the range of 7.5 to 8.5 μm/ml of erythrocytes, which is higher than that reported for humans. No daily rhythm has been detected for 2,3-DPG or pH blood levels.

With the use of arterial and venous chronically catheterized monkeys, P_{O_2}, P_{CO_2} and pH will be determined simultaneously in addition to lactate, pyruvate and hematocrit values.

G. *Monkey thyroid metabolism.*

During this report period an attempt has been made to develop a radioimmunoassay (RIA) method for the determination of thyroid stimulating hormone (TSH) in monkey plasma. The procedure involved obtaining a pure preparation of TSH (supplied by NIH) and using it with radioactive iodine (^{125}I) to produce labeled TSH (TSH*) according to the method of Greenwood et al. (Biochem. J. 1963). The labeled TSH is then separated from free iodine (I) by chromatography on a sephadex column (G-100). This procedure supplies labeled TSH to be used for RIA that can last for a few months. An initial trial at radioiodination of TSH did not yield a good separation of the TSH from free ^{125}I and I, but a second trial yielded a very good separation as well as TSH* of a relatively high specific activity.

Conflicting results were obtained when the labeled TSH* was utilized in preliminary RIA trials with monkey plasma. However, these results indicated that procedural corrections should be made in regard to the following:

1. Volume of TSH* added to the RIA tubes.
2. Volume of anti TSH (TSH antibody) added to the RIA tubes.
3. Velocity of centrifugation of the precipitate in the RIA tubes.

The latest results obtained from RIA on standard TSH indicate good progress with this technique, and a detailed assay will be carried out on samples of monkey plasma.

Serum thyroxine levels were also determined in a manner described previously in Status Report No. 18.

H. *Growth in the pig-tailed monkey.*

An updated summary of body weights related to age of the pig-tailed monkey is shown in Table 9. The data from individual additional males appeared to follow in a similar manner the multiphasic growth curve for this sex previously indicated in Status Report No. 19. Also of interest, it is noted at an age of 30 to 42 months the female body weight exceeds that of the male. While this sexual difference has not been reported for other species of macaques, this trend has been noted for growing chimpanzees and the human primate. Growth measurements are being continued on 5 males and 5 females of known age.

I. *Physiological studies of seals.*

A study of body composition and blood volume changes associated with the growth and development of young harbor seals *(Phoca vitulina)* was concluded during the current reporting period. Although the work, carried out in collaboration with Dr. R. W. Elsner of the University of California at San Diego, was focused primarily on growth and erythropoiesis (Status

Report No. 22), the opportunity was taken to make some basic body composition measurements on the animals.

Harbor seals are usually born during March and April, and the first set of measurements were made in mid-May, shortly after capture. The measurements were then continued at 6-week intervals for a period of 10 months. The infant seals were captured shortly after weaning and before having taken to the sea. One of the aspects of the study concerned rearing half of the animals in a dry enclosure, in contrast to a more natural deep diving tank, to determine whether some of the unique physiological characteristics of marine mammals, e.g., extended breath-holding ability and large blood volume, are inborn or the result of "training" and adaptation to life in the oceans. The results of all the measurements are tabulated in Tables 10 and 11. There appear to be no clear differences in the blood parameters between seals raised in the dry or wet enclosures, suggesting that at least the unusually large oxygen carrying and storage capacity of the animals are genetically controlled rather than adaptive in nature.

The per cent body fat, computed from a measure of $^{3}H_2O$ space, shows an interesting but perhaps not unexpected change during the first year of development. At the time of weaning, the seals appear to have accumulated substantial stores of body fat. For a period after capture and while learning to feed on frozen fish, the animals lose much of their energy reserves, until the trend is reversed by an ever increasing food consumption. A similar situation probably prevails in the wild until the seal acquires proficiency in the hunt. On the other hand, the burgeoning fat stores of the animals by the 8th month of captivity may be atypical, and the result of nearly *ad libitum* access to fish with a minimum of energy expenditure.

IV. EXPERIMENTAL SURGERY

A. *Surgical team experience.*

Personnel of this laboratory, both professional staff and graduate students, have participated in training sessions in order to restore our surgical operation capability during the current report period. This surgical team has now performed 16 surgeries on 15 monkeys. Monkeys initially chosen for surgery had had previous surgeries, and were of limited usefulness for further experimentation. While experience was being gained, four animals met their demise or were sacrificed during surgery. Two more, with previous histories of rectal prolapse, died within 7 days of surgery; one having self-removed his catheters, while the other was sacrificed following onset of peritonitis. The remaining 9 monkeys were successfully prepared, and are still alive in the colony.

B. *Chronic indwelling vascular catheters.*

At the conclusion of this report period, 3 male pig-tailed monkeys have patent aorta catheters for 111 days, 98 days, and 70 days, respectively. In addition, 2 male pig-tailed monkeys possess patent aorta and pulmonary artery catheters for 55 days and 41 days. With the level of support presently available, it appears that this number of vascular catheterized animals can be adequately maintained and available when needed for experimental use. All catheters were implanted with the dacron patch technique. Sites of implantation were the thoracic descending aorta, 3 to 5 cm posterior to the junction of the common carotid, and for the venous side in the trunk of the pulmonary artery.

The longest patency time for a chronic vascular catheter in our laboratory now stands at 771 days post-surgery. A pulmonary arterial catheter surgically implanted in #399, Catesby on 25 May 1971, had its final

patent check on 6 July 1973. Four days later the catheter was found to be broken near the exteriorization from the skin. The remaining portion of this polyvinyl chloride catheter was extremely brittle and a splice could not be introduced. This monkey had also maintained a patent aorta catheter for 560 days. At the present time he appears healthy, compatible, and happy in company with a female monkey.

C. *Implantation of biobatteries.*

Two attempts have been made to surgically implant biobatteries (described under the Bioinstrumentation section of this report). The initial laparotomy was performed 24 May 1973 on #396, Lovel, and the body of the transmitter sutured securely to psoas major muscle, while the platinum wire leading to a platinum mesh screen was loosely sutured to the transversus abdominis. Telemetry signals were not received from this implant while it remained within the abdominal cavity of the monkey. In the meantime, a second biobattery was fabricated and bench tested. Surgical reentry on 12 July 1973 revealed that the magnesium alloy core had separated from the body of the transmitter. The first battery was completely removed and the second battery secured more anteriorally to avoid the action of leg musculature which may have caused the original malfunction. This monkey has been monitored daily for output of telemetry signal and the biobattery is apparently functional for at least 2 weeks following surgical implantation.

D. *Single-channel body temperature telemetry transmitters.*

A single-channel totally implantable temperature telemetry transmitter placed surgically in #412, Peter on 14 May 1971, continued to function during the time covered by this report. In addition, 3 monkeys, #175, Salisbury, #364, Lysimachus and #366, Thaliard, instrumented with similar

devices have provided continuous biorhythmic data on body temperature changes for over a one-year period.

No laparotomies with the express purpose of implanting body temperature telemetry transmitters were performed during this report period.

V. ANIMAL COLONY

A. *Clinical health.*

All but two monkeys in the colony were negative to tuberculosis testing conducted in July 1973. The test method, using ARS mammalian intradermic tuberculin, was the same as that indicated in Status Report No. 22. Two animals were not included in this test, but they have been separately isolated since their last clearance and will be subjected to a complete examination prior to movement from these quarters.

No lethal pathology of endemic proportion has been noted in the colony. Lesions resulting from monkey interaction, restraint instrumentation, or other factors have been minimal and immediate appropriate treatment has, in all cases, resulted in good recovery.

B. *Lung mite screening.*

With the cooperation of Prof. Deane Furman, Acting Division Chairman of the Division of Entomology and Parasitology on the Berkeley Campus of the University of California, developmental testing of *in vivo* techniques for lung mite detection have proceeded with rescreening of several pig-tailed monkeys during April and July 1973. In addition, lung tissue at necropsy from a monkey previously screened has been provided for examination with pepsin digestion, and an initial chemotherapy trial has been conducted on an aged female who had shown repeated evidence of lung mites with either the bronchial irrigation or bronchial swab-irrigation *in vivo* technique.

As a result of specimen samples taken during the screening of these monkeys and the subsequent culture evaluation, certain heretofore unknown aspects of the ethology of these parasites are beginning to emerge.

Sulfaquinoxaline in a known concentration was offered to #59, Ursula in her drinking water *ad libitum* over a period of one month. Initially this compound was added to several fluids to increase its palatability. Unfortunately, all of the liquid preparations eagerly consumed by the monkey were acidic enough to precipitate the sulfaquinoxaline powder. In water this did not occur, but even in rather dilute solutions the preparation produced a degree of anorexia in the subject. Vitamin K, as an anti-hemmorhagic precaution, injected into apple or zucchini slices were offered five times weekly during the test. Most of these diet additives were consumed. A post-trial screening of #59, Ursula regarding the effects of this drug, if any, are inconclusive at the present time. She has returned to the regular colony ration and regained some of the weight loss experienced during the trial.

Alternate pharmaceutical agents and methods of therapy are currently being investigated.

C. *Monkey census.*

As shown in Table 12, forty-one monkeys comprise the total non-human primate colony at the end of this report period. No acquisitions were made, and the loss of 9 animals from a total of 50 on 1 February 1973 were mostly involved with surgical procedures carried out during the report period. A summary of the surgical team experience is reported under a separate section of this report. Thirty-nine monkeys are housed within the Environmental Physiology Laboratory and 2 are in the Life Sciences Building of the University of California.

Table 1. Results from 3-day exposure of rats to 760 torr oxygen.

Data from ARC				Data from UCB									
Rat No.	Weight Initial	Weight Final	Hematocrit	Hemoglobin g%	Haptoglobin binding cap.	Plasma protein g%	Albumin g%	$\alpha_1 + \alpha_2$	β_1? g%	β_2? g%	Fibrinogen g%	γ g%	Albumin/ Globulin
C-1	224	231	42.4	14.7	74	5.68	2.49	0.84	0.61	0.87	0.20	0.65	0.78
C-2	222	233	36.1	12.3	65	5.51	2.39	0.82	0.57	0.91	0.20	0.61	0.77
C-3	232	232	40.8	13.3	73	5.36	2.43	0.83	0.62	0.89	0.19	0.40	0.83
C-4	225	224	42.8	13.7	66	5.46	2.40	0.78	0.59	0.85	0.23	0.61	0.79
C-5	216	219	41.0	13.1	72	5.34	2.33	0.86	0.64	0.81	0.24	0.45	0.78
C-6	215	224	38.0	12.6	68	5.14	2.19	0.78	0.56	0.82	0.17	0.62	0.74
C-7	228	234	41.8	14.0	66	5.39	2.44	0.76	0.60	0.89	0.23	0.47	0.82
C-8	227	236	40.7	13.3	71	5.24	2.27	0.77	0.57	0.86	0.20	0.57	0.76
Mean				13.4	69	5.39	2.37	0.80	0.60	0.86	0.21	0.55	0.78
E-9	214	184	50.8	16.9	101	5.83	2.34	1.04	0.51	0.95	0.62	0.35	0.67
E-10	235	204	49.6	16.8	93	5.41	2.35	1.07	0.41	1.03	0.43	0.11	0.77
E-11	225	198	52.8	17.5	65	4.69	2.08	0.72	0.48	0.74	0.31	0.36	0.83
E-12	216	187	51.7	17.6	42	5.06	2.06	0.83	0.44	0.89	0.37	0.46	0.69
E-13	230	204	55.4	17.8	32	5.16	2.14	0.86	0.40	0.84	0.46	0.46	0.71
E-14	228	192	51.2	17.3	54	4.89	2.22	0.88	0.41	0.72	0.36	0.31	0.83
E-15	225	196	58.5	19.5	24	4.52	2.02	0.83	0.36	0.65	0.33	0.33	0.84
E-16	224	196	47.5	16.2	55	4.72	2.16	0.71	0.34	0.74	0.37	0.40	0.84
Mean				17.4	58	5.04	2.17	0.87	0.42	0.82	0.41	0.35	0.77

C - Control animals

E - Experimental animals

? - Arbitrary division, because of uncertainty of designation of these electrophoretic bands in rat plasma.

Table 2. Hourly respiratory gas exchange measurements during a 4-day metabolic balance trial on Monkey #307 (Angelo).

Date	Time	Pod Hour	Liters/hr Oxygen Consumption (STPD)	Liters/hr Carbon Dioxide Production (STPD)	Respiratory Quotient
19 Mar 73	1200	1	4.36	3.96	0.91
		2	4.83	4.49	0.93
		3	5.05	4.62	0.91
		4	4.67	4.08	0.87
		5	5.29	4.32	0.82
		6	4.43	4.23	0.95
		7	6.64	5.52	0.83
		8	5.41	4.48	0.83
		9	5.80	5.12	0.88
		10	4.84	4.20	0.87
		11	4.85	4.21	0.87
		12	4.85	4.41	0.91
20 Mar 73	2400	13	4.48	3.69	0.82
		14	4.38	3.79	0.87
		15	4.62	3.69	0.80
		16	4.33	3.79	0.87
		17	6.44	6.34	0.99
		18	5.10	4.71	0.92
		19	6.34	5.98	0.94
		20	3.83	3.29	0.86
		21	4.38	4.13	0.94
		22	4.39	3.80	0.87
		23	2.71	2.31	0.85
		24	3.34	2.85	0.85
20 Mar 73	1200	25	3.64	3.35	0.92
		26	3.89	3.65	0.94
		27	4.38	4.14	0.94
		28	4.72	4.51	0.96
		29	4.71	4.51	0.96
		30	4.72	4.52	0.96
		31	4.61	4.37	0.95
		32	4.97	4.73	0.95
		33	4.63	4.53	0.98
		34	4.96	4.73	0.95
		35	6.22	5.88	0.95
		36	4.47	3.92	0.88

(continued)

Table 2 (continued)

Date	Time	Pod Hour	Liters/hr Oxygen Consumption (STPD)	Liters/hr Carbon Dioxide Production (STPD)	Respiratory Quotient
21 Mar 73	2400	37	4.72	4.27	0.91
		38	4.75	4.45	0.94
		39	4.88	4.44	0.91
		40	4.64	4.15	0.89
		41	4.75	4.30	0.91
		42	4.64	4.30	0.93
		43	4.10	3.65	0.89
		44	4.88	4.29	0.88
		45	3.61	3.22	0.89
		46	4.75	3.96	0.83
		47	4.40	3.81	0.87
		48	4.15	3.56	0.86
21 Mar 73	1200	49	4.41	3.66	0.83
		50	4.40	3.66	0.83
		51	4.38	3.79	0.87
		52	4.63	4.43	0.96
		53	4.24	3.80	0.90
		54	4.39	3.80	0.87
		55	3.95	3.50	0.89
		56	3.86	3.51	0.91
		57	4.51	4.11	0.91
		58	4.72	4.37	0.93
		59	4.22	3.93	0.93
		60	4.41	3.81	0.86
22 Mar 73	2400	61	4.38	4.09	0.93
		62	4.39	4.10	0.93
		63	4.39	4.10	0.93
		64	3.43	3.25	0.95
		65	3.98	3.83	0.96
		66	4.66	4.47	0.96
		67	3.66	3.51	0.96
		68	3.86	3.22	0.83
		69	3.25	3.06	0.94
		70	-	-	-
		71	3.95	3.80	0.96
		72	4.24	3.50	0.83

(continued)

Table 2 (continued)

Date	Time	Pod Hour	Liters/hr Oxygen Consumption (STPD)	Liters/hr Carbon Dioxide Production (STPD)	Respiratory Quotient
22 Mar 73	1200	73	4.65	4.45	0.96
		74	5.14	4.80	0.93
		75	5.54	5.29	0.95
		76	5.05	4.75	0.94
		77	5.54	5.05	0.91
		78	5.15	4.90	0.95
		79	4.65	4.30	0.93
		80	4.75	4.41	0.93
		81	4.65	4.11	0.88
		82	5.41	5.06	0.94
		83	4.90	4.61	0.94
		84	4.48	4.13	0.92
23 Mar 73	2400	85	4.26	3.97	0.93
		86	4.17	3.73	0.89
		87	4.52	3.82	0.85
		88	4.66	4.07	0.87
		89	4.42	3.97	0.90
		90	4.42	3.82	0.86
		91	3.23	2.93	0.91
		92	3.52	3.27	0.93
		93	2.73	2.62	0.96
		94	3.87	3.66	0.95
		95	4.11	3.91	0.95
		96	4.00	3.81	0.95
23 Mar 73	1200				

Table 3. Daily oxygen consumption and carbon dioxide production during a 4-day metabolic balance trial on Monkey #307 (Angelo).

Day	Liters/24 hrs Oxygen Consumption (STPD)	Liters/24 hrs Carbon Dioxide Production (STPD)	Respiratory Quotient
1	115.4	102.0	0.88
2	110.2	101.2	0.92
3	100.6	91.2	0.91
4	107.8	99.4	0.92
Mean	108.5	98.5	0.91

Table 4. Output analysis of pod contents[1] for #307, Angelo, 4.1 days (19-23 March 1973).

Weight of freeze dried matter, in g	729.1
Weight of blotting paper, in g	624.5
Net weight of freeze dried urine and feces, in g	104.6
Net weight of petroleum ether extract, in g	2.431
Net weight of freeze dried and defatted urine and feces, in g	102.17
Per cent ash in freeze dried and defatted urine and feces	32
Per cent ash in freeze dried and defatted urine and feces including blotting paper[2,3]	4.5

Analyses (based on freeze dried and defatted matter) in grams per 4.1 days

	Calculated Intake	Kjeldahl	Ashed	
			K_2CO_3 added	No K_2CO_3
Nitrogen	22.21	18.18		
Phosphorus	3.24	2.28	2.33	2.19
Chloride	2.95		2.82	2.52
Sulfur	0.37[4]		0.35	
Calcium	5.74	3.31	5.46	
Magnesium	0.91	0.79	0.78	
Sodium	1.42	1.58	1.54	
Potassium	4.49	4.58		
Iron	0.21		0.13	

[1] Based on intake of 789 tablets of PMC 5040 with an original wt of 631.2 g and a freeze dried and defatted wt of 568.0 g.

[2] The defatted contents were ground 3 times in a Wiley mill.

[3] The 624.5 g paper contributed only 0.3 g ashes, or less than 1% of total ashes. No correction was made in the elemental analyses for the contribution due to paper.

[4] This value obtained by ashing 5.0 g of ground PMC tablets at 550°C without K_2CO_3 and subsequently reducing sulfate to sulfide in H_2 gas at 1050°C using 14.8 mg of ash.

Table 5. Metabolic balance study on a monkey in pod for 3.98 days.

A. Analysis of Purina Monkey Chow Pellets

Weight of (116) pellets[1], in g	99.106
Weight of (116) pellets after freeze-drying, in g	91.919
Weight of water, in g	7.187
Per cent water in original pellets	7.25
Per cent fat in the freeze-dried pellets	2.2
Weight of ash[2] from 25.0 g freeze-dried and defatted pellets	1.675
Per cent ash	6.7

Total animal intake in g per 3.98 days

PMC pellets	1157
Weight of pellets after freeze-drying and defatting	896.86
Fat (2.2% of freeze-dried pellets), in g	19.2

	Kjeldahl[3]	Ashed[3,4]
Nitrogen	35.756	
Phosphorus	5.450	5.432
Chloride		3.980
Sulfur[5]		0.816
Calcium	[8.718][6]	10.473
Magnesium	[1.066][6]	1.430
Sodium	2.269	2.269
Potassium	6.365	
Iron		0.359

1. A total of 116 pellets were removed for analysis from the food provided the monkey #307, Angelo during the 3.98 days in the pod. For every ten pellets deposited in the pellet dispenser, one pellet was taken for analysis. During the 3.98 days, the monkey consumed a total of 1157 pellets.
2. Ashes obtained from heating 25.0 g of freeze-dried, defatted and powdered pellets (in a Wiley mill) in a kiln at about 550°C. No K_2CO_3 was added.
3. Calculation based on freeze-dried and defatted powdered pellets.
4. Two ml of 2% K_2CO_3 were added, plus sufficient water to enable the powder to be thoroughly wetted and mixed.
5. The recovery of "sulfur" from Na_2SO_4 varies at this time from 85% to 96%. No correction for the ash analysis can be applied until the reason for above variance is established.
6. These values in brackets are only for comparison. They can not be used, because it is known *a priori* that recovery is incomplete.

Table 6. Metabolic balance study on a monkey in pod for 3.98 days.

B. Analysis of pod contents

Weight of freeze-dried matter, in g	797.7
Weight of blotting paper, in g	617.3[1]
Net weight of freeze-dried urine and feces, in g	180.40
Net weight of petroleum ether extract, in g	2.87
Net weight of freeze-dried and defatted urine and feces, in g	177.53
Weight of ash from 10.0 g freeze-dried and defatted urine, feces and paper (without K_2CO_3), in g	0.640
Per cent ash	6.4

Following analyses are in grams per 3.98 days

	Kjeldahl[2]	Ashed[2,3]
Nitrogen	20.931	
Phosphorus	5.951	6.011
Chloride		3.725
Sulfur[4]		0.641
Calcium	[7.569][5]	9.586
Magnesium	[1.257][5]	1.329
Sodium	1.890	1.900
Potassium	5.354	
Iron		0.319

1. This weight is that of the blotting paper at ambient humidity. The freeze-dried weight of the paper remains to be assessed. If, as expected, the water-free paper will weigh less, then, by difference, the dry mass excreted by the animal will be larger. This will not affect the "output-input" balance as presently calculated.
2. Calculation based on freeze-dried and defatted pod contents including the blotting paper. The error introduced by including the paper is considered negligible.
3. Two ml of 2% K_2CO_3 added to 5 g powder obtained from grinding the dry pod contents in a Wiley mill three times. Sufficient water was also added for proper mixing with the K_2CO_3.
4. The recovery of "sulfur" from Na_2SO_4 varies at this time from 85% to 96%. No correction for the ash analysis can be applied until the reason for above variance is established.
5. These values, in brackets, are only for comparison. They can not be used, because it is known *a priori* that recovery is incomplete.

Table 7. Metabolic balance study on a monkey in a pod for 3.98 days. (#307, Angelo, 24-27 April 1973)

C. Output-intake ratio

	$\frac{\text{Output}}{\text{Intake}} \times 100$	Per cent retained by monkey
Nitrogen	58.5	41.5
Phosphorus	87.3	12.7
Chloride	94.0	6.0
Sulfur	79.0	21.0
Calcium	92.0	8.0
Magnesium	93.0	7.0
Sodium	84.0	16.0
Potassium	84.0	16.0
Iron	89.0	11.0
Fat	15.0	85.0

Table 8. Body weights of male pig-tailed monkeys initially and immediately following four days of pod restraint.

Monkey No.	Body Wt in kg		Difference
	Before	After	
411	10.02	9.65	- 0.37
290	10.96	11.01	+ 0.05
200	5.57	5.56	- 0.01
307	11.54	12.12	+ 0.58
174	13.17	13.22	+ 0.05
314	9.00	8.95	- 0.05
337	13.75	13.00	- 0.75
341	12.23	11.98	- 0.25
410	12.52	12.51	- 0.01
409	11.40	11.13	- 0.27

Table 9. Body weights of known age laboratory-reared male and female pig-tailed monkeys.

Age in Months	Males			Females		
	No.	Mean Wt. (kg)	Std. Dev. (kg)	No.	Mean Wt. (kg)	Std. Dev. (kg)
6	9	1.36	0.07	8	1.22	0.15
12	9	1.85	0.14	8	1.70	0.16
18	7	2.49	0.12	7	2.25	0.17
24	6	2.84	0.14	7	2.62	0.17
30	6	3.09	0.23	7	3.11	0.21
36	6	3.38	0.18	7	3.53	0.26
42	5	3.64	0.08	6	3.83	0.40
48	4	4.35	0.43	5	4.10	0.38
54	4	5.08	0.36	5	4.58	0.40
60	4	5.41	0.54	4	4.57	0.20
66	4	6.05	0.56	4	4.83	0.11
72	3	6.71	0.76	4	5.12	0.37
78	2	7.72	0.95	4	5.40	0.20
84	2	8.27	1.03	3	5.37	0.28
90	2	8.80	1.00	3	5.36	0.26
96	2	9.20	0.78	1	5.56	
102	1	9.98				
108	1	10.09				
114	1	10.12				

Table 10. Body composition measurements on young harbor seals *(Phoca vitulina)* reared in a dry (non-diving) enclosure.

		15 May 1972	26 Jun 1972	08 Aug 1972	18 Sep 1972	30 Oct 1972	11 Dec 1972
Body Weight (kg)	GL	22.7	17.7	17.3	18.2	25.9	27.7
	RL	15.4	15.0	22.7	27.7	34.1	34.5
	Rasp	∿	∿	15.4	17.7	24.1	32.7
	$\bar{X}$	19.1	16.4	18.5	21.2	28.0	31.6
Total Body Water (liters)	GL	11.01	9.66	9.92	11.48	13.16	14.12
	RL	10.60	10.36	15.59	16.26	17.21	15.89
	Rasp	∿	∿	10.63	10.94	13.16	15.73
	$\bar{X}$	10.81	10.01	12.05	12.89	14.51	15.25
% Total Body Water	GL	48.5	54.6	57.3	63.1	50.8	51.0
	RL	68.8	69.1	68.7	58.7	50.5	50.0
	Rasp	∿	∿	69.0	61.8	54.6	48.1
	$\bar{X}$	58.7	61.9	65.0	61.2	52.0	49.7
Lean Body Mass (kg)	BL	15.1	13.2	13.6	15.7	18.0	19.4
	RL	14.5	14.2	21.4	22.3	23.6	23.6
	Rasp	∿	∿	14.6	15.0	18.0	21.5
	$\bar{X}$	14.8	13.7	16.5	17.7	19.9	21.5
% Lean Body Mass	GL	66.4	74.8	78.5	86.4	69.6	69.9
	RL	94.2	94.7	94.1	80.4	69.2	68.5
	Rasp	∿	∿	94.5	84.7	74.8	65.9
	$\bar{X}$	80.3	84.8	89.0	83.8	71.2	68.1
Body Fat (kg)	GL	7.6	4.5	3.7	2.5	7.9	8.3
	RL	0.9	0.8	1.3	5.4	10.5	10.9
	Rasp	∿	∿	0.8	2.7	6.1	11.2
	$\bar{X}$	4.3	2.7	1.9	3.5	8.2	10.1
% Body Fat	GL	33.6	25.2	21.5	13.6	30.4	30.1
	RL	5.8	5.3	5.9	19.6	30.8	31.5
	Rasp	∿	∿	5.5	15.3	25.2	34.1
	$\bar{X}$	19.7	15.3	11.0	16.2	28.8	31.9

Table 10. Body composition measurements on young harbor seals *(Phoca vitulina)* reared in a dry (non-diving) enclosure (continued).

		15 May 1972	26 Jun 1972	08 Aug 1972	18 Sep 1972	30 Oct 1972	11 Dec 1972
RBC Volume (ml)	GL	1325	1521	1316	1552	1552	1751
	RL	993	1361	1662	2121	1996	2042
	Rasp	~	~	1124	1281	1425	1745
	$\bar{X}$	1159	1441	1367	1651	1658	1846
RBCV/kg BW	GL	58	86	76	85	60	63
	RL	65	91	73	77	59	59
	Rasp	~	~	73	72	59	53
	$\bar{X}$	62	89	74	78	59	58
RBCV/kg LBM	GL	88	115	97	99	86	90
	RL	69	96	78	95	85	87
	Rasp	~	~	77	85	79	81
	$\bar{X}$	79	106	84	93	83	86
Plasma Volume (ml)	GL	1106	1117	1282	1382	1458	1579
	RL	994	1406	1825	2076	2451	2160
	Rasp	~	~	902	1173	1402	1881
	$\bar{X}$	1050	1262	1336	1544	1770	1873
PV/kg BW	GL	49	63	74	76	56	57
	RL	65	94	80	75	72	63
	Rasp	~	~	59	66	58	58
	$\bar{X}$	57	79	71	72	62	59
PV/kg LBM	GL	73	85	94	88	81	81
	RL	69	99	85	93	104	92
	Rasp	~	~	62	78	78	87
	$\bar{X}$	71	92	80	86	88	87
Blood Volume (ml)	GL	2431	2638	2599	2934	3010	3330
	RL	1987	2767	3487	4197	4447	4204
	Rasp	~	~	2026	2454	2827	3626
	$\bar{X}$	2209	2703	2704	3195	3428	3720
BV/kg BW	GL	107	149	150	161	116	120
	RL	129	185	154	152	130	122
	Rasp	~	~	132	139	117	111
	$\bar{X}$	118	167	145	151	121	118
BV/kg LBM	GL	161	200	191	187	167	172
	RL	137	195	163	188	188	178
	Rasp	~	~	139	164	157	169
	$\bar{X}$	149	198	164	180	171	173

Table 10. Body composition measurements on young harbor seals *(Phoca vitulina)* reared in a dry (non-diving) enclosure (continued).

		15 May 1972	26 Jun 1972	08 Aug 1972	18 Sep 1972	30 Oct 1972	11 Dec 1972
Whole Body Hematocrit (%)	GL	54.5	57.7	50.6	52.8	51.6	52.6
	RL	50.0	49.2	47.7	50.5	44.9	48.6
	Rasp	∿	∿	55.5	52.2	50.4	48.1
	$\bar{X}$	52.3	53.5	51.3	51.8	49.0	49.8
Venous Hematocrit (%)	GL	58.8	61.0	56.7	58.1	59.1	57.5
	RL	55.7	52.7	50.9	49.3	52.1	55.8
	Rasp	∿	∿	61.7	53.5	54.3	52.4
	$\bar{X}$	57.3	56.9	56.4	53.6	55.2	55.2
WBH/VH Ratio	GL	0.93	0.95	0.92	0.91	0.87	0.92
	RL	0.90	0.93	0.94	1.02	0.86	0.87
	Rasp	∿	∿	0.92	0.98	0.93	0.92
	$\bar{X}$	0.92	0.94	0.93	0.97	0.89	0.90
Hemoglobin (g/100 ml)	GL	22.0	22.9	20.6	21.2	21.7	21.0
	RL	19.8	18.9	18.0	17.5	19.1	20.6
	Rasp	∿	∿	22.2	19.7	20.5	19.6
	$\bar{X}$	20.9	20.9	20.3	19.5	20.4	20.4
MCHC (%)	GL	37.4	37.5	36.3	36.5	36.7	36.6
	RL	35.5	35.9	35.4	35.5	36.6	36.9
	Rasp	∿	∿	36.0	36.8	37.8	37.4
	$\bar{X}$	36.5	36.7	35.9	36.3	37.0	37.0

Table 11. Body composition measurements on young harbor seals *(Phoca vitulina)* reared in a wet (diving) enclosure.

		16 May 1972	27 Jun 1972	07 Aug 1972	19 Sep 1972	31 Oct 1972	12 Dec 1972	22 Jan 1973	26 Feb 1973
Body Weight (kg)	TL	19.1	15.9	21.3	21.8	22.7	31.8	32.7	35.7
	BL	15.4	20.5	25.0	27.2	30.9	35.4	38.2	41.8
	BR	13.6	20.5	25.0	26.3	29.5	32.2	37.7	35.9
	TR	17.3	15.0	19.1	23.2	26.8	34.1	∿	∿
	$\bar{X}$	16.4	18.0	22.6	24.6	27.5	33.4	36.2	37.8
Total Body Water (liters)	TL	10.07	9.34	13.04	13.06	12.96	14.00	15.05	16.25
	BL	8.97	12.12	15.80	16.30	16.20	17.09	18.74	19.50
	BR	7.60	11.62	14.63	14.92	15.59	16.58	19.60	19.34
	TR	9.55	9.52	12.05	13.62	14.78	15.52	∿	∿
	$\bar{X}$	9.05	10.65	13.88	14.48	14.88	15.80	17.80	18.36
% Total Body Water	TL	52.7	58.7	61.2	59.8	57.1	44.0	46.0	45.5
	BL	58.2	59.1	63.3	59.9	52.4	48.3	49.1	46.7
	BR	55.9	56.7	58.5	56.7	52.8	51.5	52.0	53.9
	TR	55.2	63.5	63.1	58.7	55.1	45.5	∿	∿
	$\bar{X}$	55.5	59.5	61.5	58.8	54.4	47.3	49.0	48.7
Lean Body Mass (kg)	TL	13.8	12.8	17.8	17.9	17.8	19.2	20.6	22.2
	BL	12.3	16.6	21.7	22.3	22.2	23.4	25.7	26.8
	BR	10.4	15.9	20.0	20.4	21.3	22.7	26.8	26.5
	TR	13.1	13.1	16.5	18.7	20.2	21.2	∿	∿
	$\bar{X}$	12.4	14.6	19.0	19.8	20.4	21.6	24.4	25.2
% Lean Body Mass	TL	72.0	80.4	83.8	81.9	78.2	60.3	63.0	62.3
	BL	79.7	81.0	86.7	82.0	71.9	66.2	67.3	64.0
	BR	76.6	77.7	80.1	77.7	72.3	70.5	71.1	73.8
	TR	75.6	87.0	86.4	80.4	75.5	62.3	∿	∿
	$\bar{X}$	76.0	81.5	84.3	80.5	74.5	64.8	67.1	66.7
Body Fat (kg)	TL	5.3	3.1	3.5	3.9	4.9	12.6	12.1	13.5
	BL	3.1	3.9	3.3	4.9	8.7	12.0	12.5	15.0
	BR	3.2	4.6	5.0	5.9	8.2	9.5	10.9	9.4
	TR	4.2	2.0	2.6	4.5	6.6	12.9	∿	∿
	$\bar{X}$	4.0	3.4	3.6	4.8	7.1	11.8	11.8	12.6
% Body Fat	TL	28.0	19.6	16.2	18.1	21.8	39.7	37.0	37.7
	BL	20.3	19.0	13.3	18.0	28.1	33.8	32.7	36.0
	BR	23.4	22.3	19.9	22.3	27.7	29.5	28.8	26.2
	TR	24.4	13.0	13.6	19.6	24.5	37.7	∿	∿
	$\bar{X}$	24.0	18.5	15.8	19.5	25.5	35.2	32.8	33.3

Table 11. Body composition measurements on young harbor seals *(Phoca vitulina)* reared in a wet (diving) enclosure (continued).

		16 May 1972	27 Jun 1972	07 Aug 1972	19 Sep 1972	31 Oct 1972	12 Dec 1972	22 Jan 1973	26 Feb 1973
RBC Volume (ml)	TL	1146	1198	1414	1578	1724	1858	1893	2104
	BL	952	1592	1869	2043	2142	2252	2308	2651
	BR	938	1511	1701	1918	2174	2264	2442	2353
	TR	1143	1148	1455	1768	1985	2060	~	~
	$\bar{X}$	1045	1362	1610	1827	2006	2109	2214	2369
RBCV/kg BW	TL	60	75	66	72	76	58	58	59
	BL	62	78	75	75	69	64	60	63
	BR	69	74	68	73	74	70	65	66
	TR	66	77	76	76	74	60	~	~
	$\bar{X}$	64	76	71	74	73	63	61	63
RBCV/kg LBM	TL	83	94	79	88	97	97	92	95
	BL	77	96	86	96	96	96	90	99
	BR	90	95	85	94	102	100	91	89
	TR	87	88	88	95	98	97	~	~
	$\bar{X}$	84	93	85	93	98	98	91	94
Plasma Volume (ml)	TL	1007	1010	1273	1255	1312	1499	1598	1770
	BL	928	1336	1306	1427	1613	1683	1740	1868
	BR	802	1343	1488	1539	1791	1738	2070	2004
	TR	1122	1137	1435	1667	1798	2017	~	~
	$\bar{X}$	965	1207	1376	1472	1629	1734	1803	1881
PV/kg BW	TL	53	64	60	58	58	47	49	50
	BL	60	65	52	53	52	48	46	45
	BR	59	66	60	59	61	54	55	56
	TR	65	76	75	72	67	59	~	~
	$\bar{X}$	59	68	62	61	60	52	50	50
PV/kg LBM	TL	73	79	72	70	74	78	78	80
	BL	75	81	60	64	73	72	68	70
	BR	77	85	74	75	84	72	77	76
	TR	86	87	87	89	89	95	~	~
	$\bar{X}$	78	83	73	75	80	79	74	75
Blood Volume (ml)	TL	2153	2208	2687	2833	3036	3357	3491	3874
	BL	1880	2928	3175	3470	3755	3935	4048	4519
	BR	1740	2854	3189	3457	3965	4002	4512	4357
	TR	2265	2285	2926	3435	3783	4007	~	~
	$\bar{X}$	2010	2569	2994	3299	3635	3843	4017	4250
BV/kg BW	TL	113	139	126	130	134	106	107	109
	BL	122	143	127	128	122	111	106	108
	BR	128	139	128	131	134	124	120	121
	TR	131	152	153	148	141	120	~	~
	$\bar{X}$	124	143	134	134	133	115	111	113
BV/kg LBM	TL	156	173	151	158	171	175	169	174
	BL	153	176	146	156	169	168	158	169
	BR	167	180	160	170	186	168	168	164
	TR	173	174	177	184	187	192	~	~
	$\bar{X}$	162	176	159	167	178	176	165	169

Table 11. Body composition measurements on young harbor seals *(Phoca vitulina)* reared in a wet (diving) enclosure (continued).

		16 May 1972	27 Jun 1972	07 Aug 1972	19 Sep 1972	31 Oct 1972	12 Dec 1972	22 Jan 1973	26 Feb 1973
Whole Body Hematocrit (%)	TL	53.2	54.3	52.6	55.7	56.8	55.4	54.2	54.3
	BL	50.6	54.4	58.9	58.9	57.0	57.2	57.0	58.7
	BR	53.9	52.9	53.3	55.5	54.8	56.6	54.1	54.0
	TR	50.9	50.2	50.7	51.5	52.5	50.5	∿	∿
	X	52.2	53.0	53.9	55.4	55.3	54.9	55.1	55.7
Venous Hematocrit (%)	TL	57.3	59.6	58.5	61.0	61.1	57.5	57.1	56.7
	BL	56.7	58.0	61.4	63.0	60.4	60.5	63.8	64.1
	BR	58.7	56.9	58.2	61.7	58.3	63.1	59.0	57.3
	TR	56.3	56.6	56.0	54.0	55.0	52.4	∿	∿
	X	57.3	57.8	58.5	59.9	58.7	58.4	60.0	59.4
WBH/VH Ratio	TL	0.93	0.91	0.90	0.91	0.93	0.96	0.95	0.96
	BL	0.89	0.94	0.96	0.94	0.94	0.95	0.89	0.92
	BR	0.92	0.93	0.92	0.90	0.94	0.90	0.92	0.94
	TR	0.90	0.89	0.91	0.95	0.95	0.96	∿	∿
	X	0.91	0.92	0.92	0.93	0.94	0.94	0.92	0.94
Hemoglobin (g/100 ml)	TL	20.9	21.9	20.6	21.8	23.2	20.9	20.0	20.3
	BL	20.5	20.4	21.5	22.3	22.3	21.9	22.8	23.6
	BR	22.3	20.3	21.2	22.5	21.5	22.9	21.5	20.9
	TR	20.8	20.9	20.3	19.6	19.0	18.4	∿	∿
	X	21.1	20.9	20.9	21.6	21.5	21.0	21.4	21.6
MCHC (%)	TL	36.5	36.7	35.2	35.7	37.9	36.3	35.0	35.8
	BL	36.2	35.2	35.0	35.4	36.7	36.2	35.7	36.8
	BR	38.0	35.7	36.4	36.5	34.6	36.3	36.4	36.5
	TR	36.9	36.9	36.3	36.3	37.9	35.1	∿	∿
	X	36.9	36.1	35.7	36.0	36.8	36.0	35.7	36.4

Table 12. Census of non-human primates *(M. nemestrina)* for the period 1 February 1973 to 31 July 1973.

	Feb	Mar	Apr	May	June	July
Number at start of month	50	48	43	42	42	42
Acquisitions	0	0	0	0	0	0
Losses	2	5	1	0	0	1
Number at end of month	48	43	42	42	42	41