Blood chemistry homeostasis during prolonged fasting in the northern elephant seal

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COSTA, DANIEL P., AND C. LEO ORTIZ. *Blood chemistry homeostatis during prolonged fasting in the northern elephant seal.* Am. J. Physiol. 242 (Regulatory Integrative Comp. Physiol. 11): R591–R595, 1982.—Serum electrolytes, 3 enzymes, and 11 metabolites were monitored for 32–68 days in weaned, naturally fasting elephant seal pups. Serum glucose, urea nitrogen, and creatinine levels declined as the fast progressed, whereas total protein, albumin, and globulin levels remained nearly constant. By contrast, triglycerides, cholesterol, uric acid, and bilirubin were quite variable and no definite trends were apparent. Alkaline phosphatase activity appeared to increase during fasting, while serum glutamic oxalacetic transaminase and lactate dehydrogenase remained fairly uniform. Comparisons of averaged blood chemistry values from singly and multiply sampled fasting pups to that of four nursing pups showed significant differences in the levels of blood urea nitrogen, creatinine, cholesterol, bilirubin, and albumin, but sampling uncertainty limited physiological interpretation. Electrolyte levels in all animals were maintained within narrow limits under all conditions with little interindividual variation. These results further document the remarkable homeostasis achieved during prolonged fasting in elephant seals and support the hypothesis that fat is the primary energy substrate during the protracted natural fasts characteristic of this species.

Mirounga angustirostris; metabolic substrate levels; natural fasting

EXTENDED PERIODS of complete food and water abstinence are part of the normal life history of many marine mammals (1, 8). During the breeding season adult male northern elephant seals *Mirounga angustirostris* are capable of fasts that may exceed 3 mo (14). Furthermore, females give birth, nurse, and wean their pups over a month-long period of total abstinence, during which their offspring may gain from 100 to 150 kg solely on a diet of mother's milk (15, 24). Once weaned, pups may fast for 2–3 mo until they depart the rookeries and presumably begin feeding (23). Previous studies of fasting elephant seal pups (19, 20) investigated water, energy, and urea flux and demonstrated that fat metabolism supplies most of the energy and water requirements of these animals with little overall protein oxidation. These reports, however, did not investigate important hematologic parameters that might provide further insights into other physiological adaptations underlying these remarkable fasts.

Although there exists an extensive literature dealing with alterations in metabolism and blood chemistry profiles in humans and laboratory mammals under clinical or experimental fasting conditions (e.g., 3, 6, 26, 28), comparable data from animals undergoing extended fasts in their natural habitat are understandably meager. One notable exception is the work of Nelson et al. (17, 18) on captive but naturally over-wintering bears that serves as an excellent model of natural fasting in terrestrial carnivores.

In the present study, blood chemistry profiles were monitored for up to 68 days in individual weaned elephant seal pups during prolonged food and water abstinence prior to their departure to feed at sea. The parameters presented here are those most often reported in both human clinical and experimental literature and were chosen to maximize the potential comparative value of this investigation. Of the serum enzymes examined lactate dehydrogenase (LDH) and serum glutamic oxalacetic transaminase (SGOT) activity often serve as indices of tissue trauma and other pathological states and were monitored to assess any undue trauma associated with the capture procedure or other abnormalities in the experimental animals. These data are compared to those of several nursing pups on the same rookery.

MATERIALS AND METHODS

Blood samples were collected from weaned and nursing northern elephant seal pups during the breeding season (Dec.–Mar.) on Año Nuevo Island, 30 km north of Santa Cruz, CA. The breeding behavior and population dynamics of the northern elephant seal on this rookery have been monitored for more than a decade (12, 13).

A total of 20 animals were blood sampled during the study. Four newly weaned pups were sampled and weighed 4–6 times over periods ranging from 32 to 68 days postweaning. Within 3 ± 1 days of weaning each animal was tagged and individually marked with peroxide to aid in future identification, since nearly 800 weaned animals were present on the rookery at the end of the study. An additional six weaned pups were sampled and weighed once during the study period, and although their exact weaning dates were unknown it was estimated from their weight, dentition, and pelage that most had been fasting in excess of 30 days. The mean weight of these six animals was 134 ± 26 kg at the time of sampling. The four suckling pups used in the study were sampled during the 3rd wk of nursing.

In addition to the blood samples collected from healthy fasting weaned seals, samples were taken from two or-

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phaned pups who had been injured and were in an emaciated condition. These two animals had been orphaned within the first 2 wk of nursing.

Capture, restraint, and blood sampling procedures have been described previously (19, 20). After sampling, whole blood was allowed to clot, spun at 4,000 rpm for 10 min (IEC, clinical centrifuge). Serum was decanted into glass storage vials and frozen at −75°C until analysis, 24–48 h later. To assess possible changes in blood chemistry resulting from minor stress induced by the capture-restraint procedure itself, blood profiles of four animals sampled on the same day by two separate techniques were compared. Each animal was approached while asleep and rapidly sampled (~15 s) using the method of Bonnell and Selander (2). One to three hours later the same animals were resampled using our standard procedure. Results obtained using the two methods did not differ significantly.

All analyses were run in duplicate on an automatic clinical analyzer (Programachem model 1040, American Monitor, Indianapolis, IN). In some cases, Na^+, K^+, and glucose concentrations were confirmed by conventional flame photometry (Advanced Instruments) and standard colorimetric, glucose oxidase-peroxidase assay (Sigma), respectively.

RESULTS

Table 1 summarizes the average levels of 13 serum components from both fasting and nursing animals. The mean values for weaned pups were computed by combining all data from both singly and multiply sampled individual animals, i.e., approximately 32–34 blood samples. Although the mean levels of blood urea nitrogen (BUN), creatinine, cholesterol, bilirubin, and albumin differ significantly between nursing and fasting animals, the method of combining data from all fasting pups, the small sample ($n = 4$), and generally higher sample variability in nursing pups precludes conclusive physiological interpretation of these differences. At least some of the sample variability in nursing pups, most notably triglycerides levels, probably reflects differences in the interval between the pups' last suckling bout and time of sampling, since samples were taken opportunistically. It is interesting to note that with the exception of glucose most of the clinical values for weaned pups are within or somewhat above those for the normal 48-h postabsorptive human.

Electrolyte levels under all conditions were maintained within narrow limits (Table 2). The average serum concentrations in nursing and weaned pups even after prolonged food and water abstinence were nearly identical, and both inter- and intraindividual variations through time were remarkably small. Elevated levels of electrolytes and protein, both clinical signs of dehydration, were never observed despite fasts that exceeded 2 mo.

Serum glucose (Fig. 1) and BUN (Fig. 2) levels declined during the fasting period in the four repeatedly sampled individuals. Least-square linear regression analysis of the data showed highly significant ($P \leq 0.01$, Student's t test) negative correlations for both parameters ($r = -0.67$ and -0.79 for glucose and BUN, respectively). Parallel decreases in creatinine concentration were also noted but were somewhat more variable. For example, creatinine fell from 1.01 ± 0.29 (SD) (range 0.79–1.37) to 0.70 ± 0.05 (SD) (range 0.65–0.75) $mg \cdot dl^{-1}$ over 56 days of fasting. Total protein, albumin, and globulins were maintained at nearly constant levels throughout the fast. By contrast, a high degree of both intra- and interindividual variability was observed in the levels of triglycerides, cholesterol, uric acid, and bilirubin, and no overall trends for these components were obvious.

Of the serum enzymes, only alkaline phosphatase activity appeared to change through time, increasing from 25 ± 6 (range 17–30) during the 1st wk to 63 ± 25 (range 46–92) $IU \cdot l^{-1}$ after 8 wk of total fasting. LDH and SGOT activity remained fairly constant during most of the fast, however, their activity increased transiently during the 6th and 7th wk.

Glucose and urea nitrogen levels did not differ significantly in individual animals sampled on the same day with the two sampling techniques (see MATERIALS AND METHODS). Mean glucose and urea nitrogen concentrations were 163 ± 24 and 28 ± 13 $mg \cdot dl^{-1}$ vs. 168 ± 32 and 32 ± 14 $mg \cdot dl^{-1}$ ($n = 8$) for rapidly sampled and normally

TABLE 1. *Summary of serum components in fasting and nursing pups*

Serum Component	Fasting Weaned Pups			Nursing Pups			Normal Postabsorptive Human Range
	n	Mean ± SD	Range	n	Mean ± SD	Range	
Glucose, $mg \cdot dl^{-1}$	14	160 ± 15	137–185	4	176 ± 25	152–210	60–110
Urea nitrogen, $mg \cdot dl^{-1}$	14	23 ± 7	12–39	4	33 ± 8*	25–35	5–25
Creatinine, $mg \cdot dl^{-1}$	10	1.12 ± 0.24	0.89–1.49	4	0.74 ± 0.32*	0.5–1.21	0.5–1.5
Uric acid, $mg \cdot dl^{-1}$	10	1.31 ± 0.35	0.81–1.83	4	0.89 ± 0.59	0.27–1.70	2–7
Triglycerides, $mg \cdot dl^{-1}$	10	147 ± 41	77–195	4	254 ± 98	159–383	30–135
Cholesterol, $mg \cdot dl^{-1}$	10	319 ± 24	273–358	4	235 ± 57†	192–314	150–250
Bilirubin, $mg \cdot dl^{-1}$	10	0.25 ± 0.12	0.11–0.43	4	1.04 ± 0.75*	0.38–1.9	0.1–1.0
Total protein, $g \cdot dl^{-1}$	10	7.66 ± 0.33	7.22–8.30	4	7.23 ± 1.18	6.10–8.48	6–8
Albumin, $g \cdot dl^{-1}$	10	3.61 ± 0.19	3.31–3.85	4	4.04 ± 0.16†	3.83–4.23	3.5–5.5
Globulin, $g \cdot dl^{-1}$	10	4.07 ± 0.23	3.46–4.52	4	3.19 ± 1.17	2.06–4.25	2.5–3.5
Alkaline phosphatase, $IU \cdot l^{-1}$	10	37 ± 14	29–84	4	71 ± 38	25–111	9–35
SGOT, $IU \cdot l^{-1}$	10	19 ± 4	12–24	4	16 ± 5	11–22	0–19
LDH, $IU \cdot l^{-1}$	10	84 ± 13	65–105	4	83 ± 10	68–89	24–78

Values are mean ± SD of all samples taken during the study. SGOT, serum glutamic oxalacetic transaminase; LDH, lactate dehydrogenase. Normal postabsorptive range for humans is included for comparison. Significant difference (Student's t test) between mean values at * $P \leq 0.05$ † $P \leq 0.01$.

restrained animals, respectively. Orphaned pups exhibited elevated SGOT (74 ± 22 IU·l^{-1}), LDH (240 ± 41 IU·l^{-1}), and glucose (204 ± 8 mg·dl^{-1}) compared to nursing or weaned animals; whereas triglycerides (65 ± 1 mg·dl^{-1}), cholesterol (123 ± 27 mg·dl^{-1}), total protein (6.5 ± 0.3 g·dl^{-1}), and globulin (2.3 ± 0.2 g·dl^{-1}) were lower. No significant differences in other serum components were noted.

The pattern of weight loss in the four serially sampled animals was nearly identical to that observed in previous studies (19). The daily weight loss averaged 0.52 ± 0.1 (range 0.43–0.61) kg·day^{-1}.

DISCUSSION

Clearly, the most significant finding of this study is the remarkable degree of homeostasis exhibited by elephant seal pups during protracted periods of complete food and water abstinence. The progressive decline in glucose and urea nitrogen levels suggests reduced protein catabolism as observed in previous kinetic studies (19, 20). These data are consistent with the hypothesis that in fasting elephant seals fat catabolism supplies nearly all energy and water requirements, whereas protein catabolism is of minor importance as an energy source.

Since survival under the physiologically austere conditions of complete abstinence demands maximum conservation of body water and rigid control of electrolytes, plasma protein, and thus plasma osmolality, the observed constancy of these parameters in fasting but otherwise active young elephant seals is not unexpected. Equally precise electrolyte and osmotic homeostasis has been observed in food- and water-deprived hibernating bears [Nelson et al. (18)], California sea lions (25), prairie dogs (21), and therapeutically fasted but water-supplemented humans (22). It is significant to note that osmotic and electrolyte homeostasis in both bears and prairie dogs subjected to food and water deprivation during the winter months is substantially better than that observed under similar conditions during the summer. This suggests that marine mammals, which routinely undergo protracted fasts during the breeding season, may have evolved seasonally variable homeostatic mechanisms not unlike those found in hibernating terrestrial species, and that fasting may be physiologically distinct from starvation or experimentally induced fasts (16). In contrast to normal weaned pups the elevated LDH and SGOT levels in orphaned pups probably indicate muscle, liver and/or heart damage (7) resulting from trauma associated with starvation or injury. Orphaned pups often sustain pro-

TABLE 2. *Summary of serum electrolytes in fasting and nursing pups*

Serum Electrolytes	Fasting Weaned Pups			Nursing Pups			Normal Post-operative Human Range
	n	Mean ± SD	Range	*n*	Mean ± SD	Range	
Na^+, meq·l^{-1}	10	147 ±2.0	142–150	4	146 ±3.34	143–150	138–144
K^+, meq·l^{-1}	10	5.0 ±0.3	4.6–6.0	4	4.8 ±0.4	4.4–5.3	4.5–5.5
Cl^{-1}, meq·l^{-1}	14	104 ±3.1	99–108				98–106
Ca^{2+}, mmol·l^{-1}	10	2.97 ±0.10	2.9–3.1	4	2.91 ±0.21	2.6–3.1	2.25–2.74
Phosphorus (total), mmol·l^{-1}	10	2.26 ±0.06	2.1–2.4	4	2.18 ±0.48	1.5–2.7	0.65–1.45

Values are mean ± SD of all samples taken during study.

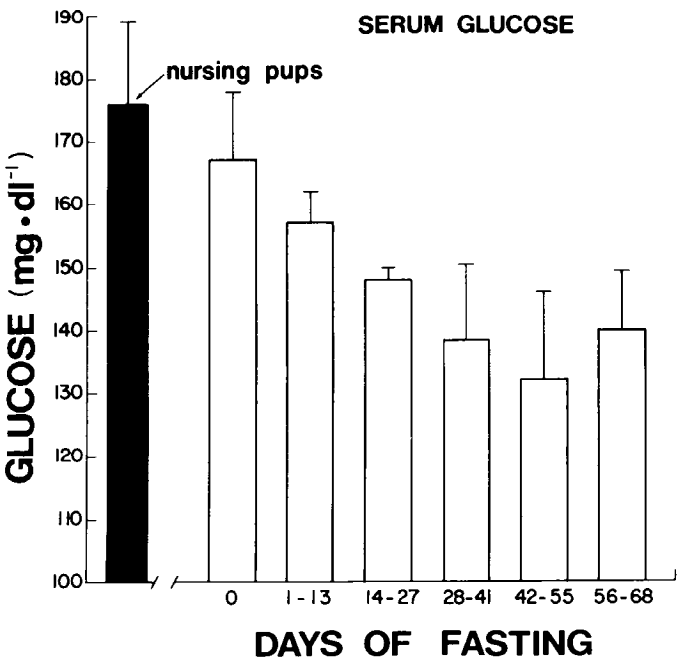

FIG. 1. Serum glucose concentration plotted as a function of length of fast in 4 repeatedly sampled fasting weaned pups. Prefasting levels in 4 nursing pups are included for comparison. *Bars*, ±SD; $r = -0.67$, $P \leq 0.01$, Student's t test.

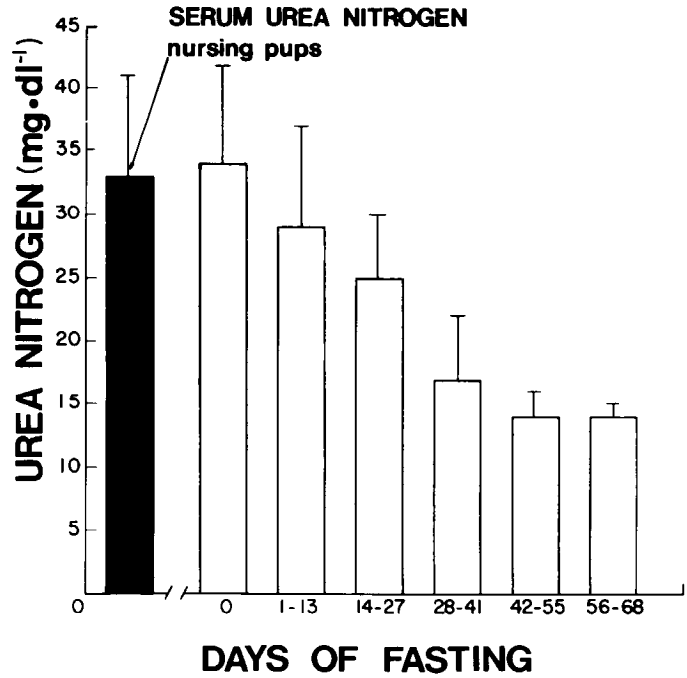

FIG. 2. Serum urea nitrogen concentration plotted as a function of length of fast in 4 repeatedly sampled weaned pups. Prefasting levels measured in 4 nursing pups are included for comparison. *Bars*, ±SD; $r = -0.79$, $P \leq 0.01$, Student's t test.

found physical abuse when attempting to suckle lactating alien females (13).

The gradual decline in both serum glucose and urea nitrogen (Figs. 1 and 2) is characteristic of other fasting mammals including humans (4). These observations almost certainly reflect a decrease in gluconeogenic activity and increased protein sparing. Preliminary studies (10) indicate that glucose turnover in fasting elephant seal pups is substantially lower than that observed in humans (27) and dogs (5) during prolonged fasts. What is somewhat paradoxical in the case of the elephant seal is that the serum glucose concentration remains some twofold higher than predicted for a mammal of its mass and metabolic rate (29) despite prolonged fasting. This "fasting hyperglycemia" may be a result of the high-fat diet consumed during suckling (24). Hyperglycemia, reduced glucose tolerance, and an impaired insulin sensitivity have been observed in fasting rats following high-fat diet regimes (32, 33). Furthermore, it appears that marine mammals in general, whether fasting or feeding, exhibit serum glucose concentrations exceeding those predicted by body size (30). The adaptive significance of this finding is unclear, but other investigators have presented plausible hypotheses relating it to the aquatic environment, diving physiology, and low-carbohydrate diets (25, 30). Since the glucose and insulin levels in "clinically normal" marine mammals resemble those seen in several forms of human diabetes (25, 31) additional data on carbohydrate metabolism and its hormonal regulation in marine mammals would be of considerable interest.

The serum cholesterol concentration in elephant seals though somewhat variable was substantially higher than that seen in fasting rabbits (28). These authors reported that cholesterol levels rose from approximately 30 mg·dl^{-1} to well over 100 mg·dl^{-1} during 32 days of fasting, whereas the lowest value obtained in this study during fasting was 273 mg·dl^{-1}. It is interesting to note that in fasting rabbits despite the rise in serum cholesterol concentration no net synthesis or degradation occurred, the increase was attributable to release of cholesterol stored in the lipid droplet (28). Elevated cholesterol appears to be characteristic of several fasting pinnipeds [California sea lion (25), southern elephant seal (11), and the northern fur seal (9)] as well as over-wintering bears (17). It would be instructive to know whether the "closed system" dynamics of cholesterol seen in forced-fasted rabbits also obtains for mammals undergoing natural fasts.

Although we have emphasized the physiological and metabolic adaptations underlying homeostasis during fasting, it must be recognized that these fasts are an integral part of the early development of young elephant seals, in sharp contrast to most mammals. Indeed, most immature mammals are incapable of prolonged fasting without sustaining profound and sometimes irreversible physiological damage. Further research may reveal additional adaptations which enable young elephant seals to not only survive but remain robust in the face of complete protracted nutritional deprivation.

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REFERENCES

1. Bartholomew, G. A. A model for the evolution of pinnipeds polygyny. *Evolution* 24: 546–559, 1970.
2. Bonnell, M. L., and R. K. Selander. Elephant seals: genetic variation and near extinction. *Science* 184: 908–909, 1974.
3. Cahill, G. F., Jr., M. G. Herrera, A. P. Morgan, J. S. Soeldner, J. Steinhe, P. L. Levy, G. A. Reichard, Jr., and D. M. Kipnis. Hormone-fuel inter-relationships during fasting. *J. Clin. Invest.* 45: 1751–1769, 1966.
4. Cahill, G. F., E. B. Marliss, and T. T. Aoki. Fat and nitrogen metabolism in fasting man. In: *Adipose Tissue: Regulation and Metabolic Function.* New York: Academic, 1970, p. 181–185.
5. Cowan, J., M. Vranic, and G. A. Wrenshall. Effect of preceding diet and fasting on glucose turnover in normal dogs. *Metabolism* 18: 319–330, 1969.
6. Frey, B. M., R. Mordasini, F. J. Frey, E. Wegmuller, G. Schlierf, and J. Hodler. Dysproteinaemia during total fasting. *Metabolism* 28: 363–369, 1979.
7. Geraci, J. R., and D. J. St. Aubin. Tissue sources and diagnostic value of circulating enzymes in cetaceans. *J. Fish. Res. Board Can.* 36: 158–163, 1979.
8. Harrison, R. J., and G. L. Kooyman. General physiology of the pinnipedia. In: *The Behavior and Physiology of Pinnipeds*, edited by R. J. Harrison. New York: Appleton-Crofts, 1968, p. 211–296.
9. Hunter, L., and S. H. Madin. Clinical blood values of the northern fur seal. *J. Wildl. Dis.* 12: 526–530, 1976.
10. Keith, E. O., S. Pernia, R. Condit, and C. L. Ortiz. Metabolism and recycling of glucose in northern elephant seals. *Bienn. Conf. Biol. Mar. Mammals 3rd Seattle, WA*, 1979.
11. Lane, R. A. B., R. J. H. Morris, and J. W. Sheedy. A haemotological study of the southern elephant seal. *Comp. Biochem. Physiol. A* 42: 841–850, 1972.
12. Le Boeuf, B. J. Male-male competition and reproductive success in elephant seals. *Am. Zool.* 14: 163–176, 1974.
13. Le Boeuf, B. J., and K. T. Briggs. The cost of living in a seal harem. *Mammalia* 41: 167–195, 1977.
14. Le Boeuf, B. J., and R. S. Peterson. Social status and mating activity in elephant seals. *Science* 163: 91–93, 1969.
15. Le Boeuf, B. J., R. J. Whiting, and R. F. Gantt. Perinatal behavior of northern elephant seal females and their young. *Behaviour* 43: 121–156, 1972.
16. Mrosovsky, N., and D. F. Sherry. Animal anorexias. *Science* 207: 837–842, 1980.
17. Nelson, R. A., J. D. Jones, H. W. Wahner, D. B. McGill, and C. F. Code. Nitrogen metabolism in bears: urea metabolism in summer and in winter sleep and role of urinary bladder in water and nitrogen conservation. *Mayo Clin. Proc.* 50: 141–146, 1975.
18. Nelson, R. A., H. W. Wahner, J. D. Jones, R. D. Ellefson, and P. E. Zollman. Metabolism of bears before, during, and after winter sleep. *Am. J. Physiol.* 224: 491–496, 1973.
19. Ortiz, C. L., D. P. Costa, and B. J. Le Boeuf. Water and energy flux in elephant seal pups fasting and under natural conditions. *Physiol. Zool.* 51: 166–178, 1978.
20. Pernia, S. D., A. Hill, and C. L. Ortiz. Urea turnover during prolonged fasting in the northern elephant seal. *Comp. Biochem. Physiol. B* 65: 731–734, 1980.
21. Pfeiffer, E. W., L. N. Reinking, and J. D. Hamilton. Some effects of food and water deprivation of metabolism in black-tailed prairie dogs, *Cynomys ludovicianus. Comp. Biochem. Physiol. A*

63: 1–4, 1979.
22. RAPOPORT, A., G. L. A. FROM, AND H. HUSDAN. Metabolic studies in prolonged fasting. I. Inorganic metabolism and kidney function. *Metabolism* 14: 31–46, 1965.
23. REITER, J., N. L. STINSON, AND B. J. LE BOEUF. Northern elephant seal development: the transition from weaning to nutritional independence. *Behav. Ecol. Sociobiol.* 3: 337–367, 1978.
24. RIEDMAN, M., AND C. L. ORTIZ. Changes in milk composition during lactation in the northern elephant seal. *Physiol. Zool.* 52: 240–249, 1979.
25. RIDGWAY, S. H. Homeostasis in the aquatic environment. In: *Mammals of the Sea: Biology and Medicine*, edited by S. H. Ridgway. Springfield, IL: Thomas, 1972, p. 590–747.
26. STEELE, R., B. WINKLER, I. RATHGEB, C. BJERKNES, AND N. ALTSZULER. Plasma glucose and free fatty acid metabolism in normal and long-fasted dogs. *Am. J. Physiol.* 214: 313–319, 1968.
27. STREJA, D. A., G. STEINER, E. B. MARLISS, AND M. VRANIC. Turnover and recycling of glucose in man during prolonged fasting. *Metabolism* 26: 1089–1098, 1977.
28. SWANER, J. C., AND W. E. CONNOR. Hypercholesterolemia of total starvation: its mechanism via tissue mobilization of cholesterol. *Am. J. Physiol.* 229: 365–369, 1975.
29. UMMINGER, B. L. Body size and whole blood sugar concentrations in mammals. *Comp. Biochem. Physiol. A* 52: 455–458, 1975.
30. UMMINGER, B. L. Mechanisms of cold adaptation in polar marine mammals. In: *Adaptations Within Antarctic Ecosystems. Proceedings of the 3rd SCAR Symposium on Antarctic Biology*, edited by B. A. Llano. 1977, p. 397–409.
31. VALENZUELA, S., A. FLORES, AND C. L. ORTIZ. Regulation of metabolism during prolonged natural fasts in the northern elephant seal: insulin and glucagon. *Annu. Minority Biomed. Supp. Symp. 9th Albuquerque, NM*, 1981.
32. ZARAGOZA, N., AND J. P. FELBER. Studies on the metabolic effects induced in the rat by a high fat diet. *Horm. Metab. Res.* 2: 323–329, 1970.
33. ZARAGOZA, N., AND J. P. FELBER. Studies of the metabolic effects induced in the rat by a high fat diet. II. Disposal of orally administered (^{14}C)-glucose. *Horm. Metab. Res.* 4: 25–30, 1972.