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Changes in blood properties of fasting and feeding harp seal pups, Phoca groenlandica, after weaning

by

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ABSTRACT

Physical blood properties and blood biochemistry were monitored in captive harp seal pups throughout the post-weaning fast and during the onset of feeding. One group was fasted for a period of 10 weeks and three other groups commenced feeding after fasts of 8 days, 29 days and 36 to 44 days respectively.

Calcium and phosphorus levels in fasting pups were lower than in feeding pups. Circulating levels of alkaline phosphatase (Alk. Phos.), serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxalo-acetic transaminase (SGOT), and creatinine phosphokinase (CPK) increased with the onset of feeding. Other monitored blood parameters exhibited no significant differences between feeding and fasting animals, and were within the range of normal values previously reported for harp seals.

Harp seal pups commence feeding within about six weeks after weaning. During this period they appear capable of maintaining homeostasis with respect to many blood parameters, prior to acheiving nutritional self-sufficiency.

INTRODUCTION

Several research facilities, public zoos and aquaria collect immature phocid seals for research or public display. These animals are often young of the year which

frequently refuse to eat when brought into captivity. For numerous animals, including sheep and cattle (Alden 1970), it has been found that prolonged periods of fasting, particularly at a young age, are permanently detrimental to future growth and development and possibly to survival. As a precaution against this, seals are often force-fed milk substitutes (Cornell 1975) or fish (Ronald et al. 1970).

In recent study, Worthy et al. (in press) maintained newly-weaned harp seal pups, Phoca groenlandica, in captivity to quantify weight losses during the normal post-weaning fast (Stewart and Lavigne 1980) and subsequent growth after the onset of feeding (Innes et al., 1981). The general health of the animals during the experiment was monitored by weekly determinations of physical blood properties and levels of various circulating enzymes and metabolites. The blood data obtained provided an opportunity to examine changes in certain blood properties during the post-weaning fast and at the onset of feeding in relation to normal values observed previously in immature (Englehardt 1979) and adult harp seals (Ronald et al. 1968, Geraci 1970, Geraci 1971, Geraci et al. 1979).

MATERIALS AND METHODS

Twenty weaned male harp seal pups, aged approximately 12 days (Stewart and Lavigne 1980, Worthy et al. in press) were collected in the Gulf of St. Lawrence on 17 March 1980 and transported to Guelph, Ontario, where they arrived on 20 March.

Four treatment groups were maintained in tanks of artificial salt water at a temperature of 10 C. One group, initially comprised of nine seals, was not offered food for the duration of the 10 week study; a 'fed' group initially consisted of five animals which were offered food after an 8 day fast; 'recovery group 1' consisted of three animals which fasted for 29 days before feeding (one of them voluntarily) and 'recovery group 2' consisted of three

animals, one of which fasted voluntarily for 36 days, one of which fasted voluntarily for 44 days prior to the onset of feeding, and another which was force-fasted for 29 days and then self-fasted for a further 15 days before commencing to feed. As part of the growth study (Worthy et al. in press) 1 feeding and 2 fasting animals were sacrificed bi-weekly for detailed analysis of carcass composition.

Food consisted of whole Pacific herring, Clupea pallasi, which was initially cut into 2.5 cm pieces. Once animals were feeding regularly, vitamin and mineral supplements were included as part of the diet (Table 1). Since the animals were maintained in salt water, no NaCl supplements were given. Animals were offered food twice daily. Any fish which were not consumed were reweighed, subtracted from the intake record and discarded.

Blood samples were obtained from the hind venous plexus of restrained animals (Ronald et al. 1969, Geraci 1971) immediately upon arrival in Guelph and weekly thereafter. Animals were fasted for at least 18 hours prior to bleeding using a Vacutainer assembly (Becton Dickinson and Co., Canada, Ltd., Clarkson, Ontario). Blood was placed into potassium ethylene-diamine tetra-acetic acid (K-EDTA) and lithium heparin tubes for routine hematology and sodium and potassium determinations respectively , and into serum tubes for blood chemistry. Blood samples for glucose determination were placed in serum tubes containing sodium fluoride. All tubes, except the EDTA tubes, were centrifuged within one hour of sampling, the serum or plasma was removed and then placed on dry ice for future analysis. The EDTA tubes were kept cool (5 C) in a refrigerator, and hematological analysis was done within 3 days of sampling (Geraci and Engelhardt, 1974).

Packed cell volume (PCV) was obtained using the micro-hematocrit technique (Simmons 1976). Red and white blood cell counts were determined with a Coulter Counter using 'Isoton' to dilute the sample (Coulter Electronics

Inc, Hialeah, Florida). 'Zaponin' (Coulter Electronics Inc., Hialeah, Florida) was used to produce erythrocytolysis facilitating white cell counts and hemoglobin determination. Hemoglobin was measured on a Hemoglobinometer (Coulter Electronics Inc.). Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC) were calculated from the RBC count, hematocrit and hemoglobin values (Simmons 1976). Sodium and potassium levels in heparinized plasma were measured using a flame spectrophotometer (Radiometer, Copenhagen).

Total protein and blood glucose were measured using colorimetric techniques (Technical Bulletins no. 540 and, Sigma Chemical Co., St. Louis, Missouri).

Serum levels of calcium, phosphorus, blood urea nitrogen (BUN), alkaline phosphatase (Alk. Phos.), uric acid, serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxalo-acetic transaminase (SGOT), lactate dehydrogenase (LDH), creatinine phosphokinase (CPK), and creatinine levels were measured on an auto-analyzer (KDA, American Monitor, Indianopolis; Ontario Veterinary College, University of Guelph).

Data were examined by using an Analysis of Covariance (Statistical Analysis System , version 79.38, general linear models procedure) on each parameter, with the value of the parameter at the initial sampling as the covariate. Fisher's (protected) LSD procedure ($P = 0.05$) was used to test for significant differences between adjusted means obtained from the ANCOVA (Kleinbaum and Kupper 1978). If no significant differences were noted between treatments the data for that week were pooled.

RESULTS

Red and white cell numbers, hemoglobin and PCV remained constant throughout the study, regardless of duration of

fast (Table 2). Consequently, there was no significant differences in MCV, MCH, or MCHC (Table 2).

No significant differences were noted in the levels of LDH, creatinine, BUN, uric acid and total proteins ($P < 0.05$) (Table 3). Levels of uric acid in the non-feeding animals often appeared higher than those of feeding animals, but again these differences were not statistically significant ($P < 0.05$).

Blood glucose values also showed no significant differences between treatments at any point in the study ($P < 0.05$). The levels did show a decline over the initial 3 weeks of the study, from a mean of 173.1 ± 2.81 (± S.E.) mg/dl to a level fluctuating around 150 mg/dl (Fig. 1).

Levels of sodium and potassium showed no significant differences between the four treatments (Table 3), although fasting animals often had circulating sodium levels below 140 mEq/l.

In contrast blood calcium levels appeared to be related to feeding regimen, with levels in non-feeding animals significantly lower than those of feeding animals ($P < 0.05$) (Fig. 2). The same relationship was noted for blood phosphorus levels, and although not statistically significant ($P > 0.05$), levels in the fasted group animals were approximately 50% of those observed in feeding animals at age 85 days.

SGOT showed significant differences ($P < 0.05$) between treatments at age 43 days and between ages 57 and 85 days, with the 'fed' group always having the highest value. SGPT levels were always lower in the non-feeding animals and increased upon commencement of feeding ($P < 0.05$) (Fig. 3). CPK showed some significant differences ($P < 0.05$) (Fig. 4), with the 'fed' group always having the highest values. Again, there was an increase in the level of CPK with the commencement of feeding.

Alkaline phosphatase (Alk. Phos.) levels in feeding animals remained constant, whereas those in non-feeding animals declined significantly ($p < 0.05$) (Fig. 4). When animals commenced feeding Alk. Phos. levels again increased into the same range as the feeding animals.

DISCUSSION

In contrast to most other mammals, weaning in the harp seal is quite abrupt. The mother does not usually initiate the pup into the water, nor does she teach it to swim or forage. Soon after weaning, the pup moults its natal coat and enters the water either of its own accord or because of the break-up of the pack ice. Evidence suggests that these animals then undergo a fast which in some animals may last up to 6 weeks (Worthy et al. in press).

During this time the pup must contend with the thermal properties of water in order to thermoregulate. The animal compensates for the high thermal conductivity of water by having a well developed insulative layer of blubber. It has been suggested that since maintenance of the blubber layer is a priority, the lean body mass, including protein, may be a major source of energy in these fasting seals (Stewart and Lavigne 1980, Bailey et al. 1980, Worthy et al. in press).

Circulating levels of BUN, uric acid, creatinine and total proteins remained constant during the post-weaning fast and with the onset of feeding. Observed values were similar to normal ranges reported previously for both harp seals and ringed seals (Phoca hispida)(Geraci 1970, Geraci 1971, Geraci et al. 1979, Englehardt 1979, Ronald et al. in prep.). Uric acid levels were often higher in non-feeding animals, similar to those found in non-feeding harp seals by Geraci et al. (1979). Similarly blood glucose levels were maintained within the reported range for captive adult harp seals (Ronald et al. in prep.). The initially high levels of glucose found in all animals may indicate that newly-weaned animals have elevated glucose levels.

Alternatively, elevated cortisol levels resulting from the stress of transport may account for the initial elevation in glucose levels.

The experimental seals in the present study also maintained both their erythrocyte and leukocyte counts, packed cell volumes and hemoglobin levels within ranges reported for both recently captured young animals and adults (Ronald et al. 1969, Geraci 1971, Englehardt 1979, Ronald et al. in prep.). Consequently the animals also maintained MCV, MCH, and MCHC within normal ranges (Geraci 1971).

Mean levels of sodium for feeding animals were within the reported normal range (147 to 161 mEq/l) but potassium levels were in the low end of the reported normal range (3.35 to 6.05 mEq/l) (Geraci 1970, Englehardt 1979, Ronald et al. in prep.). Levels of sodium were often very low in non-feeding animals, with a large number of readings between 135 and 146 mEq/l. Likewise, levels of potassium were often low, with readings between 2.7 and 3.2 mEq/l. Geraci (1972) reported that hyponatremia resulted from insufficient salt intake with superimposed physiologic and pathologic stresses. Mild to severe disturbances of the CNS and death generally resulted when plasma sodium concentration dropped below 145 mEq/l. However, Geraci et al. (1979) found young, thin, free-ranging ringed seals with sodium levels ranging from 140 to 145 mEq/l, with a mean of 143 mEq/l. If the major source of these salts is food, pups undergoing the normal post-weaning fast may be adapted to tolerate decreased levels for some time until the onset of feeding.

Feeding animals maintained levels of calcium and phosphorus similar to reported levels for captive adult harp seals (Geraci 1971, Ronald et al. in prep.). Fasting animals showed declining calcium and phosphorus levels throughout the fast. Serum calcium is a highly regulated ion and bone mineral is readily mobilized to maintain levels (Kaneko and Cornelius 1970), but this is not so with phosphorus which tends to be mobilized from soft tissue.

Qualitative observations of the distal bones of the flippers during the latter part of the study indicated extensive bone demineralization, with the digits being soft and flexible (Worthy unpubl. observations).

Alkaline phosphatase levels in feeding animals remained high, whereas fasting animals showed a continual decline throughout the study. Circulating levels of Alk. Phos. in young growing animals are primarily due to enzymes released during bone formation. Decreasing levels of Alk. Phos. have been reported during fasting (McDaniel and Dempsey 1962, Bide and Dorward 1970, Healey 1974). Healey (1974) related the level of Alk. Phos. to differing rates of osteogenesis and noted that levels also decrease with maturity. Decreasing levels of Alk. Phos. and circulating calcium indicate that bone demineralization is probably taking place extensively and this may result in decreased growth and therefore smaller adults if animals fast for prolonged periods of time.

It has been shown that the levels of SGPT in the liver of a three week old harp seal pup are about one third of the levels found in adults (St. Aubin 1976). During normal maturation, with a proper diet, blood levels of SGPT increase, paralleling the rise in liver concentrations (Ronald et al. in prep.). This could account for the continuing low levels of SGPT exhibited by fasting animals and the intermediate levels of the recovery group 1 and recovery group 2 animals in the present study (Fig. 3a). All values of SGPT measured in non-feeding animals were below levels reported by Geraci (1970) for captive adult harp seals, whereas feeding animals were within this range. Fasting animals had lower levels than 2 to 12 month old captive pups (Ronald et al. in prep.).

SGOT levels in the muscle of adults are 50% higher than the levels reported in three week old pups (St. Aubin 1976).

Increasing muscle mass results in increasing blood levels of SGOT (St.Aubin 1976), as indicated by the 'fed' group animals in the present study. SGOT levels reported by Geraci (1970) in captive adult harp seals ranged from 68.0 to 144.0 IU/l and only the 'fed' group animals in the present study were in this range constantly.

LDH values exhibited by the experimental seals were within the reported range for both adult (Geraci 1970) and young harp seals (Ronald et al. in prep.).

CPK is an enzyme which is quick to respond to 'stress' (St. Aubin et al. 1979) and within a short time blood levels can increase dramatically. This may explain the higher CPK levels in the feeding animals, which tended to struggle more than fasted animals during the blood sampling periods.

On day 54 of the fast, one fasting animal died. Analysis of a blood sample obtained 30 minutes prior to death showed increased levels of SGOT (92.3 IU/l), LDH (444 IU/l), CPK (59 IU/l), creatinine (2.5 IU/l), BUN (87.1 IU/l) and uric acid (2.9 IU/l) compared to other animals. Measurement of total bilirubin showed a level of 1.3 mg/dl, of which 0.6 mg/dl was conjugated. This compares with normal observed values of total bilirubin of approximately 0.2 mg/dl in harp seals (Geraci 1970, Worthy unpubl. observations, Ronald et al. in prep.), and ringed seals (Geraci et al. 1979). Elevated LDH levels have been implicated in a number of hepatic disorders (Kaneko and Cornelius 1970). High levels of SGOT indicate skeletal and cardiac muscle damage (St. Aubin and Geraci 1977) and high SGOT and LDH combined have been connected with myocardial infarction (Latner 1975). Levels of these parameters observed just prior to death are indicative of jaundice, possibly resulting from liver damage, and muscle breakdown. Elevation of BUN, uric acid and creatinine may have resulted from kidney damage or disease (Latner 1975). Liver, kidney and possibly heart

damage could have resulted from the depletion of core proteins in the terminal stages of starvation.

During fasts, several marine mammals (Tursiops truncatus and Lagenorhyncus obliquidens) (Ridgeway 1972), and weaned pups of Mirounga leonina (Bryden 1972) and P. groenlandica (Stewart and Lavigne 1980, Worthy et al. in press) lose muscle at least as fast as body fat and may meet glucose requirements through protein utilization (Ridgeway 1972). Animals that maintain blood glucose levels by utilizing protein as a precursor or alternatively using protein directly as an energy source, must rid their body of nitrogenous wastes. This could result in marked water losses. The seals in the present study did not become dehydrated; they maintained a normal PCV in blood and normal water levels in both carcass and viscera (Worthy et al. in press). Fasting harbour seals, P. vitulina, apparently do not drink seawater (Depocas et al. 1971), however otariids fasting in warm climates have been reported to do so (Gentry 1981). Fasting pups in this study must then be able to derive sufficient water metabolically and from free water in the tissues, with the possible ingestion of small amounts of salt water. Muscle tissue, being approximately 72% water in both feeding and fasting harp seal pups (Worthy unpubl. results), could serve as an immediate source of pre-formed water, in addition to being an energy source. Catabolism of blubber, which results in 1.07 g of water per gram of fat catabolized (Prosser and Brown 1961), would also provide metabolic water. It thus appears that fasting harp seal pups maintain water balance and meet caloric requirements by metabolizing blubber and protein in a ratio which prevents the rapid depletion of either, thereby allowing for the maintenance of an effective insulative layer and prolonging the use of the core musculature as an energy source.

Field data suggest that harp seal pups begin feeding on their own within about six weeks of weaning (Worthy et al.,

in press). All of our experimental animals remained in good physiological condition throughout the first six weeks of the post-weaning fast, and all but one animal appeared capable of maintaining homeostasis for the duration of the ten week experiment.

The post-weaning fast is a normal part of the life cycle in numerous pinniped species. It would appear that most concerns about healthy young phocid seals refusing to eat upon initial arrival at a facility are unfounded.

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Table 1: Daily vitamin supplements given to all feeding animals.

One vitamin B capsule (1)	
(thiamine hydrochloride)	100 mg
One Novo-B capsule (2)	
vitamin B	10 mcg
thiamine mononitrate	35 mcg
riboflavin	15 mcg
niacinamide	50 mcg
pyridoxine HCl	5 mg
calcium d'Pantothenate	20 mg
ascorbic acid	300 mg
One vitamin A capsule (2)	25000 IU
One halibut liver oil capsule (2)	
vitamin A	5000 IU
vitamin D	400 IU
One vitamin E capsule (1)	
(73.5 mg of d-alpha tocopheryl acetate)	100 IU
One Latan capsule (3)	
vitamin A	5000 IU
vitamin B	1000 IU
vitamin B	2 mcg
thiamine mononitrate	3 mcg
riboflavin	3 mg
niacinamide	20 mg
pyridoxine	3 mg
calcium carbonate	250 mg
folic acid	2 mg
iron (ferrous fumarate)	66 mg

(1) ICN Canada Ltd., Montreal, Canada.
(2) Novopharm Ltd., Toronto, Canada.
(3) Ayerst Laboratories, Montreal, Canada.

Table 2: The mean (± S.D.) and range for the pooled data for packed cell volume (PCV), hemoglobin (HMG), red cell count (RBC), white cell count (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin content (MCHC). Reported normal values are shown.

	Units	n	$\bar{x}$ ± S.D.	Observed Range	Reported Range	Source
RBC	(10^6/mm)	158	5.2 ± 0.56	4.0 - 6.9	3.8 - 6.9	1,2,3,4
WBC	(/mm)	158	10976 ± 3974	3892 - 26104	3800 - 37565	1,2,3,4
HMG	(g/dl)	158	20.0 ± 1.92	15.4 - 24.1	15.8 - 28.8	1,3,4
PCV	(%)	158	54.8 ± 4.6	42.5 - 68.0	38.5 - 68.0	1,3,4
MCV	( )	158	106.0 ± 11.3	77.5 - 141.3	88.0 - 138.0	1,4
MCH	( )	158	38.7 ± 4.8	28.5 - 54.8	30.0 - 56.0	1,4
MCHC	(%)	158	36.6 ± 2.0	33.1 - 43.2	33.0 - 47.0	1,4

1. Geraci, 1971. ..... pups up to 6 months old
2. Englehardt, 1979. ..... 3 month old pups
3. Ronald et al. 1969. ..... captive young animals
4. Ronald et al. in press ... pups up to 2 months old

Table 3: The mean (± S.D.) and range for the pooled data for sodium, potassium, total proteins, creatinine, blood urea nitrogen (BUN), uric acid and lactate dehydrogenase (LDH). Reported normal values are shown.

	Units	n	$\bar{x}$ ± S.D.	Observed Range	Reported Range	Ref.
Total Proteins	(mg/dl)	145	5.5 ± 0.46	4.1 - 6.8	5.3 - 7.9	1,2,4 *
BUN	(mg/dl)	139	38.8 ± 11.7	15.9 - 71.5	20 - 69	1,3
Uric Acid	(mg/dl)	139	1.3 ± 0.39	0.5 - 2.8	0.3 - 2.6	1,3
Creatinine	(mg/dl)	139	0.9 ± 0.26	0.3 - 1.5	1.1 - 1.7	1,4
LDH	(IU/l)	139	159 ± 47.4	57 - 294	134 - 187	1,4
Sodium	(mEq/l)	159	153.5 ± 8.1	135 - 166	140 - 161	1,2
Potassium	(mEq/l)	159	3.8 ± 0.42	2.7 - 4.8	3.3 - 6.1	1,2

1. Geraci, 1970. ..... captive adults
2. Englehardt, 1979. ..... 3 month old pups
   10 month old pups *
3. Geraci et al. 1979. ..... captive young adults
   (Phoca hispida).
4. Ronald et al. in press. .. up to 2 month old pups

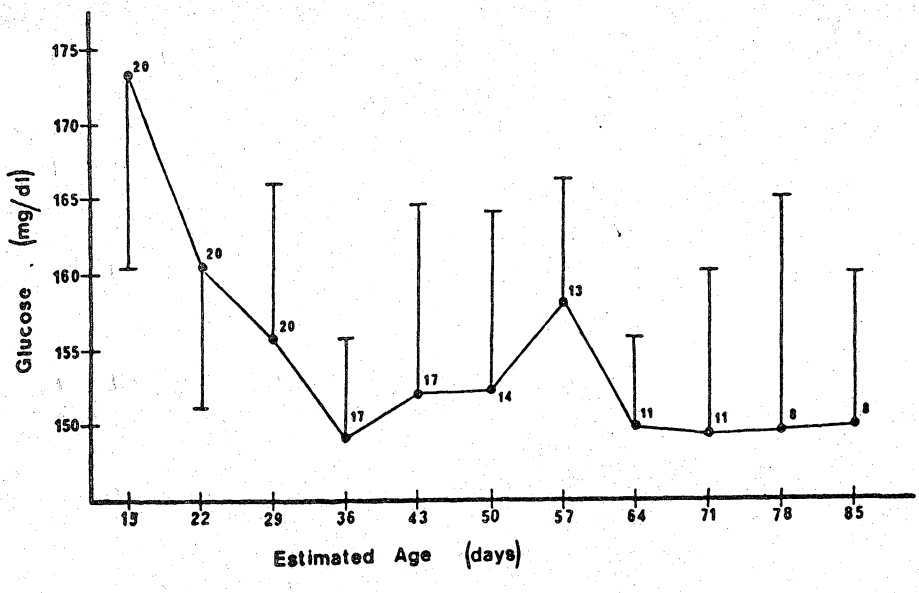

Fig. 1: The means (± S.D.) of the pooled data for blood glucose levels of all treatments for each sampling period .

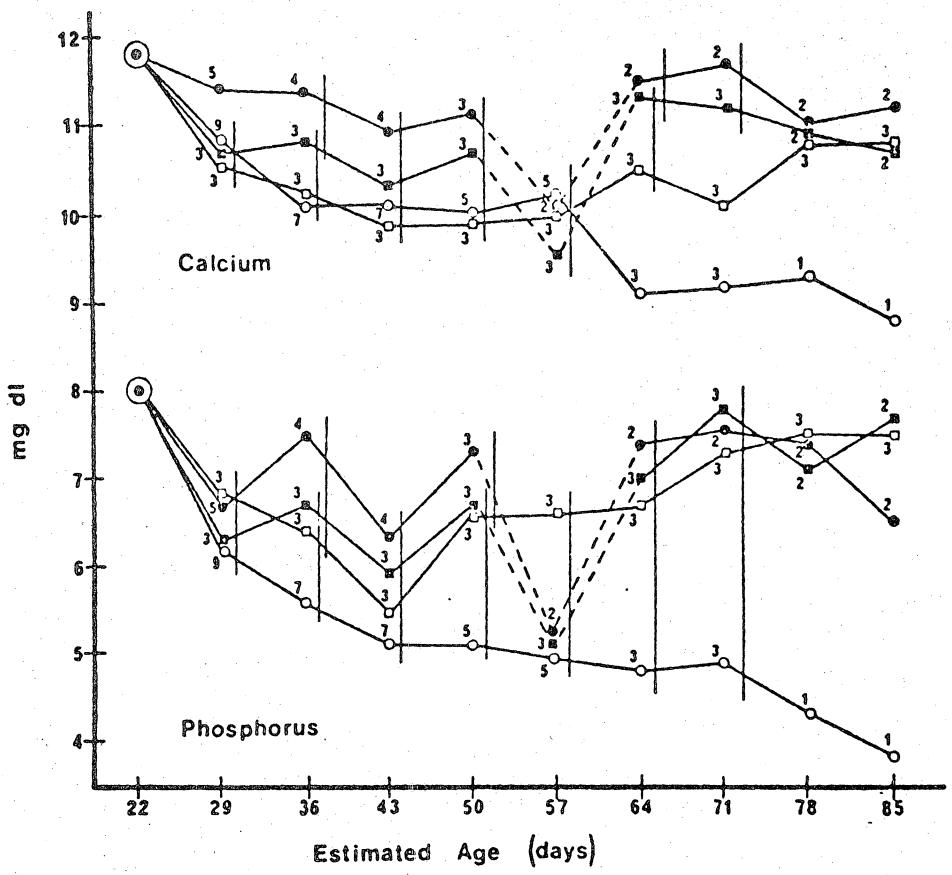

Fig. 2: The adjusted mean value for calcium and phosphorus (obtained from an Analysis of Covariance, using the initial value as the covariate) for each treatment. Points joined by bars are not significantly different using Fisher's (protected) LSD procedure ($P < 0.05$).

- 'fed' group
- recovery group 1
- recovery group 2
- 'fasted' group

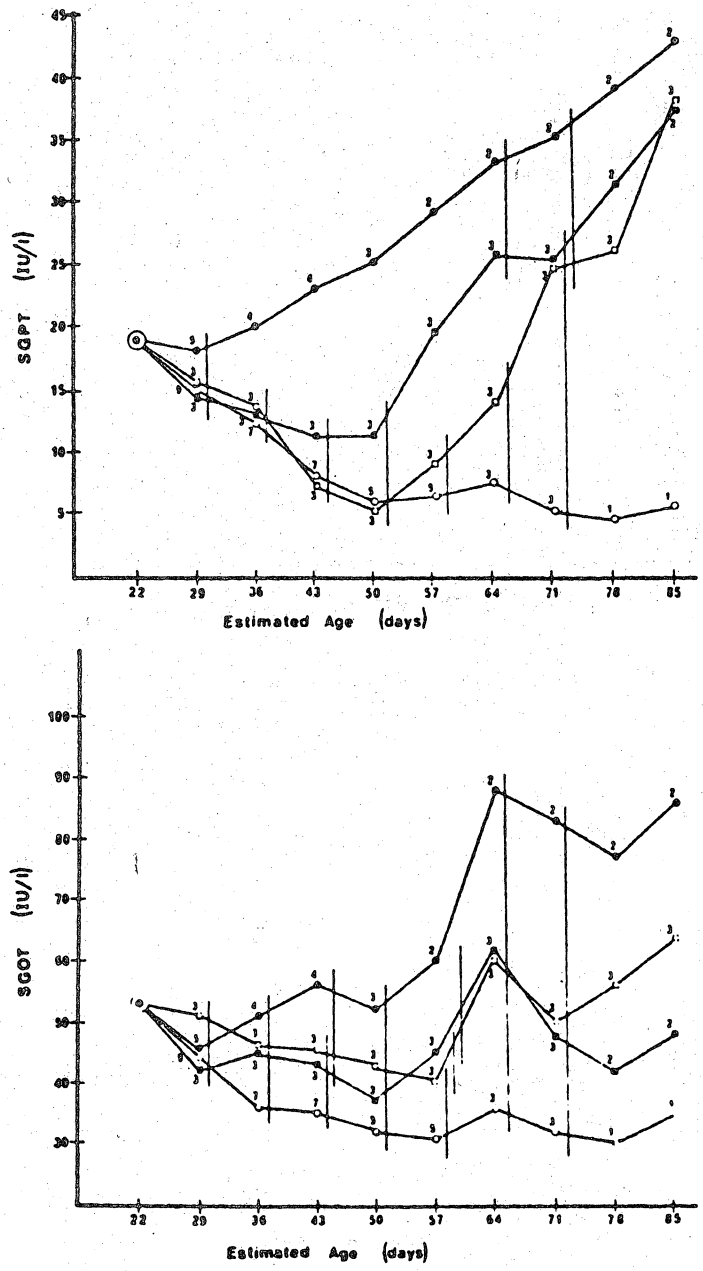

Fig. 3: The adjusted mean value for SGOT and SGPT (obtained from an Analysis of Covariance, using the initial value as the covariate) for each treatment. Points joined by bars are not significantly different using Fisher's (protected) LSD procedure ($P < 0.05$).

- ⊙ 'fed' group
- ⊠ recovery group 1
- □ recovery group 2
- ○ 'fasted' group

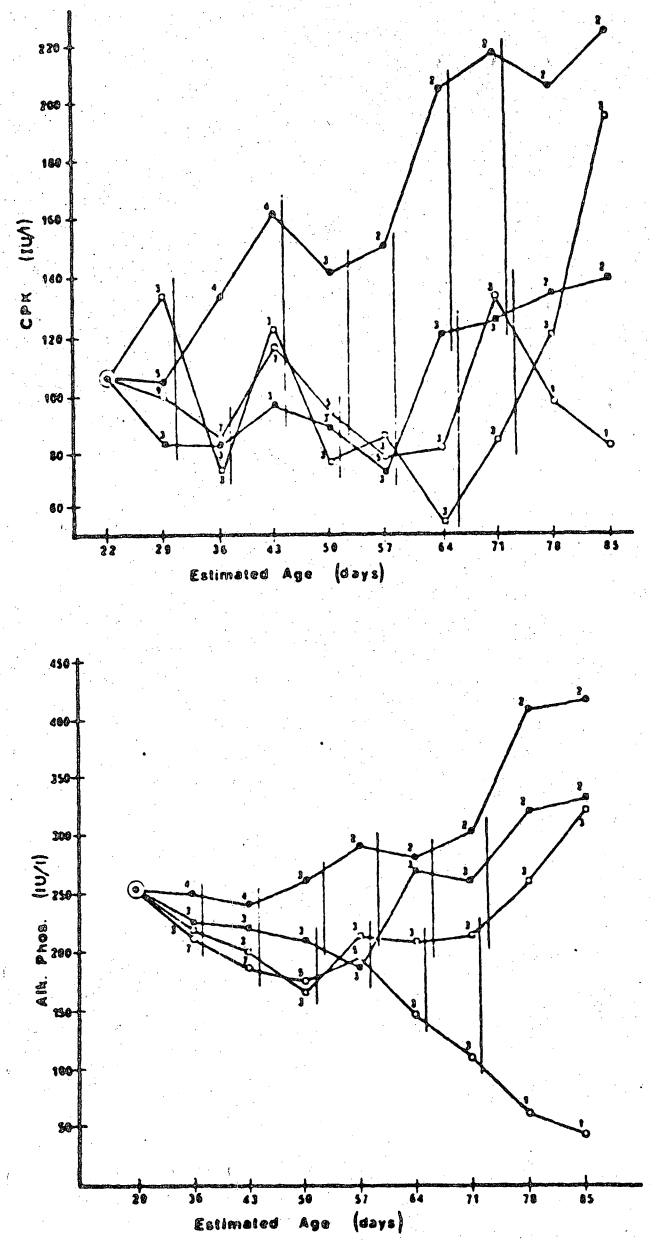

Fig. 4: The adjusted mean value for CPK and Alk. Phos. (obtained from an Analysis of Covariance, using the initial value as the covariate) for each treatment. Points joined by bars are not significantly different using Fisher's (protected) LSD procedure ($P < 0.05$).

● 'fed' group
■ recovery group 1
□ recovery group 2
○ 'fasted' group