Protein Energy Malnutrition and Fat Mobilization in Neonatal Calves

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Abstract

Fat stores and organ weights were assessed in calves at birth (n = 5) and after seven days of milk (n = 5) or electrolyte (n = 5) feeding.

Compared to newborn calves, milk-fed calves had a significant ($p < 0.05$) redistribution of fat from perirenal area to bone marrow. The thymus also involuted during milk feeding.

In electrolyte-fed calves there was a significant loss of perirenal and bone marrow fat. The visible omental, mesenteric and subcutaneous fat stores were depleted. Epicardial fat stores were not visibly affected.

There was a high correlation between bone marrow crude fat and bone marrow dry matter (R = 0.92). This suggests that dry matter estimations can be used to assess bone marrow fat stores. Perirenal fat may be intermediate in type between brown and white adipose tissue because it is mobilized in response to fasting, and formalin fixed perirenal fat did not contain detectable levels of thermogenin.

Key words: Calf, thymus, organ weights, adipose tissue, bone marrow fat, thermogenin, malnutrition.

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Résumé

Apport insuffisant de protéines énergétiques et mobilisation des réserves adipeuses, chez des veaux naissants

Cette expérience visait à déterminer la localisation des réserves adipeuses et le poids des organes, chez cinq veaux naissants, ainsi que chez dix autres, âgés de sept jours, dont cinq avaient reçu du lait et cinq, des électrolytes, depuis leur naissance.

Comparativement aux nouveau-nés, les veaux nourris au lait affichèrent une redistribution significative ($p < 0,05$) de leurs réserves adipeuses, de la région périrénale à la moelle osseuse, ainsi qu'une involution thymique.

Ceux qui recevaient des électrolytes subirent une perte appréciable de leurs réserves adipeuses périrénales et myéloïdes, ainsi qu'un puisement de celles de l'épiploon, du mésentère et du tissu sous-cutané; celles de l'épicarde ne subirent toutefois aucune atteinte visible.

On enregistra une relation étroite entre le gras de la moelle osseuse et sa matière sèche (R = 0,92). Cette constatation suggéra la possibilité d'utiliser la détermination de la matière sèche de la moelle osseuse pour en connaître la teneur en tissu adipeux. Le gras périrénal représente probablement un type intermédiaire entre le tissu adipeux blanc et le brun, parce que le jeûne en provoqua la mobilisation et que sa fixation dans la formaline ne se solda pas par la détection de thermogénine.

Mots clés: veaux, thymus, poids des organes, tissu adipeux, gras de la moelle osseuse, thermogénine, maldigestion.

Introduction

Body fat stores are the major source of endogenous energy (1) and are used as one of the determinants in assessing nutritional reserves (2). The amount of adipose tissue present at birth is important to the survival of the newborn animal (1, 3, 4, 5), since it is a major determinant of the length of time the newborn can survive without suckling (1,5). In addition, body fat stores (adipose tissue) function as shock absorbers and aid in thermal insulation (6).

The amount of body fat present at birth is influenced by factors such as species, breed, sex, maternal nutrition and the amount of placentation (1, 4). In a variety of newborn mammals, including the calf, most of the adipose tissue present at birth has been identified as brown adipose tissue (BAT) (7, 8). This tissue has a thermoregulatory function by way of nonshivering thermogenesis (7). In response to norepinephrine, fatty acids in BAT are oxidized in situ, for purposes of heat production (7).

Protein energy malnutrition (PEM) is not uncommon in neonatal calves and veterinarians are frequently required to assess body condition (body fat stores) and occasionally the weight of various organs. In newborn calves normal values are poorly documented and no values are available for the amount of marrow fat. The effects of PEM and cold exposure on fat mobilization have been studied in neonatal lambs (3, 5) and in several species of wildlife (9, 10).

However, no information is available as to the rates of redistribution and disappearance which take place in neonatal calves.

Protein energy malnutrition resulting in fat mobilization, atrophy of lymphoid tissue and increased susceptibility to infection have been described in man and are of particular importance in Third World countries (11, 12, 13). The purpose of this study was to compare the effects of PEM and milk feeding on body fat stores and organ weights in neonatal calves. In addition we wanted to establish the range of values for the major fat depots and selected organ weights in newborn calves.

Materials and Methods

Fifteen healthy, newborn, male, Holstein calves were obtained from six dairy farms in the Saskatoon area. These farms were selected on the basis of adequate management and feeding practices. All calves were obtained during their first day of life and had received approximately four liters of colostrum.

Upon arrival at the Western College of Veterinary Medicine each calf was weighed and blood samples were collected. Animals were assigned randomly to one of three groups: Group 1 (newborn), Group 2 (milk-fed), and Group 3 (electrolyte-fed).

Calves in group 1 were necropsied on day 1. All calves in group 2 and 3 were housed on straw in individual pens within an isolation room. The guidelines of the Canadian Council on Animal Care "Guide to the Care and Use of Experimental Animals" Vol. I were followed. Room temperature was maintained at 14-16°C and a daily 12 hour photoperiod was established. Calves in group 2 were fed warm, whole cow's milk twice each day for seven days and necropsied on day 8. The amount of milk fed per day was equal to 10% of animal's body weight.

Calves in group 3 were fed a warm balanced electrolyte plus glycine solution with glucose (Ion Aid, Syntex Agribusiness Ltd., Calgary) twice a day for a seven day period and necropsied at day 8. During the seven day feeding period each calf in group 3 consumed a total of 377 g of electrolytes plus glycine and 200 g of glucose (glucose fed at 50% of manufacturers recommended level). The calculated daily energy intake for groups 2 and 3 was 10.5-14.5 MJ and 1.1 MJ, respectively.

Calves in groups 2 and 3 were observed at least four times per day. All

TABLE I
Bodyweight on Day 1 (All Groups) and on Day 8 for Milk-Fed and Electrolyte-Fed Calves with & Change

		Bodyweight* (kg)		
Group	N	Day 1	Day 8	% Change*
Newborn	(5)	43.9± 4.9	—	
Milk-fed	(5)	42.5± 4.1	44.1± 4.0	+3.9± 0.9[a]
Electrolyte-fed	(5)	45.2± 5.5	41.4± 5.1	-8.4± 1.7[b]

* Mean± SD
[ab]Values in a column with different superscripts are significantly different ($P < 0.05$)

TABLE II
Absolute and Relative Weight of Thymus and Spleen in Neonatal Calves

		Thymus		Spleen	
Group	N	Weight*	Weight/BW**	Weight*	Weight/BW**
Newborn	(5)	185± 46[a]	0.42± 0.09[a]	95± 15	0.22± 4
Milk-fed	(5)	128± 14[b]	0.30± 0.05[b]	117± 23	0.27± 6
Electrolyte-fed	(5)	89± 49[b]	0.21± 0.10[b]	90± 8	0.22± 4

* Mean± SD (gram)
** Organ/BW ratio × 10^2, Mean± SD (%)
[ab]Values in a column with different superscripts are significantly different ($P < 0.05$)

TABLE III
Perirenal Fat (per Kidney) and Femur Bone Marrow Fat (per Femur) in Neonatal Calves

		Weight of Fat*	
Group	N	Kidney	Femur
Newborn	(5)	108± 20[a]	1.9± 0.7[b]
Milk-fed	(5)	79± 12[b]	2.9± 1.0[a]
Electrolyte-fed	(5)	36± 6[c]	0.7± 0.3[c]

* Mean± SD (gram)
[abc]Values in a column with different superscripts are significantly different ($P < 0.05$)

TABLE IV
Percentage of Dry Matter, Crude Fat (Ether Extraction) and Nonfat Residue in Femurs of Neonatal Calves

		Percentage Present in Bone Marrow		
Group	N	Dry Matter	Crude Fat	Nonfat Residue
Newborn	(5)	31.5± 2.3[a]	21.1± 1.5[b]	10.5± 2.7
Milk-fed	(5)	38.5± 5.8[a]	28.6± 5.6[a]	8.1± 2.8
Electrolyte-fed	(5)	18.4± 2.1[b]	6.6± 3.0[c]	11.8± 2.7

* Mean ≥ SD
[abc]Values in a column with different superscripts are significantly different ($P < 0.05$)

TABLE V
Selected Organ Weights (Mean± SD) in Newborn and Eight Day Old Calves (gram)

		Prescapular Lymph Node	Kidney	Thyroid Glands	Adrenal Glands
Newborn	(5)	4.9± 1.96	86± 13	11.3± 2.9	3.74± .56
Milk-fed	(5)	5.2± 1.14	86± 17	10.4± 1.7	3.78± .26
Electrolyte-fed	(5)	4.6± 1.54	89± 12	9.0± 2.0	3.62± .45

animals remained bright, active and maintained their body temperature within normal range.

Prior to necropsy calves were weighed, stunned with a captive bolt pistol and exsanguinated by severing the brachial or femoral arteries. A complete postmortem examination was performed. The thymus, prescapular lymph nodes, thyroid glands, adrenal glands, spleen, kidneys and perirenal fat were freed from surrounding structures, weighed, and selected portions were fixed in 10% neutral buffered formalin for future study. The weight of paired organs (right and left prescapular lymph nodes, kidneys, and perirenal fat) was averaged. In cases in which the weight of the forestomachs, abomasum and their contents exceeded 700 g, (the empty forestomachs and abomasum weigh about 700 g), the weight in excess of 700 g was deducted from the body weight at the time of necropsy. Formalin fixed perirenal fat was analyzed for thermogenin by competitive ELISA assay.

Fat deposits in the omentum, mesentery, pericardial sac and in the coronary groove of the heart were assessed visually. The femur was cut longitudinally in a mid-sagittal plane and length was measured on cut surface. Bone marrow was removed from the diaphyseal medullary cavity of both femurs. Marrow from the left femur was used for determination of percentage of dry matter content. Marrow contents were weighed and incubated at 68°C for 48-72 hours in order to allow water to evaporate, and periodically weighed until no further weight reduction was present. The amount of residual dry matter was expressed as a percentage of the initial weight and consisted of crude fat and nonfat bone marrow residue. The percentage crude bone marrow fat present in the right femur was determined by the ether extraction method. Bone marrow contents were weighed, oven dried and then extracted with petroleum ether in a Soxhlet extractor. The solvent was then evaporated from the extract, and the fat residue was weighed and expressed as a percentage of the initial weight. Nonfat residue values were calculated by subtracting crude fat values from dry matter values.

All data were subjected to a one-way analysis of variance with significance at $P < 0.05$. In instances of a significant treatment effect, the Student-Newman-Keuls procedure was used to detect differences among means at $P < 0.05$.

Results

There was no significant difference in the initial body weights of the three groups. At eight days of age electrolyte-fed calves had lost significantly more weight than their milk fed counterparts (Table I).

At necropsy, calves from all groups had grossly a similar skeletal size but electrolyte-fed calves were gaunt. Newborn and milk-fed calves had abundant deposits of firm adipose tissue around the kidneys (Figures 1 and 2). In electrolyte-fed calves there was a small amount of perirenal adipose tissue present, which was darker than the perirenal adipose tissue from newborn and milkfed calves (Figure 3). Fat in the mesentery and omentum (Figure 4) of newborn and milk-fed calves was soft and white, and the amount of subcutaneous fat was sparse. Electrolyte-fed calves had complete depletion of subcutaneous, omental (Figure 5), and mesenteric fat deposits. Fat present on the parietal surface of the pericardial sac in newborn (Figure 6) and milkfed calves was white and firm, whereas in electrolyte fed calves this fat was yellow (Figure 7), with a gelatinous appearance interpreted as serous atrophy. The amount and appearance of fat present in the coronary groove and extending down the paraconal and subsinosal interventricular grooves was similar in calves of all groups.

Red marrow fat (adipose tissue intermixed with hemopoietic tissue) was clearly visible, firm and red in the medullary cavity of the femurs in newborn (Figure 8) and milk-fed calves. In electrolyte-fed calves the medullary cavity was filled with tissue which was uniformly gelatinous and red. (Figure 9). There were significant differences between groups in thymic (Table II), perirenal, and femoral fat weights (Table III). Newborn calves had a significantly larger thymus gland, less femoral fat, and more perirenal fat than older, milk fed, calves. The only significant difference between milk-fed and electrolyte-fed calves was the absolute weight of the fat depots (Table III) and percentage dry matter and crude fat in femur bone marrow (Table IV). The loss of thymic weight in electrolyte-fed calves did not attain significance at the $P = 0.05$ level. There was a tendency for splenic weight (Table II) to vary between treatment groups ($P < 0.06$). The weights of other selected tissues did not change with treatment (Table V).

The correlation coefficient (R) in electrolyte fed calves between percentage femoral crude fat and amount of perirenal fat was 0.92 ($p = 0.013$). The calculated coefficient of determination (R^2) was 0.85. The loss of bone marrow fat was accompanied by a parallel decrease in bone marrow dry matter with little change in the amount of nonfat solids (Table IV). The correlation coefficient between percentage crude fat and percentage dry matter of femur bone marrow, in all 15 calves was 0.96, and the calculated R^2 was 0.92. The following formula describes the relation between bone marrow crude fat and dry matter content in newborn and neonatal calves: $\%CF = (\%DM \times 1.2) - 15.95$.

Femur lengths (mm) for group 1-3, were 210.0 ± 8.0, 210.4 ± 3.0, and 211.4 ± 3.4, respectively. The femur lengths did not change significantly with feeding regime. The correlation coefficient between femur length and newborn body weight is 0.71 ($p < 0.002$), the calculated R^2 was 0.50. None of the calves in any of the groups had gross evidence of any disease process.

Discussion

Milk-fed calves had an average weight gain of 230 g/day, which is consistent with target values for dairy calves.

The amount of fat present at birth is an important determinant of how long an apparent healthy calf can survive in the face of malnutrition (1). The results of electrolyte feeding show that the amount of fat present at birth in healthy calves is more than adequate to provide energy for at least seven days of severe malnutrition, in a thermoneutral zone (14-16 degrees C). There was still an amount of adipose tissue left after seven days of fasting in these calves that will be capable of providing energy for an additional period of time.

This information can become important when a necropsy is performed on a two to four day old calf in which all fat depots have been depleted. Our data suggests that this calf was not only malnourished but also either in a severe catabolic state (as a result of cold, wind exposure or disease) or that it had poor fat reserves at birth. Herd outbreaks of this problem may indicate chronic fetal malnutrition.

Fat deposition occurs in the fetus mainly during the third part of pregnancy (14). In the human fetus, fat represents 80% of the caloric accretion in the last few weeks of gestation (15). Thus it is nutrition of the fetus during this terminal phase of gestation which affects neonatal fat deposits. Fetal

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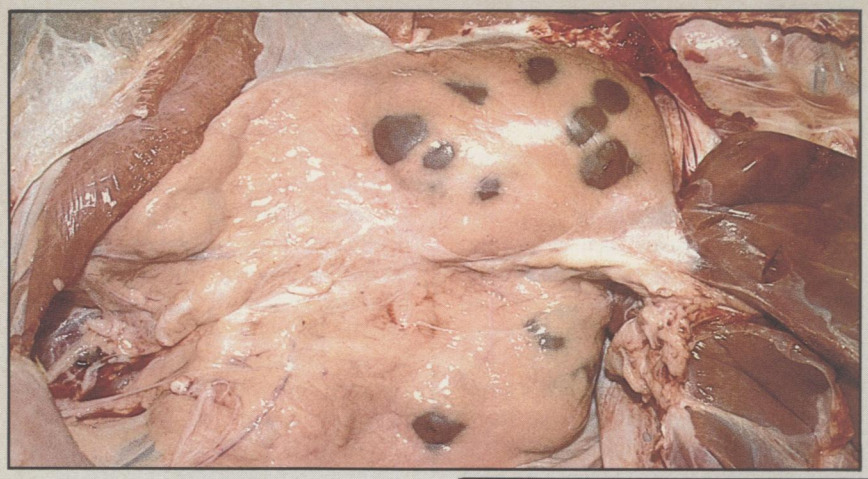

Figure 1. A close-up of the kidneys and surrounding adipose tissue in a newborn calf in left lateral position, the liver to the right, pelvic inlet to the left. The kidneys have an ample covering of slightly brown fat.

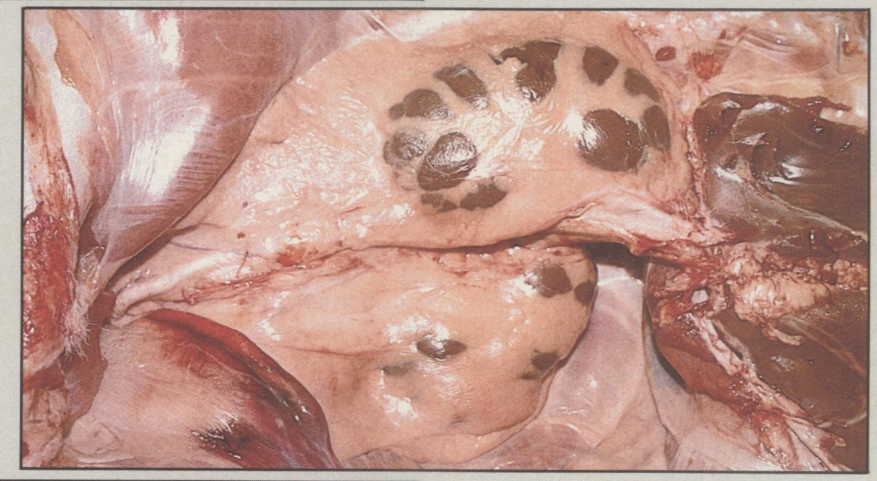

Figure 2. A close-up of the kidneys and surrounding adipose tissue in an eight day old milk-fed calf. Note the increase in exposure of the kidneys and moderate amount of fat depletion.

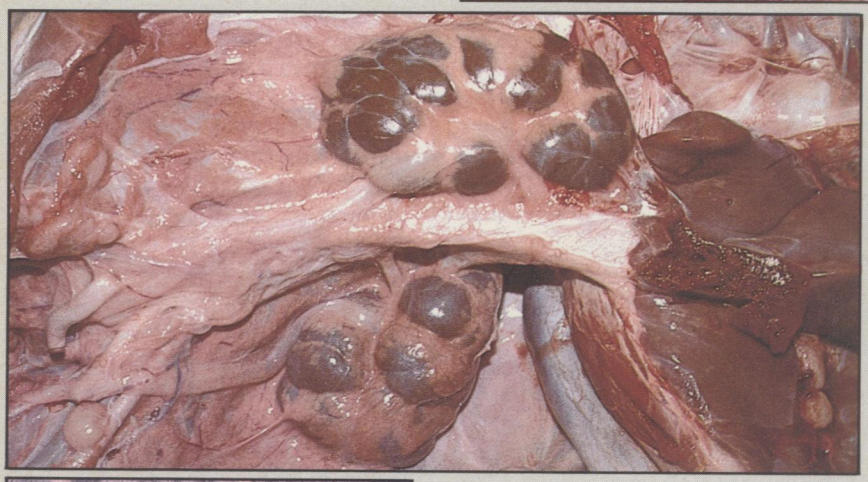

Figure 3. A close-up of the kidneys and surrounding adipose tissue in an eight day old electrolyte-fed calf. Note the marked depletion of fat. The residual fat is dark.

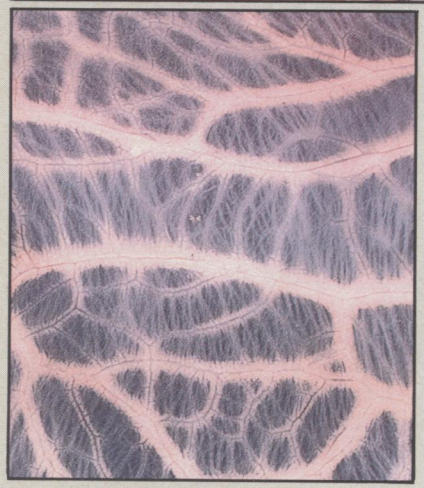

Figure 4. A single sheet of omentum in a newborn calf. Note the amount of adipose tissue present surrounding the vasculature.

Figure 5. A single sheet of omentum in an eight day old electrolyte-fed calf. Note the complete absence of adipose tissue.

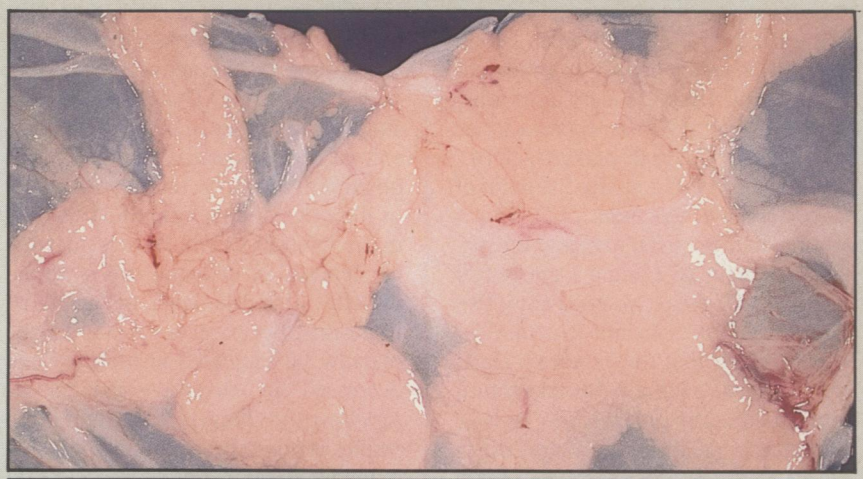

Figure 6. A close-up of the parietal surface of the pericardial sac in a newborn calf. Note the abundance and color of adipose tissue present.

Figure 7. A close-up of the parietal surface of the pericardial sac in an eight day old electrolyte-fed calf. Note the marked loss of adipose tissue and change in color.

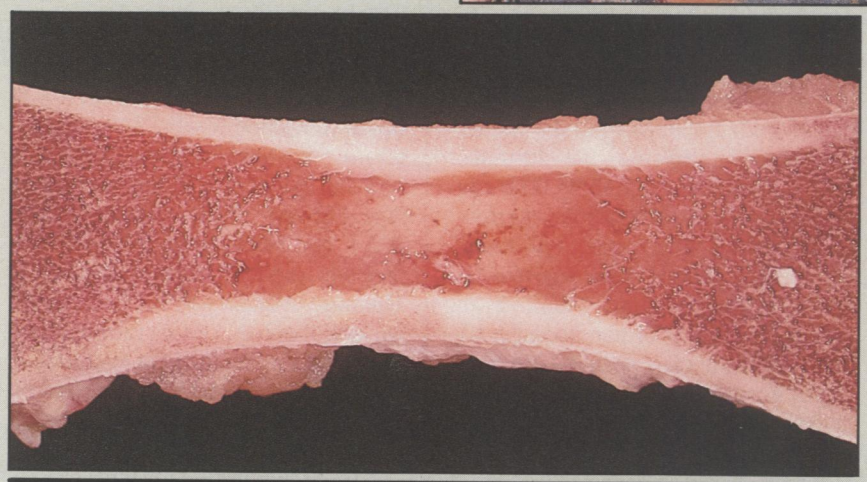

Figure 8. Diaphyseal medullary cavity in the femur of a newborn calf, which closely resembles that of an eight day old milk-fed calf. Note the presence of fat.

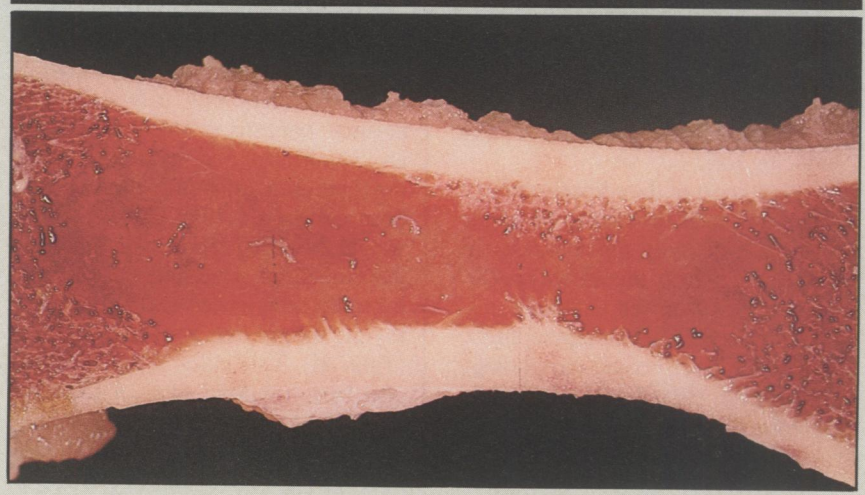

Figure 9. Diaphyseal medullary cavity in the femur of an eight day old electrolyte-fed calf. Note the absence of fat and gelatinous appearance of bone marrow.

(Continued from page 367)
malnutrition may result from either placental insufficiency or maternal malnutrition (4, 16). Adequate nutritional intake during gestation appears to be necessary for full placental growth (4).

A newborn calf contains 2.8 gram of fat per 100 g of wet tissue (17). This is intermediate between man and the pig, which contain 16.1 and 1.1 gram/100 gram of wet tissue, respectively (18). The above figures include structural fat which means that the newborn piglet has very little available for fat mobilization and relies on glycogen in liver and muscle as an endogenous energy source (19). Omentum and mesentery have been reported to be the first visible sites of fat depletion, probably due to the small amounts present (8) and the close proximity of the adipose cell to the vascular tissue (6). In the present study there was complete depletion of fat at these sites after seven days of electrolyte feeding. In the newborn calf perirenal fat is the largest visible depot, and subcutaneous fat is extremely sparse (8, 20). In our study, feeding of electrolytes alone markedly reduced the amount of perirenal fat present.

It is noteworthy that no grossly visible change took place in the amount and color of epicardial fat of calves subjected to seven days of PEM. In lambs, it has been suggested that the fat present at this site is chemically different from other fat depots (21), which could explain why epicardial fat is mobilized last (22).

In our study partial serous atrophy of fat was evident macroscopically on the pericardial sac and in bone marrow of electrolyte-fed calves. The term serous atrophy of fat is used to describe the watery, translucent appearance of atrophic adipose tissue (23), in animals in which fat has been mobilized. This condition is synonymous with older terms such as mucoid degeneration, myxomatous degeneration, mucoid atrophy of fat (24) and gelatinous transformation of fat (25). It usually is detected most easily in bone marrow, coronary groove of heart, pericardial sac, and around the kidneys. Transmission electron microscopy and histochemical analysis have determined that the gelatinous material is extracellular and consists mainly of glycosaminoglycans (acid mucopolysaccharides) with some collagen and fibrin which is deposited by activated interstitial mesenchymal cells (23, 25).

In a previous evaluation of adipose tissue in premature and newborn calves (20), the intramedullary cavity was not identified as a site of fat deposition. Our data show that newborn calves are born with a substantial amount of fat present within their femur. This differs from the situation in rabbits in which bone marrow fat develops postnatally (26). Based on gross appearance, intramedullary fat in the neonate is identified as red marrow fat (27). Femoral bone marrow fat deposits in neonatal calves varied directly with the level of nutrition. Using the ether extraction method, bone marrow fat increased in milk-fed and decreased in electrolyte-fed calves, significantly. This is consistent with studies in other species which show that red marrow fat is mobilized directly in response to malnutrition and expanding hemopoiesis, while yellow marrow fat (in the adult) remains stable until the final stages of starvation (28).

In milk-fed calves, a redistribution of fat took place with a significant decrease of perirenal fat and increase of bone marrow fat. Therefore it seems that bone marrow has a priority for fat deposition in neonatal calves, which are gaining weight. Bone marrow is a three component system, comprised of water, fat and nonfat residue (29). Our data showed that the bone marrow in the femur of neonatal calves contains 8-11% nonfat residue which was not affected by age or fasting (Table IV). With a R^2 of 0.92 between bone marrow dry matter and crude fat, one can calculate crude fat levels from dry matter content by applying the formula: $\%CF = (\%DM \times 1.2) - 15.95$. This can be useful in calculating bone marrow crude fat when no ether extraction method is available. Bone marrow fat levels are used in mature wildlife as an indicator of degree of starvation (9, 10, 29). Possibly in early neonatal deaths of calves we can use bone marrow crude fat levels, together with amount of perirenal fat to assess body condition. However further work is needed in this area. Neonatal animals can contain either brown or white fat. White fat is similar to that found in adults and is an energy store. Brown fat has a heat producing role.

At birth most of the adipose tissue of the calf has been thought to be of the brown fat type, only subcutaneous fat was thought to be of the white type (8). This was based on the metabolic response to exogenous norepinephrine, and electron microscopic morphology (8). However, the color is only slightly darker than white adipose tissue (WAT) and the light microscopic appearance is similar to WAT. This is in contrast to rodents, where brown fat is distinctly brown and the adipocyte has a multilocular appearance with light microscopy (7).

Perirenal fat is the major fat depot in the calf, it develops early during gestational life and is thought to consist of BAT, which is mainly used for thermogenesis (1). However, in electrolyte-fed calves, perirenal fat appeared to be used as a source of energy. Group 3 calves were housed in a thermoneutral environment, (14-16°C), and were in a negative nutritional balance. The 67% loss of perirenal fat in electrolyte fed calves is likely due to fatty acid oxidation for ATP production elsewhere, rather than being used for heat production locally. The lipid stored in perirenal tissues of the lamb has also been reported to be depleted by starvation in a thermoneutral environment (30, 31). The above findings suggested that BAT can be used as a source of energy when need arises in preruminants.

Recently it has been realized that BAT contains a polypeptide known as thermogenin, which uncouples respiration from ATP synthesis within the mitochondria (32). Thermogenin has been found in BAT mitochondria of several rodents, rabbits, harpseal, Norwegian lemming and man (33). Preliminary evaluation revealed that no thermogenin could be detected in formalin fixed perirenal fat of newborn calves (detection level < 5 ng thermogenin). Possibly adipose tissue in neonatal calves may be intermediate in type between BAT of rodents and WAT, and be of the much lower thermogenic capacity than rodent BAT. This might explain why BAT of rodents, but not of calves, can be distinguished from WAT under the light microscope. It would also explain why perirenal fat in calves can be mobilized to meet nutritional demands.

The thymic to body weight ratio in our experimental animals was in agreement with unpublished data of 24 newborn Jersey bull calves, used as negative controls in recent BVD studies (34). It appeared that the weight of the thymus in apparently healthy newborn calves is $0.42 \pm 0.09\%$ of body weight. Milk-fed calves maintained in a thermoneutral zone and on adequate nutrition experienced a 30% decrease in thymic weight over a seven day period (Table II). This initial weight loss has also been reported in man (35) and should be considered when interpreting the significance of thymic size in neonatal calves. The thymus of electrolyte-

fed calves lost a greater proportion of its weight than did any other organ (Table II). The thymus is extremely sensitive to levels of nutrition and has been called the barometer of nutrition (36). Failure to demonstrate significant differences in thymic weight between groups 2 and 3 was probably due primarily to the small number of calves and the large amount of individual variation. Thymic size in neonatal calves could be important, since precocious involution of the thymus may result in decreased immune competence and has been related to increased susceptibility to disease (11).

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