Dietary Factors Influencing the Development of the Ruminant Stomach

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Forty-six Holstein (36 bulls, 10 heifers) calves were fed diets consisting largely of milk, grain, hay, or a mixture of hay and grain. Calves were slaughtered at birth, 4, 7, 10, 13, and 16 weeks of age and observations made on the development of the stomach compartments. Calves fed milk alone showed little change, but calves receiving dry feed had a noticeable increase in volume and tissue deposition of the fore-stomachs with age, as well as a more extensive papillary development of the rumen wall. Initial changes were evident at 4 weeks and increased steadily. It is postulated that chemical entities, probably end products of microbial digestion of feed, rather than coarse materials stimulate the development of fore-stomach tissue.

THE FORE-STOMACHS OF THE NEW-BORN RUMINANT are rudimentary and essentially nonfunctional. Sisson and Grossman (*23*) reported that the reticulo-rumen constitutes about one third of the total stomach capacity at birth and that it increases to about 85% at maturity. The adult bovine rumen is reported to have a capacity of 20 to 60 gallons. In addition, the rumen microbiological activity makes it possible for ruminants to obtain a large part of their energy requirements from coarse fibrous feeds (*7*), to produce sufficient B vitamins for the body's needs (*5*), and to synthesize much useful proteinaceous material from nonprotein nitrogen (*20*).

Though there has been extensive research on ruminants of all ages, there is only a limited amount of critical knowledge as to the most satisfactory way to develop the fore-stomachs of calves and to the importance that should be attached to this development. Several widely different, satisfactory systems of feeding dairy calves are in use at present, but the relationship between these feeding methods, the rate and extent of rumen development, and the mature capacity of the cow to consume and digest roughage is not well understood. The factors influencing the microbiological, physiological, and biochemical development of rumen function recently have been reviewed by Flatt (*9*).

The diet is an important factor causing structural changes of the ruminant fore-stomachs after birth (*6*, *22*). Calves subsisting solely on liquid diets have been shown to have undeveloped rumens (*8*, *12*, *15*, *21*, *28–30*).

The data of Trautmann (*25*) demonstrated that though fore-stomach development was retarded with prolonged, exclusive milk feeding, some growth was apparent, suggesting that diet was not the only factor responsible for tissue growth. McCandlish (*15*) observed that the mucous membranes, folds, and papillae were normal in milk-fed calves which had received grain for 30 days, though the musculature was not well developed. The abomasum was normal while the omasum was undersized. By supplementing a grain ration with cod liver oil and alfalfa ash, Mead and Regan (*19*) produced normal growth in calves. They reported that on post-mortem examination at 2 years of age, the rumens lacked fill but otherwise appeared normal. Grain was reported (*11*) to cause development of normal rumens, though no experimental evidence was presented. Many have believed that coarse bulky feed is essential for the proper development of the rumen (*2*, *3*, *12*, *15–17*). Blaxter (*6*) reported that animals given roughage, in addition to milk, developed larger rumens than milk-fed calves growing at the same rate. There was no increase in stomach tissue weight, for the roughage had increased the capacity of the fore-stomachs by stretching the tissues.

Trautmann (*25*) demonstrated that roughage was not required for the structural development of the pillars, compartments, and papillae of the rumen. He showed clearly, however, that adding plant food (not specifically identified) increased the size of the fore-stomachs, the degree of papillary development, and the amount of elastic tissue present in the rumen mucosa. Trautmann's later studies on regeneration of the rumen and reticulum (*26*) and omasum (*27*) suggested that solid feed hastened this process.

It is obvious from the published data that the type of diet markedly affects the rate and character of fore-stomach development. Data on the pattern of normal development are limited, however. Blaxter and coworkers (*6*) reported a 54-fold increase in the volume of the omasum for calves fed roughage from birth. Using wet tissue weight of the rumens of calves fed milk, hay, and dry calf starter, Kesler, Ronning, and Knodt (*13*) indicated that the most rapid change occurred between 4 and 6 weeks of age. Marshall, Arnold, and Becker (*18*) stated that the weight and volume of rumen tissue exceeded that of the abomasum between the 7th and 30th day of age. Flatt (*9*) has recently summarized the early research in Germany which reported the capacities of the various stomach compartments with increases in age. Diet was not controlled, and, in most instances, the cattle examined were smaller than modern animals. These data show a gradual increase in the proportion of the total capacity occupied by the rumen and reticulum. The ratio of reticulo-rumen to omasum-abomasum was 9 to 1 in adult cattle as compared to 8 to 1 at 12 months of age, 4 to 1 at 6 months of age, and 1 to 3 at birth. Lagerlof (*14*), using cross-sectioning techniques, observed that the rumen of normally fed animals had attained its final relative size and position in 8 to 9 months even though at 3 months of age it was not strikingly different from the adult. Grossman (*10*) concluded that 4 to 6 months are required for the fore-stomachs to approach adult proportions, though final proportions would not be achieved until 1.5 years of age.

Because of the paucity of information critically relating widely different diets to the fore-stomach development of young calves, the following study was initiated.

Experimental Procedure

Animals and Treatments Thirty purebred Holstein bull calves were obtained from the Cornell herd after having suckled the dam for at least 2 days. They were brought to the experimental barn and assigned at random to one of the three following dietary treatments.

Group 1, Milk. Ten calves were fed milk in nipple pails from the Cornell Holstein herd at the following approximate percentage of live weight per day: arrival to 2 weeks, 9%; 3 to 4 weeks, 10%; 5 to 6 weeks, 10.5%; 7 to 8 weeks, 11%; 9 to 10 weeks, 11.5%; 11 to 16 weeks, 12.0%. This schedule satisfied the total digestible nutrient (TDN) requirements of the Morrison standard for calves of this age making normal growth. In addition to milk, the calves received gradually increasing amounts of a mineral solution until at 4 weeks of age they were consuming 10 ml. per 100 pounds of live weight daily. One liter of the mineral solution contained the following compounds: $FeSO_4.7H_2O$, 37.33 grams; $CuSO_4.5H_2O$, 2.946 grams; $CoCl_2.6H_2O$, 0.303 gram; and $MnSO_4.4H_2O$, 3.045 grams. Magnesium oxide was fed at the rate of 3.3 grams per 100 pounds of body weight after 4 weeks of age. In most instances it appeared to cause some scouring and frequently had to be reduced to one half of this amount. After 4 weeks of age the calves received vitamin D in the form of irradiated yeast (9000 I.U. per gram) at the rate of approximately 1000 I.U. per 100 pounds of body weight per day.

Group 2, Grain. Ten calves each received a total of 350 pounds of Holstein milk fed in an open pail according to the schedule prescribed by Turk and Burke (*24*). In addition a commercial calf starter was offered free choice from the 8th day of age. One ton of calf starter contained the following ingredients: yellow corn meal, 405.5 pounds; wheat bran, 300 pounds; crushed oats, 400 pounds; linseed oil meal, 140 pounds; soybean oil meal, 280 pounds; dehydrated alfalfa meal, 140 pounds; cane molasses, 100 pounds; dried skim milk, 100 pounds; corn distiller's dried solubles, 100 pounds; irradiated yeast, 0.5 pound; dicalcium phosphate, 10 pounds; ground limestone, 10 pounds; iodized salt, 10 pounds; and vitamin A feeding oil, 4 pounds.

Group 3, Hay. Ten calves each received 350 pounds of Holstein milk fed in an open bucket as above and in addition a 2 to 1 mixture by weight of high quality, second cutting alfalfa–brome grass hay chopped to 1-inch lengths and the above calf starter from the 8th to the 56th day of age. Thereafter they were given only chopped hay fed free choice.

Two calves in each group were designated at random to be slaughtered at 4, 7, 10, 13, or 16 weeks of age. Experiments involving the calves slaughtered at 16 weeks were conducted between February and July 1952, while those involving the 4- to 13-week slaughter groups were conducted between September 1952 and April 1953.

Group 4, Hay and Grain. In the spring of 1955 four grade Holstein bull calves were purchased from local auction sales as "bob" veals and assigned to Group 4 (hay and grain). They each received 350 lbs. of Holstein milk fed in an open pail. A mixture of second cutting mixed bird's-foot trefoil–timothy hay and a commercial calf starter in the ratio of 3 grain to 2 hay was fed after 21 days of age. All calves were slaughtered after they had received dry feed for 12 weeks.

Group 5, Newborn. Two purebred Holstein bull calves were obtained from the Cornell herd when they were less than 24 hours old and 10 grade Holstein female calves were purchased from local auction sales as "bob" veals. These animals were slaughtered immediately and because data from all were similar, they have been grouped together and considered as newborn calves.

Calves in all groups were given 12 grams of sulfathalidine upon arrival at the experimental barn. They were housed in individual tie stalls equipped with raised wooden platforms to eliminate the consumption of bedding. Each stall contained a feed box, and an iodized salt block and calves in Group 2 had access to a mineral mixture containing equal parts of dicalcium phosphate and iodized salt. Water was offered *ad libitum* after the 14th day of age. Sulfathalidine was used to treat cases of scours that did not respond to a reduction in feed. Calves were fed twice daily at 5 A.M. and 4 P.M.

Observations A daily record of feed consumption was kept while body weight, height at the withers, and circumference of the heart girth and barrel were determined weekly. Chemical composition of the feeds is shown in Table I.

The calves were slaughtered at the proper ages and several observations made on the stomach compartments. As rapidly as possible after stunning and bleeding, the gastrointestinal tract was detached from the carcass. The various components were separated by ligatures and cut apart into the reticulo-rumen, omasum, abomasum, and intestines, care being taken to prevent any loss of ingesta. Each separate part with its contents was weighed.

The volume displacement of each stomach compartment was determined as follows: A glass tube was fitted into the esophagus and the reticulo-rumen immersed in a 22-gallon can of water. The organ was then filled with water until the level of the water in the tube was 2 inches above the water level of the can with the reticulo-rumen free of air pockets and completely submerged. The can was filled to overflowing, the reticulo-rumen was removed, and the amount of water required to replace the organ was measured. The volume displacement of the abomasum was measured in a similar manner with the glass tube inserted through the pyloric sphincter. The omasum was immersed in a vessel filled with water and the displacement measured. No attempt was made to increase the internal pressure. In most instances the measurement of the volumes of all three compartments was completed within 1 hour from the time of stunning.

All of the parts of the tract were opened, washed free of their contents, and weighed. It was then possible to calculate the ingesta-free body weight and minimize the variation due to differences in fill.

Table I. Chemical Composition of Feeds Fed to Young Dairy Calves

Feed	Group	H_2O, %	Protein, %	Ether Extract, %	Crude Fiber, %	N.F.E. %	Ash, %
Starter	2 and 3	10.03	20.24	3.88	7.49	52.83	5.53
Hay	3[a]	7.95	16.13	2.98	25.57	40.64	6.73
Hay	3[b]	6.89	14.26	3.22	27.44	42.22	5.97
Hay	4	7.29	13.22	2.63	29.14	43.04	4.68

[a] 16-week slaughter group.
[b] 4- through 13-week slaughter group.

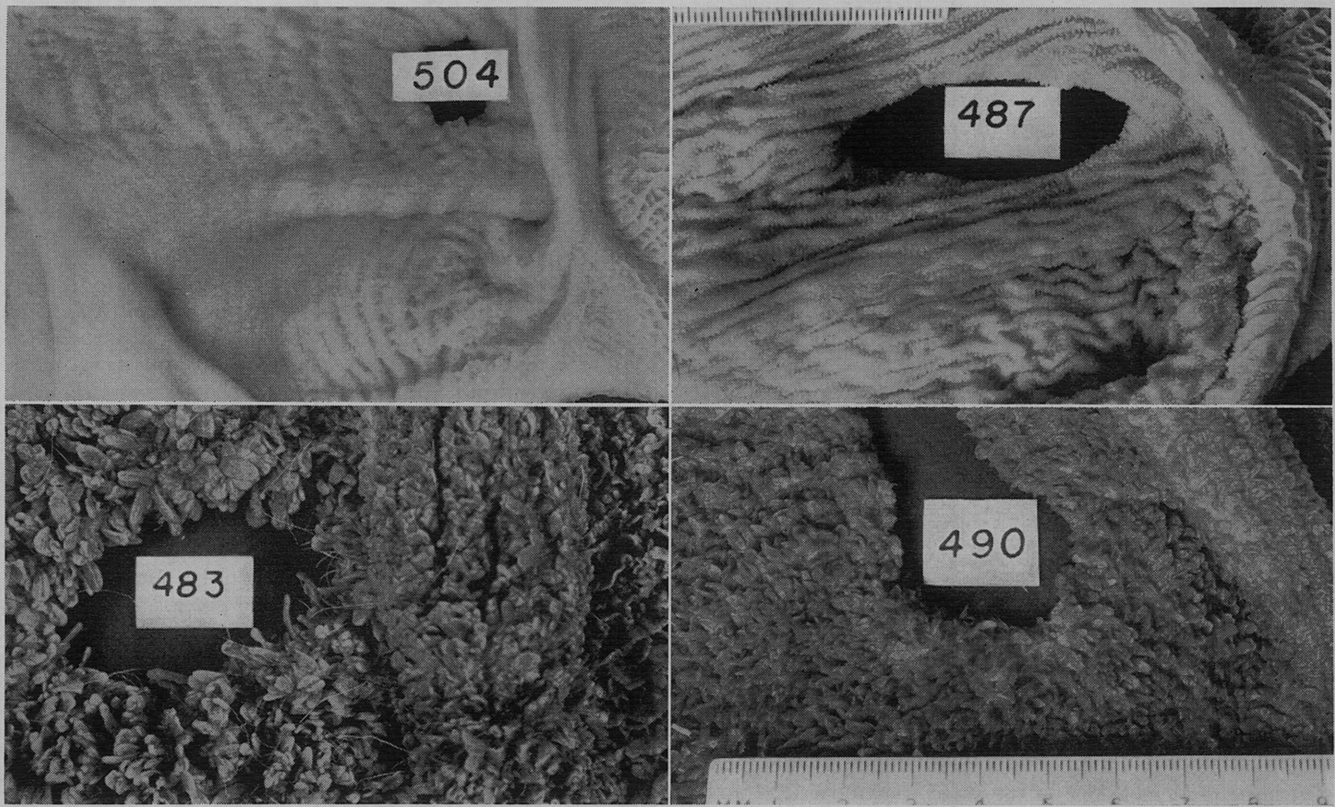

Figure 1. Photographs of rumen wall showing ventralmost portion of cranial dorsal sac just caudal to rumino-reticular fold

Rumino-reticular fold and reticulum are visible in right portion of each picture. 504 newborn, 487 milk-fed to 13 weeks (group 1), 483 grain-fed to 13 weeks (group 2), 490 hay-fed to 13 weeks (group 3)

Table II. Final Ingesta-Free Body Weight of Dairy Calves Receiving Several Diets[a]

Diet	Age, Weeks: Newborn	4	7	10	13	16
	Body Weight, Lb.					
Milk	78[b]	103	105	163	205	259
Grain	...	107	125	143	231	242
Hay	...	78	131	115	118	143
Hay and grain	...	...	...	...	207[c]	...

[a] Average of 2 observations except where noted.
[b] Average of 12 observations.
[c] Average of 4 observations.

Table III. Volume Displacement of Stomach Compartments of Dairy Calves Receiving Several Diets and Slaughtered at Various Ages

(Liters/45.4 kg. ingesta-free body weight)[a]

Group	Diet	Age, Weeks: Newborn	4	7	10	13	16
		Reticulo-rumen					
1	Milk	2.0[b]	3.7	4.2	3.4	3.2	9.4
2	Grain	...	5.8	11.3	14.4	13.3	9.3[d]
3	Hay	...	4.4	10.6	22.2	31.4	23.8
4	Hay and grain	...	...	...	...	13.6[e]	...
		Omasum					
1	Milk	0.05[c]	...	0.07[d]	0.12	0.08	0.10[d]
2	Grain	...	...	0.15[d]	0.39	0.38	0.76
3	Hay	...	0.14[d]	0.38[d]	0.67	0.98	1.34
4	Hay and grain	...	...	...	...	0.86[e]	...
		Abomasum					
1	Milk	2.7[b]	1.9	2.3	1.5[d]	1.4	2.0
2	Grain	...	1.8	1.8	1.4[d]	1.1	1.4[d]
3	Hay	...	2.0	1.6	1.4	3.2	2.0[d]
4	Hay and grain	...	...	...	...	1.5[e]	...

[a] Average of 2 observations except where noted.
[b] Average of 12 observations.
[c] Average of 11 observations.
[d] One observation.
[e] Average of 4 observations.

The various stomach compartments were trimmed of excessive fatty tissue and fascia, and in turn lyophilized in a Stokes freeze-drying vacuum chamber. Material was considered dry when the vacuum reached 100 microns. Following drying, they were allowed to stand in the laboratory for at least 48 hours, were weighed, ground through a meat grinder, and then through the 2.0-mm. sieve of a Wiley mill. Representative samples of the tissues were dried, and extracted with anhydrous diethyl ether for 48 hours, and the amount of fat-free dry matter of each of the stomach compartments was determined.

Results

Because the calves receiving hay (Group 3) consumed considerably less total energy during the experimental period than those receiving grain and milk (Groups 1, 2, and 4), there was a decided difference in the rate of gain between the experimental groups (Table II). For this reason, the observations made on the stomach capacity and fat-free dry tissue of the stomach compartments have been expressed per unit of ingest-free body weight for comparison.

The results of the volume displacement measurements per 45.4 kg. of ingesta-free body weight are reported in Table III. The determination of the volume displacement for the 16-week slaughter group was taken prior to the time the technique was perfected, which resulted in a longer time lapse between stunning and the final determination. It is possible that the lack of conformity between these data and the remainder can be explained on this basis.

It is evident that the calves receiving milk alone showed little change with increasing age in the volume per unit of body weight for either of the three stomach compartments. These organs grew, but only in proportion to the increase in body weight, thus confirming the observation of Trautmann (*25*) and Blaxter and coworkers (*6*). On the other hand, grain calves showed a marked increase in the volume of the reticulo-rumen and the omasum per unit of body weight. These changes were noticeable as early as 4 weeks of age and gradually increased. Calves receiving hay had a greater total capacity of the reticulo-rumen and omasum than either the grain- or milk-fed calves. There were variations in abomasal volume, but in general the volume change was in proportion to body weight and was unaffected by any dietary treatment.

Data from Group 4 (hay and grain) have been shown in Table II with the 13-week group. The volume of their stomach compartments was intermediate between Groups 2 and 3.

The results of the fat-free dry matter determinations have been summarized in Table IV. For the milk-fed calves there was little deposition of tissue in any of the stomach compartments except that which could be associated with increases in body weight. Calves receiving grain or hay showed a distinct increase in the amount of tissue deposited in the reticulo-rumen and omasum with respect to time. There was relatively little difference between the grain- and hay-fed calves while Group 4 calves had somewhat less tissue in the reticulo-rumen. The type of diet had little effect on the amount of abomasal tissue.

The data for the newborn calves suggest that there may be a change in the amount and capacity of the various stomach compartments from birth to 4 weeks of age, irrespective of the type of ration the animal received, which is in agreement with Auernheimer's (*3*) observations. It is apparent, however, that dry feed is essential for any radical change to occur following the initial transition from birth to young calfhood.

Diet had a marked effect on the lining of the rumen tissue as indicated by the degree of papillary development. Newborn calves exhibited a smooth epithelium with no prominent papillae. At 4 weeks of age milk-fed calves showed some papillae, but in all instances these were less than 2 mm. in height, and remained essentially unchanged for as long as 16 weeks. Calves receiving hay or grain showed marked papillary growth which was noticeable as early as 4 weeks, and became marked at 7 weeks. Papillae approached 1 cm. in height on these latter diets. There was little difference in general appearance of the papillae for calves receiving grain, hay, or the hay-grain combination. Representative pictures of the rumen papillae are shown in Figure 1.

Discussion

It is evident that dry feed has resulted in an alteration in the growth rate of the reticulo-rumen and omasum with respect to body weight. There was little change in the abomasum, however, as a result of radically different dietary treatments.

The influence of grain on fore-stomach development is somewhat surprising since it has been commonly believed that hay is required for the development of the normal rumen tissue.

It is also a common observation that cattle with large middles consume large amounts of roughage and conversely that consumption of roughage develops large middles (rumens). The relative importance of inheritance and of dietary differences is unknown. Because grain has been as effective as hay in stimulating the early growth of fore-stomach papillae and tissue, while milk was ineffective, it appears that dry feed *per se* is essential for the development of the fore-stomachs. Hay, because of its greater bulk, increased the capacity of the fore-stomachs without affecting the amount of tissue growth.

Because, in the past, the course nature of the feed has been considered as a causative agent for the rumen tissue development, and grain alone contains only about 7% of crude fiber it would appear that another explanation is needed to account for the extensive papillary development and tissue deposition occurring in the fore-stomachs of calves on a grain diet. When a calf drinks milk it usually bypasses the rumen via the esophageal groove and enters the abomasum; thus little of the milk enters the rumen. In contrast, when dry feed is consumed most of it passes directly into the rumen. Such feed, whether concentrate or roughage, is attacked by microorganisms, resulting in soluble products which may be absorbed through the rumen wall. It seems logical that chemical products resulting from microbiological breakdown of dry feed might stimulate the development of tissue. It has been well established that the rumen and omasum are absorptive organs (*1*, *4*). The papillae are presumed to be absorptive entities of these tissues and thus, teleologically speaking, materials to be absorbed conceivably could stimulate the development of organs to absorb.

A pilot study to determine the importance of roughage materials on the development of rumen papillae was conducted. One 10-day-old calf was given a total of 1.5 pounds of nylon bristles by capsule (approximately 1 inch in length) as the only dry feed in addition to its normal milk allotment. No papillary development was observed after the bristles had been in the rumen for 70 days. The amount of fore-stomach tissue was essentially the same as for the milk-fed calves. This observation lends support to the theory that chemical entities rather than coarse materials are largely responsible for the growth and development of fore-stomach tissue of young calves.

Table IV. Fat-Free Dry Matter of Stomach Compartments of Dairy Calves Receiving Several Diets and Slaughtered at Various Ages

(Grams/45.4 kg. ingesta-free body weight)[a]

		Age, Weeks					
Group	Diet	Newborn	4	7	10	13	16
		Reticulo-rumen					
1	Milk	26.8[b]	34.4	43.0	35.2	38.4	35.6
2	Grain	...	53.3	92.5	129.1	154.1	173.9
3	Hay	...	53.2	97.6	117.2	165.9	152.1
4	Hay and grain	...	...	...	...	121.0[c]	...
		Omasum					
1	Milk	7.8[b]	12.4	9.3	6.9	10.0	8.3
2	Grain	...	9.7	16.2	20.5	24.3	31.6
3	Hay	...	8.9	17.9	24.0	25.9	38.0
4	Hay and grain	...	...	...	...	36.5[c]	...
		Abomasum					
1	Milk	33.0[b]	32.2	23.3	24.0	29.5	29.2
2	Grain	...	26.4	27.7	26.9	20.8	20.3
3	Hay	...	28.8	25.5	28.5	31.0	26.0
4	Hay and grain	...	...	...	...	28.7[c]	...

[a] Average of 2 observations except where noted.
[b] Average of 12 observations.
[c] Average of 4 observations.

Literature Cited

(1) Aggazzoti, A., *Clin. Vet.* **7**, 54 (1910).
(2) Andres, J., *Schweiz. Arch.* **70**, 225 (1928).
(3) Auernheimer, O., *Z. Fleisch- u. Milchhyg.* **20**, 393 (1910).
(4) Barcroft, J., McAnally, R. A., Phillipson, A. T., *J. Exptl. Biol.* **20**, 120 (1944).
(5) Bechdel, S. I., Eckles, C. H., Palmer, L. S., *J. Dairy Sci.* **9**, 409 (1926).
(6) Blaxter, K. L., Hutcheson, M. K., Robertson, J. M., Wilson, A. L., *Brit. J. Nutrition* **6**, i (1952).
(7) Carroll, E. J., Hungate, R. E., *Appl. Microbiol.* **2**, 205 (1954).
(8) Davenport, E., Ill. Agr. Expt. Sta. Bull. **46** (1897).
(9) Flatt, W. P., Ph.D. thesis, Cornell University, Ithaca, N. Y., 1955.
(10) Grossman, J. D., *J. Am. Vet. Med. Assoc.* **114**, 416 (1949).
(11) Hastings, E. G., *Bact. Rev.* **8**, 235 (1944).
(12) Herman, H. A., Missouri Agr. Expt. Sta. Research Bull. **245** (1936).
(13) Kesler, E. M., Ronning, M., Knodt, C. B., *J. Animal Sci.* **10**, 969 (1951).
(14) Lagerlof, N., *Skand. Vet. Tidskr.* **19**, 253 (1929).
(15) McCandlish, A. C., *J. Dairy Sci.* **6**, 347 (1923).
(16) Magee, H. E., *J. Exptl. Biol.* **9**, 409 (1932).
(17) Mangold, E., "Handbuch der Ernährung und des Stoffwechsels der Landwirtschaftlichen Nutztiere," vol. **2**, Verlag Julius Springer, Berlin, 1929.
(18) Marshall, S. P., Arnold, P. T. D., Becker, R. B., *J. Dairy Sci.* **33**, 379 (1950).
(19) Mead, S. W., Regan, W. M., *Ibid.*, **14**, 283 (1931).
(20) Reid, J. T., *Ibid.*, **36**, 955 (1953).
(21) Sanborn, J. W., Utah Agr. Expt. Sta. Bull. **21** (1893).
(22) Savage, E. S., McCay, C. M., *J. Dairy Sci.* **25**, 595 (1942).
(23) Sisson, S., Grossman, J. D., "Anatomy of the Domestic Animals," W. B. Saunders Co., Philadelphia, Pa., 1938.
(24) Turk, K. L., Burke, J. D., Correll Extension Bull. **761** (1949).
(25) Trautmann, A., *Arch. Tierernähr. und Tierzucht* **7**, 400 (1932).
(26) *Ibid.*, p. 421.
(27) *Ibid.*, **9**, 575 (1933).
(28) Warner, R. G., Bernholdt, H. F., Grippin, C. H., Loosli, J. K., *J. Dairy Sci.* **36**, 599 (1953).
(29) Wiese, A. C., Johnson, B. C., Mitchell, H. H., Nevens, W. B., *J. Nutrition* **33**, 263 (1947).
(30) Wise, G. H., Petersen, W. E., Gullickson, T. W., *J. Dairy Sci.* **22**, 559 (1939).

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DIETARY ENERGY REQUIREMENTS

Effects of Caloric Intake on Nitrogen Balance and Organ Composition of Adult Rats

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Restriction in caloric intake, with or without protein in the diet, resulted in depletion in body fat and body protein and an increase in body water in adult rats. The increase in water was particularly marked in rats fed a restricted caloric intake in the presence of dietary casein. Nitrogen balance also decreased as calories were reduced, increased catabolic activity being a first response to such a restriction. The data were interpreted to mean that the animal could adapt to maintain essential tissues through shifts in metabolism, although continued deprivation in caloric intake resulted in marked loss in tissue nitrogen. There could be an optimum caloric intake for each protein intake. Differential changes of body tissues after caloric restriction demonstrated that some organs were more labile than others.

The energy requirements and the maintenance of essential tissue protein constituents are of first importance to the living system. A restriction in caloric intake results in certain metabolic adjustments that are directed toward correction of the deficit in energy intake. At the same time, essential anabolic functions are continued. Thus Allison, Anderson, and Seeley (*4, 5*) found that a marked restriction in caloric intake in dogs could prevent attainment of nitrogen equilibrium with retention of dietary nitrogen at low nitrogen intakes. Further studies by Rosenthal and Allison (*18*) and Rosenthal (*17*) suggested the following sequence of response to a caloric restriction in dogs. With sufficient fat and labile protein stores, reduced calories result in increased catabolism of labile protein stores, thus raising the excretion of urinary urea nitrogen. The retention of dietary nitrogen, as measured by the nitrogen balance index, was not altered. Upon depletion of fat and labile protein stores, the over-all catabolic activity of the animal was reduced, thereby conserving body nitrogen and reducing energy requirements. Eventually, however, the animal was forced to dip into the tissue reserves to such an extent that the nitrogen balance index was reduced and the body was badly depleted of tissue proteins.

These studies, together with numerous others (*6, 7, 11, 14*), emphasize that the response to a caloric restriction is a function of the physiological state of the animal as well as the diet. It is possible that there is an optimum caloric intake for each protein intake, the perfect balance between protein and calories resulting in an adequate development of body mass.

The following experiments were undertaken, therefore, to study more fully the responses in rats to caloric restriction, measured not only in terms of nitrogen balance but also in terms of changes that occur in various organs of the body.