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BULLETIN
OF THE
NATIONAL RESEARCH
COUNCIL

NUMBER 67

THE MINIMUM PROTEIN REQUIREMENTS
OF CATTLE

Report of Committee on
Animal Nutrition

H. H. MITCHELL

PUBLISHED BY THE NATIONAL RESEARCH COUNCIL
OF
THE NATIONAL ACADEMY OF SCIENCES
WASHINGTON, D. C.

1929

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OF CATTLE

National research council
Report of Committee on
Animal Nutrition¹

H. H. MITCHELL

¹The members of this committee of the Division of Biology and Agriculture are: Paul E. Howe, U. S. Bureau of Animal Industry, Washington, D. C., *Chairman*; F. W. Christensen, State College Station, Fargo, N. Dak.; E. B. Forbes, State College, Pa.; F. G. King, Lafayette, Ind.; L. A. Maynard, Ithaca, N. Y.; H. H. Mitchell, Urbana, Ill.; H. J. Gramlich, Lincoln, Nebr.; A. G. Hogan, Columbia, Mo.; F. B. Morrison, Ithaca, N. Y.; C. R. Moulton, Chicago, Ill.; E. G. Ritzman, Durham, N. H.

THE MINIMUM PROTEIN REQUIREMENTS OF CATTLE

H. H. MITCHELL

PRELIMINARY CONSIDERATIONS

The question of protein requirements of farm animals possesses both a scientific and a practical significance. It is obvious that the food requirements of animals and the factors modifying these requirements are legitimate subjects for scientific research whether they relate to farm animals of great economic importance to man, or to laboratory animals of no economic importance, or to man himself. In each case the problems involved are those of experimental biology, and the results obtained will aid in a better understanding of the science of nutritional physiology.

Experimental investigations with farm animals which deal with the entirely practical questions of feeding and management are more understandable, more readily explainable, and hence more intelligently applicable the more exact and extensive is the available knowledge of the food requirements of such animals. For example, the favorable effect of the addition of a nitrogenous concentrate to the ration of fattening cattle may be due to its content of protein or of phosphorus, or it may be due to some unknown specific effect of the concentrate on the digestive tract resulting in a greater desire for food and a greater consumption of it. Obviously, the decision as to which of these possible explanations is correct will have much to do with the practical application of the experimental finding to other rations than those used, and even to other nitrogenous concentrates. The greatest return from even these wholly practical investigations can be realized only when their results are as completely explainable as the control and the measurement of experimental conditions will permit. Complete and accurate information regarding the nutrient requirements of farm animals and the nutritive value of farm feeds will obviously aid in interpreting intelligently the outcome of such practical studies.

What is true of practical experimentation with farm animals is equally true of scientific experimentation. An accurate knowledge of the food requirements of the experimental subjects is not infrequently a vital prerequisite to the correct interpretation of the results secured. Much of the German experimentation on the capacity of ruminants to

synthesize protein in the paunch from urea and other simple nitrogenous compounds,¹ has been rendered largely unintelligible through lack of an accurate knowledge of protein requirements.

One of the most practical contributions of the science of nutrition to the feeding of farm animals is the formulation of feeding standards applying to different classes of animals and to a variety of conditions. How practical these standards have proved to be and how greatly they have modified feeding practices cannot be told. Whether or not their value in increasing the financial profits of feeding operations is considerable, undoubtedly knowledge of feeding standards and their limitations will aid materially in the intelligent appreciation of the live stock business, particularly in the ability to cope successfully with changing conditions of feed supply and to avoid exploitation by manufacturers of commercial feeds and other products for live stock.

Feeding standards should promote maximal production with a minimum of overfeeding. They should include a factor of safety, so that a normal variation in the composition and nutritive value of feeds and in the functional capacities of animals will rarely if ever result in underfeeding. But obviously, a factor of safety cannot be scientifically included in a feeding standard until the actual minimum requirements of animals for the different nutrients have been determined. For the same reason an engineer cannot intelligently impose a factor of safety in the construction of a bridge unless a satisfactory estimate of the maximum load that the bridge will have to bear can be made. Hence, feeding standards for farm animals must ultimately be based upon satisfactory determinations of minimum animal requirements.

It may never be necessary or advisable to feed a farm animal in exact accord with its protein requirements, but when an animal is non-producing at certain seasons of the year, or when protein concentrates become relatively high in price, it may become expedient to approximate these requirements. In such cases it is clear that an exact knowledge of protein requirements, as well as of the protein values of farm feeds, becomes of immediate practical significance.

For these reasons, a study of the protein requirements of farm animals is well worth while. In a recent report of the Subcommittee on Animal Nutrition⁽¹⁾ a method of measuring such requirements was proposed, differing in all essentials from methods commonly used in previous

¹In most of these experiments, the question of the nutritive value of the urea addition depends for its answer upon the question whether the digestible crude protein of the basal ration is adequate in itself to cover the protein requirements of the animal.

studies. The present report is an attempt to apply this method to a determination of the protein requirements of beef and dairy cattle by the use of experimental data already available.

The essential features of the method of measuring protein requirements that will be exemplified in this report are as follows:

1. The protein requirements of animals, as well as the protein values of feeds, are best expressed in terms of nitrogen, or of a conventional protein, such as the ordinary $N \times 6.25$, rather than in terms of true protein. Throughout this report, the term "protein" is used in this conventional sense.

2. The problem of protein requirements must be considered as separate and distinct from the problem of the protein values of feeds and rations, if permanent progress is to be made in respect to either. Hence the attempt to measure protein requirements in terms of digestible feed protein should be abandoned. Such expressions of protein requirements have served a useful purpose in the past, and may necessarily serve for several years to come until the information required for a more rational expression becomes available; but future investigational work may well be planned along other lines.

3. Protein requirements may be conveniently and rationally expressed in terms referable to the animal rather than to its feed. Although the percentage of nitrogen in the different nitrogenous compounds of the animal body varies greatly, the need for them, or for their precursors, the dietary amino acids, may be satisfactorily measured by the total nitrogen content of the tissue constituents catabolized endogenously, in the case of maintenance, or by the total nitrogen content of the new tissues formed in growth, fattening and reproduction, or by the total nitrogen content of the milk produced in lactation. These nitrogen values may for convenience be converted into conventional protein.

4. The use of such measures of protein requirements can be made to the best advantage only in conjunction with measures of the protein values of feeds based upon (a) the total nitrogen content of the feed, (b) the wastage of nitrogen in digestion, (c) the necessary wastage of absorbed nitrogen in the process of its conversion into tissue constituents or the constituents of body secretions, and (d) the supplementary relations between the available nitrogenous constituents of the feed and those of the other feeds with which it is commonly fed.

It follows, therefore, that the results obtained in the following study relative to protein requirements are not to be compared with values already current in the literature. Heretofore, protein requirements have been expressed in two ways, either in terms of digestible crude protein or

in terms of digestible true protein. Statements thus expressed are ambiguous, since they are not related to protein of any definite or known biological value. In this report, protein requirements are expressed in terms of animal expenditures or storages of nitrogen, converted for convenience into conventional protein ($N \times 6.25$). They may be considered as representing the requirements for digestible crude protein possessing a biological value of 100. They are thus definite in their significance, minimal in the truest sense of the word, and adaptable to any protein mixture the biological value of which may be satisfactorily estimated.

THE PROTEIN REQUIREMENTS FOR MAINTENANCE

Existing protein standards for maintenance.—From a study of American and foreign investigations on farm animals in which low-protein rations were used, Armsby⁽²⁾ has concluded that the protein requirements of swine, cattle, sheep and horses are very closely the same per unit of weight. This evident similarity between animals differing so widely in size and dietary habits is probably more significant than the actual numerical requirement resulting from this study, i. e., 0.6 pound digestible crude protein per 1000 pounds live weight. It is of some significance also that Sherman⁽³⁾, in a summary of similar, though more extensive and exact, investigations on adult human subjects, also arrived at approximately the same relation of protein requirement to body weight, i. e., 0.6 gram per 1000 grams.

But there is abundant evidence that these requirements are too high, even in terms of digestible protein. Armsby himself has noted certain Danish experiments on two dry cows in which nitrogen equilibrium was attained on intakes of 0.21 pound and 0.25 pound of digestible protein per 1000 pounds live weight, and another experiment on steers in which similar quantities of digestible protein sufficed for maintenance. The long-continued feeding experiments of Perkins⁽⁴⁾ on dairy cattle may also be cited to the same effect. Subtracting the protein secreted in the milk of these cows from the digestible crude protein intake, left balances of crude protein varying from 0.43 to 0.68 pound per 1000 pounds live weight, averaging 0.52 pound. Since a 100 per cent conversion of digestible crude protein into milk protein would probably never be realized, the actual amounts of protein used for maintenance by these cows were probably distinctly less than the values above cited.¹ The recent results of Buschmann⁽⁵⁾ possess much the same significance.

¹ Much the same experience has been more recently reported by Maynard, Miller, and Krauss in Memoir 113 of the Cornell University Agricultural Experiment Station (p. 17).

Sherman also has noted many experiments on men with a much lower indicated protein requirement for maintenance than the average cited above, and has himself, in conjunction with his associates, reported experimental observations of this description. Hindhede's work has afforded further striking confirmation⁽⁶⁾ of the adequacy throughout prolonged feeding periods of amounts of protein only one-half the Sherman average. It is, therefore, clearly evident that the standard requirement of 0.6 part of digestible protein per 1000 of body weight includes a large margin of safety.

The excretion of urinary nitrogen by fasting cattle or cattle on low-protein rations.—However, if the maintenance requirement for protein is to be measured in terms referable to the animal rather than to its food, the values just considered are not directly pertinent to the problem. Attention must rather be directed to the daily excretion of urinary nitrogen of cattle on low-nitrogen or nitrogen-free rations. It is also important to consider the nitrogen excretion of fasting cattle. Forbes, Fries and Kriss⁽⁷⁾ have found the average daily urinary nitrogen of two fasting cows per 1000 pounds live weight to be 46.5 gms. and 43.6 gms., for the sixth to the ninth day of fast, inclusive. This is equivalent approximately to 0.1 gm. of urinary nitrogen per kilogram of body weight, or to 0.6 pound of crude protein per 1000 pounds of body weight. For two steers weighing approximately 600 kgms., taken from a sub-maintenance ration and fasted for 10 days, Carpenter⁽⁸⁾ found the nitrogen excretion in the urine for the last 3 or 4 days to average .059 gm. and .067 gm. per kilogram per day, equivalent to 0.37 and 0.42 part of protein per 1000 of weight.

It is, however, well known that the nitrogen excretion of fasting animals includes a considerable fraction of nitrogen representing the catabolism of tissue constituents serving as a source of energy. Since this function may be served by the non-nitrogenous nutrients, it does not represent a function of protein that should be considered in the determination of protein requirements. The protein requirement for maintenance, therefore, does not bear any constant relation to the excretion of urinary nitrogen during fasting, but it is directly measured by the excretion of urinary nitrogen during the adequate feeding of a nitrogen-free ration, i. e., what might be called specific nitrogen starvation.

Unfortunately no experimental data appear to be available on the nitrogen output of cattle under conditions of specific nitrogen starvation. It must, therefore, suffice to cite experiments in which this condition is more or less closely approximated. Thus, reference may be made to the experiments of Bull and Grindley⁽⁹⁾ on the nitrogen metabolism of steers on various rations. In these experiments, 2 steers were brought

to nitrogen equilibrium on a maintenance ration of 1 part of clover hay to 5 parts of corn. During this period, the nitrogen output of these steers averaged daily .046 gm. and .048 gm. per kilogram of weight, equivalent to 0.29 and .30 part of protein per 1000 of weight. Titus⁽¹⁰⁾ has determined the daily output of urinary nitrogen of three steers on a ration containing 40 per cent of alfalfa hay and 60 per cent of paper pulp. These values per kilogram of live weight are .042, .052, and .045 gm., or, in terms of protein per 1000 of weight, 0.26, 0.32 and 0.28.

The Laboratory for Agricultural Research in Copenhagen has published⁽¹¹⁾ the metabolism data from 2 dry cows subsisting upon a low-nitrogen ration supplying 46 to 47 gms. daily. Nitrogen equilibrium was established on this ration with average daily excretions in the urine of 14 gms. and 15 gms. of nitrogen, equivalent to .029 gm. and .034 gm. per kilogram of body weight, or 0.18 and .21 part of protein per 1000 of body weight.

Honcamp, Koudela, and Müller⁽¹²⁾ have investigated the nitrogen metabolism of 2 cows in milk on a low-nitrogen ration of oat straw, corn, potato flakes, molasses, dried beet pulp, and salts. The nitrogen intake was 85 and 89 gms. daily, and the urinary nitrogen output was 0.042 gm. and 0.035 gm. per kilogram of body weight, or .26 and .22 part of protein per 1000 of weight. If the disposal of endogenous metabolites is not influenced by the function of milk secretion, these values are comparable to similar values secured on dry cows.

The nitrogen output of 2 young growing heifers subsisting upon a low-nitrogen ration was determined by Hart, Humphrey and Morrison⁽¹³⁾. The calves weighed from 300 to 400 pounds and the ration used consisted of wheat straw, corn starch, cane sugar, calcium phosphate and salt. The average daily excretions of urinary nitrogen were equivalent to 0.036 gm. and 0.030 gm. per kilogram of weight, or 0.20 and 0.17 part of protein per 1000 of weight. In a later somewhat similar experiment upon a younger calf, Steenbock, Nelson and Hart⁽¹⁴⁾ obtained a minimum excretion of urinary nitrogen of 0.045 gm. per kilogram of weight.

The minimum excretion of urinary nitrogen per kilogram of body weight, for various farm animals and man.—As Armsby's study of the amounts of digestible feed protein per unit of body weight required for the maintenance of weight or of nitrogen equilibrium has indicated a remarkable similarity among different species of farm animals in this respect, it is of interest to compare different animals also with respect to the excretion of urinary nitrogen per unit of weight on nitrogen-free or low-nitrogen rations. Such a comparison for the larger farm animals from such available data as have been located has been made in Table 1. The data in this table for each species are arranged in the order of decreasing ratios of urinary nitrogen to body weight.

TABLE 1

RECORDED DETERMINATIONS OF THE DAILY EXCRETION OF URINARY NITROGEN BY FARM ANIMALS ON LOW-NITROGEN OR NITROGEN-FREE RATIONS

Species of animal	Body weight kgs.	Daily urinary nitrogen gms.	Urinary nitrogen per kgm. body wt. gms.	Investigator and reference	
Pig.....	22.2	1.60	0.072	McCullum and Hoagland(15)	
	14.3	0.96	0.067	" " "	
	17.7	1.09	0.062	" " "	
	19.5	1.09	0.056	McCullum and Steenbock(16)	
	16.8	0.90	0.054	" " "	
	25.0	1.32	0.053	Pfeiffer(17)	
	38.1	2.00	0.052	Mitchell and Kick(18)	
	10.9	0.54	0.050	McCullum and Steenbock(16)	
	38.1	1.88	0.049	Mitchell and Kick(18)	
	40.0	1.95	0.049	" " "	
	46.3	2.23	0.048	McCullum and Hoagland(15)	
	38.5	1.83	0.047	McCullum and Steenbock(16)	
	26.3	1.19	0.045	Pfeiffer(17)	
	37.2	1.61	0.043	McCullum and Steenbock(16)	
	68.1	2.65	0.039	" " "	
	41.0	1.54	0.038	Morgen <i>et al</i> (19)	
	74.9	2.61	0.035	McCullum and Steenbock(16)	
	Cattle.....	145	6.48	0.045	Steenbock, Nelson and Hart(14)
		385	16.32	0.042	Honcamp, Koudela and Müller(12)
177		6.33	0.036	Hart, Humphrey and Morrison(13)	
440		16.40	0.035	Honcamp, Koudela and Müller(12)	
443		15	0.034	Copenhagen investigators (11)	
168		5.03	0.030	Hart, Humphrey and Morrison(13)	
485		14	0.029	Copenhagen investigators (11)	
Sheep.....	47	3.39	0.072	Morgen, Beger and Westhauser(19)	
	38	2.59	0.068	" " " "	
	35	2.37	0.068	" " " " (20)	
	45	2.63	0.058	" " " "	
	43.5	2.39	0.055	" " " " (19)	
	45	2.41	0.054	" " " " (20)	
	35	1.81	0.052	" " " "	
	40	1.91	0.048	" " " "	
	42	1.84	0.044	" " " " (19)	
	40	1.71	0.043	" " " " (20)	
	54	2.02	0.038	" " " " (19)	
	31.9	0.99	0.031	Völtz(21)	
	33.1	1.03	0.031	Scheunert, Klein and Steuber(22)	
	43.5	1.16	0.027	" " " "	
	44.1	1.05	0.024	" " " "	

A considerable variability is thus revealed, the significance of which cannot be definitely assessed. Probably most of it is due to other factors than the functional variability of individual animals. In particular, it seems probable that the individual results represent varying degrees of success in attaining the endogenous level of urinary nitrogen excretion, because of the presence of varying amounts of absorbable nitrogen in the rations, or of an inadequate consumption of them. The same situa-

TABLE 2
RECORDED DETERMINATIONS OF THE MINIMUM ENDOGENOUS URINARY
NITROGEN OF HUMAN SUBJECTS

Day of experiment	Total urinary N gms.	Body weight kgms.	Nitrogen per kilo gms.	Investigator and reference
10	3.8	64.0	0.059	Folin(23)
4	3.76	69.7	0.054	Landergren(24)
5	3.5	70.5	0.050	Folin(23)
4	3.04	62.4	0.049	Landergren(24)
5	2.7	55.7	0.049	Folin(23)
8	3.12	63.5	0.048	Klemperer(25)
7	3.34	71.3	0.047	Landergren(24)
7	2.42	57.5	0.042	Roehl(26)
8	2.89	71.0	0.041	Kocher(27)
12	2.6	64.0	0.041	Folin(23)
8	2.51	65.0	0.040	Klemperer(25)
19	2.98	76.2	0.039	Thomas(28)
6	2.93	79.2	0.037	Kocher(27)
9	2.25	61.4	0.037	Graham and Poulton(29)
11	2.13	60.5	0.035	Robison(30)
11	1.99	57.8	0.034	Robison(30)
6	2.01	88.0	0.032	Klercker(31)
..	1.84	58.0	0.032	Sivén(32)
24	1.58	65.3	0.024	Smith(33)
71	1.75	72.5	0.024	Deuel and others (98)

NOTE.—It is interesting to note that Petrén (J. Biol. Chem., 61:355 (1924)) in the clinical treatment of diabetic patients by means of diets very low in protein and carbohydrates and rich in fat, has obtained extremely low values for the day's excretion of urinary nitrogen. When expressed per kilogram of body weight these values range from 0.023 to 0.035 gms.

tion exists with respect to similar experiments on men, the results of which are summarized in Table 2.

It is perhaps significant that in general the lowest values have been obtained with human subjects only after 10 days or more of subsistence upon the experimental diet. The lowest and most recent results were obtained on the 24th and the 71st days of feeding. The slow adjustment of the animal body to low levels of nitrogen feeding, noted in particular by Hindhede(34), is evidently a similar phenomenon. Apparently the "de-

posit protein"¹ in the tissues is only eliminated after a considerable period of nitrogen-free feeding. In the case of the pig, McCollum has found that oftentimes 2 or 3 weeks of such feeding are required before a minimal excretion of urinary nitrogen is established. Thus it is to be expected that in the search for a minimum value the success of any particular attempt may be considered the greater, the lower the result obtained becomes.

A tentative estimate of the minimum protein requirement for maintenance.—For the reasons given, the lower values reported of the ratio between urinary nitrogen and body weight are in all probability more significant than the higher values, a conclusion that receives considerable support from the fact that for pigs, sheep and cattle, as well as for men, the minimum values obtained agree within narrow limits, i. e., 0.024 gm. to 0.035 gm. per kilogram of weight. It is probably not far from the truth to conclude that the maintenance requirement of nitrogen by these species is not far from 0.030 gm. per kilogram of body weight.² This is equivalent to 0.19 pound of protein per 1000 pounds body weight.

That this value may be subject to fluctuation with age, vitality, fatness, sex, and other factors seems probable, but the extent and direction of these effects cannot be predicted definitely in the absence of experimental observations bearing directly upon these questions, except with respect to fatness. It appears reasonably certain that increasing fatness will decrease the ratio of endogenous urinary nitrogen to body weight, and since the recorded observations of this ratio do not relate to animals in a highly fat condition (in most cases quite the reverse), the minimum ratio indicated is probably well above the truth when applied to fat animals. The effect of age is not so readily predicted, but if the endogenous metabolism may be presumed to bear some relation to the basal heat production, then the young animal, particularly the very young animal, would be expected to possess a larger ratio of endogenous nitrogen to body weight than would the mature animal.

¹This term is applied by Lusk to the unorganized protein in the body which is retained in the cellular fluids in amounts proportionate to the level of protein intake. With a change in protein intake, the lag in nitrogen excretion is due to the repletion or depletion of deposit protein.

²However, there is no implication that this value has a general application to all animals. The experiments of Underhill and Goldschmidt on dogs (*J. Biol. Chem.*, 15: 341 (1913)) indicate a much higher level of endogenous metabolism for this species, the minimum approximating 0.1 gm. of urinary nitrogen per kilogram of body weight. For rabbits, similar high values have been obtained (Mendel and Rose, *J. Biol. Chem.*, 10: 226 (1911)); Meyers and Fine, *Ibid.*, 15: 305 (1913); Serio, *Biochem. Z.*, 142: 440 (1923)), while a large number of experiments in the author's laboratory have shown that for rats the minimum value ranges from 0.2 gm. to 0.1 gm. per kilogram of weight, depending upon age, apparently.

THE PROTEIN REQUIREMENT FOR GROWTH

The protein requirement for growth is measured, in terms referable to the animal rather than to its feed, by the rate of deposition of protein (nitrogen) in the tissues of the growing animal. The determination of the rate of deposition of protein involves determinations of the normal rate of growth in body weight, the protein content of animals of different weights and, by combining the two, the normal rate of growth in protein content.

Unfortunately, in the case of cattle these two determinations have not been done with any great thoroughness on the same breeds. The growth of the dairy breeds of cattle has been measured quite extensively by a number of investigators, but it appears that the chemical composition of dairy calves at different weights has not been studied, although a few determinations of the composition of mature dairy cows have been made. On the other hand, no comprehensive investigation of the growth of beef cattle, involving a large number of individuals of known age, has been discovered in the literature, although two extensive studies of the composition of beef cattle at different weights have been conducted in this country. The situation, therefore, must be approached with the expectation of making not infrequent use of somewhat debatable assumptions and estimations.

The use of mathematical methods in the study of growth.—Animal growth, in any of its numerous aspects, is a dynamic phenomenon which may be supposed to proceed in a smooth and definite manner when the influence of disturbing factors is removed. Growth is ordinarily studied in piecemeal fashion by attempting to determine the change with time of some animal measurement, such as body weight or the weight of some definite organ. If this change is depicted graphically on coordinate paper, it will under ideal conditions move along a smooth curve, often a relatively simple curve, the shape of which is defined by a simple mathematical function (equation) relating age (time) to the variable in question. More often, however, a simple mathematical equation will not describe the entire growth change, but only a fraction of it. However, for the range over which it describes the growth change, the mathematical equation is a complete expression of it.

Quantitative observations of growth changes can only rarely be made under the ideal conditions just considered, this being particularly true of the growth changes occurring in the farm animals. The confinement of large numbers of these animals under uniform environmental conditions is quite impracticable. Hence, disturbances in growth, due to weather changes, feed changes and digestive and other minor pathological affections of the animal, occur and they occasion irregularities in the mea-

surements secured that bear no definite biological significance. When such measurements are plotted upon coördinate paper it is impossible to connect them by a curved line of any simple description, even over narrow ranges of time. The description by a mathematical equation of a series of actual observations upon the time changes occurring in growing animals is thus not a simple process. A choice must be made of the mathematical function that will be used, based upon what is known of the laws of growth, or upon what function has been used with most success with similar sets of observations. The constants in the mathematical function chosen must then be determined from the observational data by some method designed to secure a satisfactory fit, so that the curve representing the final equation will pass through the plotted data in the most satisfactory manner. If the data are sufficiently extensive and their interpretation sufficiently exacting, certain criteria of the goodness of fit, based upon the theory of probability, may finally be applied.

The mathematical equation thus obtained from the observed data expresses in the most satisfactory manner the time changes that would have been observed under ideal conditions. It may therefore be used as a satisfactory substitute for the mass of data from which it was derived, in the same way, and for precisely the same reason, as an arithmetic mean (average) may be used to represent a mass of data clustering about a point rather than a curve.

The advantages of reducing a series of disconnected observations relating to growth to a continuous mathematical function more than compensate for the trouble involved. From such a function the most probable value of the variable measurement, whatever it may be, may be computed for *any instant of time*. The *rate of change* at any instant of time and the change in the measurement during any definite interval of time may also be readily computed. The original mass of data cannot, by any other method, be made to yield satisfactory information of this nature. Hence, for the most productive study of growth the application of mathematical methods is essential.

The differentiation of growth and fattening.—The problem of the food requirements for the growth of animals relates to the change with time in the amount of nutrients contained in their bodies. However, with animals that fatten readily during the growing period, the differentiation of growth from fattening is difficult if not impossible except on the quite arbitrary assumption that growth relates to the deposition of protein and the other constituents of protoplasm, but that fattening relates solely to the filling of the adipose tissue cells with inert fat. Yet an animal normally will always fatten to some extent during growth. In fact, it appears probable that growth without simultaneous deposition of

fat in the adipose tissue is impossible. Certainly this is true of what we understand by normal growth. On the other hand, it is quite probable that during fattening the filling of the adipose tissues with fat involves cellular proliferation in those tissues and hence a simultaneous deposition of protein and other protoplasmic constituents.

A mathematical analysis of the Missouri investigations on growing and fattening steers.—In this difficult situation, it is well to study in some detail the results of an investigation of the growth of cattle involving the chemical analysis of cattle of different ages growing and fattening at different rates. For this purpose the investigations on growing and fattening steers published by the Missouri Agricultural Experiment Station (^{35, 36, 37, 38}) will serve admirably. The data presented in Bulletin 55 of this station are particularly pertinent to this discussion.

The cattle in this comprehensive investigation were raised upon the same ration, consisting, except in the first few weeks of feeding, of a grain mixture and alfalfa hay, but were given different amounts of feed in accordance with a definite plan. The cattle in Group I were fed as much of this ration as they would consume. The cattle in Group II were fed to secure "maximum growth without the storage of surplus fat," as judged by the animal husbandmen in charge. The cattle in Group III were fed to induce a distinctly retarded growth, at a rate approximately half that shown by Group II. The cattle used were of the Hereford-Shorthorn cross and were all unsexed males.

The average monthly body weights of these three groups of animals up to and including the fourth year of age are given in Research Bulletin 62 of the Missouri Station (³⁹). The numbers of animals averaged at any one time were not large, never exceeding 19 and in the later months tapering down to 1 or 2, so that the weight-age curves are somewhat irregular, particularly those for Group II. In using these data to the best advantage, therefore, it is necessary to obtain a satisfactory mathematical description of them by fitting to them a suitable algebraic equation.

The weight-age relation.—The weight-age relation for growing animals under approximately constant conditions of feeding and management assumes the shape of a sigmoid curve. However, no simple equation for a sigmoid curve that has thus far been suggested will fit the entire growth curve of an animal. Robertson's (⁴⁰) growth curve, representing the course of an autocatalytic monomolecular reaction, is sigmoid in shape, but it is symmetrical about its point of inflection, whereas the weight-age curve of animals possesses a point of inflection much nearer birth than maturity. Brody (⁴¹) has suggested a mathematical function to describe animal growth from this point of inflection to maturity, based upon the assumption that following this point the

velocity of growth (gain in weight per unit time) declines with age in geometrical progression. The body weight is thus supposed to increase by successively decreasing gains in weight, in such a way that the ratio of the gain in weight during any month (or other interval of time) to the gain during the preceding month will be constant. The equation is as follows:

$$W = A - Be^{-kt} \quad (1)$$

in which W equals body weight at time t , A is a constant representing the maximum weight attained at maturity, B is a second constant whose magnitude depends upon A and upon the length of the growth period preceding the point of inflection, and k is a third constant equal to the ratio of the gain in weight per unit time to the growth yet to be made ($A - W$); e is the base of the natural system of logarithms.

Brody⁽⁴¹⁾ has calculated the constants of this equation applied to the growth data of the Missouri cattle in Group I. For the other groups of cattle, B and k have been calculated by the method of averages for a series of values for A , the final selection being that combination of constants giving a sum of residuals approximating closest to 0 algebraically. The equations thus obtained are as follows:

$$\text{Group I} \quad W = 2425 - 3520e^{-.0347t} \quad (2)$$

$$\text{Group II} \quad W = 1600 - 2057e^{-.0303t} \quad (3)$$

$$\text{Group III} \quad W = 1300 - 1481e^{-.0227t} \quad (4)$$

By these equations W is computed in pounds for any age t , expressed as months from conception. For cattle the conceptional age at birth is taken to be 9.4 months. The closeness of fit of these equations to the Missouri data for the three groups of cattle is indicated by the computations in Tables 3, 4 and 5.

For Group I, the agreement is poor between observed and calculated weights until a conceptional age of 15.4 to 16.4 months (6 to 7 months from birth) is reached. This area of bad fit represents the area before the point of inflection in the curve of growth, when the acceleration of growth changes from positive to negative in sign. An area of retarded growth from the 22nd to the 30th month is smoothed out and towards the upper end of the range the fit again becomes less good, but the percentage difference does not become great even to the final observation.

For Group II, the agreement between calculated and observed weights becomes satisfactory much earlier than for Group I. A serious depression of the observed growth in this group between the 36th and the 53rd month from conception is smoothed out, as it probably should be, since it possesses no evident biological significance.

TABLE 3
MISSOURI GROWTH DATA ON CATTLE, OBSERVED AND COMPUTED. GROUP I

t ¹ mos.	No. of animals	W obs. lbs.	W calc. lbs.	Diff. lbs.	t ¹ mos.	No. of animals	W obs. lbs.	W calc. lbs.	Diff. lbs.
9.4	..	80	- 115	...	34.4	4	1355	1358	+ 3
10.4	12	135	- 29	...	35.4	4	1395	1394	- 1
11.4	14	175	55	- 120	36.4	4	1430	1430	0
12.4	14	215	136	- 79	37.4	4	1465	1464	- 1
13.4	13	270	214	- 56	38.4	4	1500	1496	- 4
14.4	13	325	289	- 36	39.4	4	1535	1528	- 7
15.4	11	385	362	- 23	40.4	4	1565	1559	- 6
16.4	10	445	433	- 12	41.4	4	1595	1588	- 7
17.4	10	505	500	- 5	42.4	4	1620	1617	- 3
18.4	8	565	566	+ 1	43.4	4	1645	1644	- 1
19.4	8	625	630	+ 5	44.4	3	1670	1671	+ 1
20.4	8	685	691	+ 6	45.4	3	1695	1697	+ 2
21.4	6	745	750	+ 5	46.4	3	1720	1721	+ 1
22.4	6	815	807	- 8	47.4	3	1745	1745	0
23.4	6	880	862	- 18	48.4	3	1767	1769	+ 2
24.4	6	945	915	- 30	49.4	3	1788	1791	+ 3
25.4	6	1000	967	- 33	50.4	2	1802	1813	+ 11
26.4	6	1045	1017	- 28	51.4	2	1815	1833	+ 18
27.4	6	1090	1065	- 25	52.4	2	1835	1854	+ 19
28.4	5	1130	1111	- 19	53.4	2	1875	1873	- 2
29.4	5	1165	1156	- 9	54.4	2	1915	1892	- 23
30.4	5	1200	1199	- 1	55.4	1	1940	1910	- 30
31.4	4	1240	1241	+ 1	56.4	1	1955	1928	- 27
32.4	4	1275	1282	+ 7	57.4	1	1965	1945	- 20
33.4	4	1315	1320	+ 5					

¹ Age counted from conception; age at birth = 9.4 mos.

TABLE 4

MISSOURI GROWTH DATA ON CATTLE, OBSERVED AND COMPUTED. GROUP II

t ¹ mos.	No. of animals	W obs. lbs.	W calc. lbs.	Diff. lbs.	t ¹ mos.	No. of animals	W obs. lbs.	W calc. lbs.	Diff. lbs.
9.4	..	80	52	- 28	34.4	6	875	873	- 2
10.4	19	125	98	- 27	35.4	6	895	895	0
11.4	19	155	143	- 12	36.4	5	910	916	+ 6
12.4	19	185	186	+ 1	37.4	5	925	936	+ 11
13.4	18	215	228	+ 13	38.4	5	940	956	+ 16
14.4	17	255	269	+ 14	39.4	5	950	975	+ 25
15.4	16	295	309	+ 13	40.4	5	957	994	+ 34
16.4	13	335	348	+ 13	41.4	5	962	1012	+ 50
17.4	11	375	385	+ 10	42.4	5	967	1030	+ 63
18.4	11	410	421	+ 11	43.4	5	972	1047	+ 75
19.4	10	450	456	+ 6	44.4	5	977	1063	+ 86
20.4	10	490	490	0	45.4	4	987	1079	+ 92
21.4	9	525	523	- 2	46.4	4	1000	1095	+ 95
22.4	8	560	555	- 5	47.4	4	1015	1110	+ 95
23.4	8	595	599	+ 4	48.4	4	1025	1124	+ 99
24.4	8	625	617	- 8	49.4	4	1060	1138	+ 78
25.4	8	655	646	- 9	50.4	2	1100	1153	+ 53
26.4	8	685	674	- 11	51.4	2	1135	1166	+ 31
27.4	8	710	702	- 8	52.4	2	1165	1178	+ 13
28.4	7	735	729	- 6	53.4	2	1190	1191	+ 1
29.4	7	760	755	- 5	54.4	2	1208	1203	- 5
30.4	7	785	780	- 5	55.4	1	1225	1215	- 10
31.4	6	810	804	- 6	56.4	1	1240	1227	- 13
32.4	6	835	828	- 7	57.4	1	1255	1238	- 17
33.4	6	855	851	- 4					

¹ Conceptional age: age at birth = 9.4 mos.

TABLE 5

MISSOURI GROWTH DATA ON CATTLE, OBSERVED AND COMPUTED. GROUP III

t ¹ mos.	No. of animals	W obs. lbs.	W calc. lbs.	Diff. lbs.	t ¹ mos.	No. of animals	W obs. lbs.	W calc. lbs.	Diff. lbs.
9.4	..	80	103	+ 23	34.4	6	630	621	- 9
10.4	15	125	130	+ 5	35.4	6	645	636	- 9
11.4	17	155	156	+ 1	36.4	5	655	651	- 4
12.4	18	180	182	+ 2	37.4	5	665	666	+ 1
13.4	17	205	207	+ 2	38.4	5	675	680	+ 5
14.4	16	230	231	+ 1	39.4	5	685	693	+ 8
15.4	14	253	255	+ 2	40.4	5	695	707	+ 12
16.4	11	275	279	+ 4	41.4	5	707	720	+ 13
17.4	10	295	302	+ 7	42.4	5	717	733	+ 16
18.4	10	315	324	+ 9	43.4	5	727	746	+ 19
19.4	9	336	346	+ 10	44.4	5	736	758	+ 22
20.4	9	357	367	+ 10	45.4	5	746	770	+ 24
21.4	9	378	388	+ 10	46.4	5	756	782	+ 26
22.4	7	400	409	+ 9	47.4	5	766	794	+ 28
23.4	7	420	428	+ 8	48.4	5	776	805	+ 29
24.4	7	440	448	+ 8	49.4	5	790	816	+ 26
25.4	7	461	467	+ 6	50.4	5	817	827	+ 10
26.4	7	482	486	+ 4	51.4	3	843	838	- 5
27.4	7	503	504	+ 1	52.4	3	870	848	- 22
28.4	7	523	522	- 1	53.4	3	886	858	- 28
29.4	6	543	539	- 4	54.4	3	900	868	- 32
30.4	6	563	556	- 7	55.4	3	912	878	- 34
31.4	6	582	573	- 9	56.4	2	920	887	- 33
32.4	6	600	589	- 11	57.4	1	928	897	- 31
33.4	6	615	605	- 10					

¹ Conceptional age: age at birth = 9.4 mos.

The fit of the Brody curve to the data of Group III is excellent except for the birth weight (9.4 months) and for the last few months of observation. Again a depression of observed growth of no apparent biological significance is smoothed out, in this case between the 37th and the 51st month from conception.

The rate of weight change with age.—At this point, one of the great advantages of reducing a set of irregular age-weight observations to a simple mathematical expression may be demonstrated. From the original data on the growth in weight of these cattle it would be impossible to compute in a satisfactory manner the rate of gain at any time. The most direct method of making such a computation would be limited in time to the particular intervals of weighing adopted in the investigation, and would yield a set of successive values so irregular as to possess little significance, and hence little value. However, the growth equations derived from each of the three sets of data may be changed by a simple mathematical operation (differentiation) to other equations from which the rate of growth (gain per month in this case) may be computed for any age, and successive values obtained will vary in an orderly fashion, evidently representative of highly significant biological changes.

Thus, differentiation of Brody's growth curve represented by Equation (1) gives

$$\frac{dW}{dt} = kBe^{-kt} \quad (5)$$

in which $\frac{dW}{dt}$ is the rate of change of body weight with time at any age t .

The differential equations for the three groups of Missouri cattle are readily made up from Equations (2), (3), and (4) :

$$\text{Group I} \quad \frac{dW}{dt} = 122.14e^{-.0347t} \quad (6)$$

$$\text{Group II} \quad \frac{dW}{dt} = 62.33e^{-.0303t} \quad (7)$$

$$\text{Group III} \quad \frac{dW}{dt} = 33.62e^{-.0227t} \quad (8)$$

From these differential equations, the average rate of growth of the three groups of cattle has been computed at intervals of three months, with the results given in Table 6,¹ and at intervals of 100 pounds in weight, with the results given in Table 7.¹

¹ The first two values for Group I have been computed by another method, since the growth curve of Brody did not fit the data well at these ages. It was found that the weight-age data for this group from shortly after, to 9 months after, birth could be accurately described by the parabolic equation $W = -13.42t + 2.48t^2$, W being the weight in pounds and t the time in months from conception.

The values in Table 6 will be put to a practical use later when the question of the chemical composition of gains at different ages is being considered.

TABLE 6
RATES OF GROWTH OF MISSOURI CATTLE AT DIFFERENT AGES

Age ¹	Group I			Group II			Group III		
	Weight W lbs.	Rate of growth		Weight W lbs.	Rate of growth		Weight W lbs.	Rate of growth	
		Per mo. lbs.	Per day lbs.		Per mo. lbs.	Per day lbs.		Per mo. lbs.	Per day lbs.
12.4	215	48.08	1.60	186	42.83	1.43	182	25.39	0.846
15.4	381	62.96	2.10	309	39.12	1.30	255	23.72	0.791
18.4	566	64.50	2.15	421	35.72	1.19	324	22.16	0.739
21.4	750	58.13	1.94	523	32.62	1.09	388	20.70	0.690
24.4	915	52.38	1.75	617	29.79	0.993	448	19.34	0.645
27.4	1065	47.20	1.57	702	27.21	0.907	504	18.07	0.602
30.4	1199	42.53	1.41	780	24.85	0.828	556	16.88	0.563
33.4	1320	38.33	1.28	851	22.69	0.756	605	15.77	0.526
36.4	1430	34.54	1.15	916	20.72	0.691	651	14.74	0.491
39.4	1528	31.12	1.04	975	18.92	0.631	693	13.77	0.459
42.4	1617	28.05	0.935	1030	17.28	0.579	733	12.86	0.429
45.4	1697	25.27	0.842	1079	15.78	0.526	770	12.02	0.401
48.4	1769	22.78	0.759	1124	14.41	0.480	805	11.23	0.378
51.4	1833	20.52	0.684	1166	13.18	0.439	838	10.49	0.350
54.4	1892	18.50	0.617	1203	12.03	0.401	868	9.80	0.327
57.4	1945	16.67	0.556	1238	10.99	0.367	897	9.16	0.305
60.4	1269	10.04	0.335	923	8.56	0.285

¹ Counted from conception, rather than birth.

The differences between the observed weights and the weights calculated from this equation are as follows:

GROWTH OF GROUP I UP TO NINE MONTHS FROM BIRTH

t ¹ mos.	W obs. lbs.	W calc. lbs.	Diff. lbs.
9.4	80	93	+ 13
10.4	135	128	- 7
11.4	175	169	- 6
12.4	215	215	0
13.4	270	265	- 5
14.4	325	321	- 4
15.4	385	381	- 4
16.4	445	447	+ 2
17.4	505	517	+ 12

¹ Age from conception.

The differentiation of this equation gives $\frac{dW}{dt} = -13.42 + 4.96t$ from which the first two values for the rate of growth in Group I, given in Tables 6 and 7, are calculated.

TABLE 7
 RATES OF GROWTH OF MISSOURI CATTLE AT DIFFERENT WEIGHTS

Body wt.	Group I			Group II			Group III		
	Age ¹ mos.	Rate of growth		Age ¹ mos.	Rate of growth		Age ¹ mos.	Rate of growth	
		Per mo. lbs.	Per day lbs.		Per mo. lbs.	Per day lbs.		Per mo. lbs.	Per day lbs.
200	12.08	46.50	1.55	12.72	42.42	1.41	13.13	24.97	0.832
300	14.03	56.17	1.87	15.17	39.39	1.31	17.33	22.70	0.757
400	15.68	64.35	2.14	17.82	36.36	1.21	21.98	20.43	0.681
500	17.39	66.80	2.23	20.69	33.33	1.11	27.18	18.16	0.605
600	18.93	63.33	2.11	23.84	30.30	1.01	33.08	15.89	0.530
700	20.55	59.87	2.00	27.32	27.27	0.909	39.88	13.62	0.454
800	22.28	56.38	1.88	31.22	24.24	0.808	47.93	11.35	0.378
900	24.10	52.93	1.76	35.63	21.21	0.707	57.78	9.08	0.303
1000	26.06	49.45	1.65	40.73	18.18	0.606			
1100	28.16	45.97	1.53	46.75	15.15	0.505			
1200	30.42	42.50	1.42	54.13	21.12	0.404			
1300	32.87	39.04	1.30						
1400	35.56	35.56	1.19						
1500	38.51	32.10	1.07						
1600	41.81	28.63	0.954						
1700	45.53	25.16	0.829						
1800	49.81	21.69	0.723						
1900	54.84	18.21	0.607						

¹ Counted from conception, age at birth being 9.4 mos.

The change in composition with age and body weight.—In their study of the changing composition of steers with age, the Missouri investigators analyzed, in a very complete and careful manner, the carcasses of 11 steers from Group I, 10 steers from Group II, and 9 steers from Group III, at ages ranging from 3 months to 4 years from birth. From the data thus obtained, it is of great interest to establish a satisfactory mathematical description of the relations between age and nitrogen content, age and water content, age and fat content, and age and ash content in these three groups of steers.

In comparing the live and empty weights of the slaughtered animals with their attained ages great irregularities are observable within each group (see Mo. Res. Bull. 55, Table 79). This is not a matter of surprise, in view of the small numbers of animals slaughtered in each group, and is probably due to a variable food consumption. Before attempting a mathematical analysis of these data, it appears desirable to remove some of this irregularity if a legitimate plan is at hand. The percentage composition of an animal is undoubtedly more closely related to the weight attained than to the age of the animal. The differences in the composition of animals, except for the very immature animal, are

very largely due to differences in fat content, as Murray⁽⁴²⁾ and later Moulton⁽⁴³⁾ have shown. The more rapidly an animal increases in weight on a given ration the more rapidly will it fatten. These two considerations, therefore, appear to justify the conclusion above stated, and hence as the initial step in the analysis of the chemical data of the Missouri experiments, the age of each of the slaughtered animals was corrected in accordance with the weight attained, by the use of equations (2), (3), and (4). In other words, by substituting the weight of the animal, W , in the age-weight equation for its group, the corrected age, t , was determined. The observed ages and the corrected ages of the animals slaughtered are compared in Table 8.

TABLE 8

THE OBSERVED AND CORRECTED AGES OF THE MISSOURI STEERS SUBMITTED
TO CHEMICAL ANALYSIS

Group I			Group II			Group III		
Steer No.	Observed age mos.	Corrected age mos.	Steer No.	Observed age mos.	Corrected age mos.	Steer No.	Observed age mos.	Corrected age mos.
556	3.0	4.4	554	3.0	3.2	555	3.0	3.2
557	5.6	7.1	552	5.2	4.7	548	5.3	4.6
547	8.2	7.3	550	8.5	6.4	558	8.4	5.3
541	10.7	11.6	538	10.9	8.5	540	11.1	9.8
505	10.6	11.0	503	11.4	14.7	531	20.4	16.7
532	17.7	19.5	523	26.2	24.6	525	26.3	30.1
504	20.9	20.3	507	33.5	32.1	524	40.4	39.1
515	33.6	33.5	526	40.0	36.6	509	44.7	61.7
527	39.5	43.8	502	44.6	40.0	500	47.9	71.3
513	44.5	45.3	512	47.7	49.1			
501	47.0	49.2						

The only great discrepancies between observed and corrected ages relate to Steers 509 and 500 in Group III, which for some unexplained reason do not fit in well with the growth data given for this group of animals in Missouri Research Bulletin 62. The corrected ages given in Table 8 are the ages at which the steers would have attained their slaughter weights if they had increased in weight at the average rate for their group.

In obtaining mathematical functions describing satisfactorily the relations between corrected age and the content of the steers in moisture, nitrogen, fat and ash, Brody's equation was found generally applicable

and was therefore used throughout. The equations found to give the best fit¹ to the various sets of data are as follows:

Water content:

$$\text{Group I} \quad W = 350 - 643e^{-.0581t} \quad (9)$$

$$\text{Group II} \quad W = 275 - 546e^{-.0641t} \quad (10)$$

$$\text{Group III} \quad W = 250 - 334e^{-.0391t} \quad (11)$$

Nitrogen content:

$$\text{Group I} \quad W = 16.50 - 38.59e^{-.0728t} \quad (12)$$

$$\text{Group II} \quad W = 16.25 - 25.59e^{-.0449t} \quad (13)$$

$$\text{Group III} \quad W = 15.50 - 18.23e^{-.0246t} \quad (14)$$

Fat content:

$$\text{Group I}^2 \quad W = 800 - 967e^{-.01325t} \quad (15)$$

$$\text{Group II} \quad W = 500 - 522e^{-.00481t} \quad (16)$$

$$\text{Group III} \quad W = 350 - 357e^{-.00284t} \quad (17)$$

Ash content:

$$\text{Group I} \quad W = 28.50 - 50.28e^{-.0511t} \quad (18)$$

$$\text{Group II} \quad W = 32.50 - 41.81e^{-.0286t} \quad (19)$$

$$\text{Group III} \quad W = 23.50 - 29.34e^{-.0286t} \quad (20)$$

In each case *W* is the total weight in kilograms of the constituent in question at the age *t*, the latter being counted in months from conception. The fit of these equations to the experimental data is fully revealed in Tables 9, 10, 11 and 12, in which observed and calculated values for each steer are compared.

The fits appear to be good with the occasional exception of the youngest one or two steers in a group, the general tendency being to underestimate the nutrient content of these animals.

Using the equations just given, the average composition of the steers at regular intervals of time may be computed (see Table 13), and from these figures the percentage composition of the steers (see Table 14).

¹The constants were chosen in such a way that the sum of the squared residuals was the least.

²The relation of the fat content of the cattle to their corrected age was found to be very nearly linear, particularly for Group I, less so for Group II, and still less so for Group III. With the Group I cattle, in fact, an appreciably better fit was obtained by the use of the linear equation:

$$W = 7.932t - 100.6$$

than by the use of the exponential equation of Brody. Hence, in Table 11 the calculated fat contents of the cattle of Group I have been obtained from the linear relation.

TABLE 9
OBSERVED AND CALCULATED WATER CONTENT OF MISSOURI CATTLE AT DIFFERENT AGES

Group I				Group II				Group III						
Steer No.	Body weight kgms.	Age ¹ mos.	Water content		Steer No.	Body weight kgms.	Age ¹ mos.	Water content		Steer No.	Body weight kgms.	Age ¹ mos.	Water content	
			Obs. kgms.	Calc. kgms.				Obs. kgms.	Calc. kgms.				Obs. kgms.	Calc. kgms.
556	112	13.8	65	62	554	89	12.6	53	32	555	85	12.6	51	46
557	201	16.5	99	103	552	116	14.1	64	54	548	101	14.0	58	57
547	204	16.7	105	106	550	147	15.8	76	77	558	108	14.7	60	62
541	328	21.0	166	161	538	183	17.9	100	102	540	155	19.2	89	92
505	313	20.4	149	153	503	276	24.1	143	159	531	218	26.1	126	128
532	514	28.9	243	230	523	392	34.0	209	213	525	315	39.5	170	179
504	531	29.7	242	235	507	460	41.5	243	237	524	366	48.5	205	200
515	740	42.9	313	297	526	494	46.0	242	246	509	455	71.1	228	229
527	848	53.2	313	321	502	516	49.4	256	252	500	482	80.7	237	236
513	861	54.7	319	323	512	567	58.5	260	262					
501	891	58.6	325	329										

¹ Counted from conception, age at birth being taken as 9.4 mos.

TABLE 11
OBSERVED AND CALCULATED FAT CONTENT OF MISSOURI CATTLE AT DIFFERENT AGES

Group I				Group II				Group III						
Steer No.	Body weight kgms.	Age, ¹ mos.	Fat content		Steer No.	Body weight kgms.	Age, ¹ mos.	Fat content		Steer No.	Body weight kgms.	Age, ¹ mos.	Fat content	
			Obs. kgms.	Calc. kgms.				Obs. kgms.	Calc. kgms.				Obs. kgms.	Calc. kgms.
556	112	13.8	9.3	8.9	554	89	12.6	5.6	8.4	555	85	12.6	3.1	5.8
557	201	16.5	36	30.3	552	116	14.1	10.3	11.9	548	101	14.0	5.7	7.1
547	204	16.7	27	31.8	550	147	15.8	16.6	15.9	558	108	14.7	7.6	7.8
541	328	21.0	59	65.9	538	183	17.9	21.0	20.7	540	155	19.2	15.9	12.1
505	313	20.4	67	61.2	503	276	24.1	37.9	34.8	531	218	26.1	19.1	18.7
532	514	28.9	119	129	523	392	34.0	48.6	56.4	525	315	39.5	30.1	31.1
504	531	29.7	137	135	507	460	41.5	78.6	72.1	524	366	48.5	32.9	39.1
515	740	42.9	239	240	526	494	46.0	84.3	81.3	509	455	71.1	62.6	58.4
527	848	53.2	350	327	502	516	49.4	73.6	88.0	500	482	80.7	68.7	66.3
513	861	54.7	322	333	512	567	58.5	117.0	105.7					
501	891	58.6	355	364										

¹ Computed from conception, the age at birth being taken as 9.4 mos.

TABLE 13
THE COMPOSITION OF MISSOURI STEERS AT DIFFERENT AGES

Age ¹ mos.	Group I				Group II				Group III						
	Body wt. kgms.	Water kgms.	Fat kgms.	Nitro- gen kgms.	Ash kgms.	Body wt. kgms.	Water kgms.	Fat kgms.	Nitro- gen kgms.	Ash kgms.	Body wt. kgms.	Water kgms.	Fat kgms.	Nitro- gen kgms.	Ash kgms.
6	173	87	21.6	3.9	5.6	140	72	14.9	3.4	5.6	116	67	8.5	3.0	4.6
9	257	129	45.3	6.4	8.9	191	107	21.9	5.0	7.8	147	87	11.4	3.9	6.2
12	340	164	69.1	8.4	11.6	238	137	28.7	6.4	9.8	176	105	14.3	4.7	7.6
15	415	194	92.9	10.0	14.0	280	161	35.5	7.7	11.7	203	121	17.1	5.5	8.9
18	483	219	116	11.2	16.1	319	181	42.1	8.8	13.4	229	135	19.9	6.2	10.1
21	544	240	141	12.3	17.9	355	197	48.7	9.7	15.0	252	148	22.7	6.9	11.2
24	599	258	164	13.1	19.4	387	211	55.1	10.5	16.4	274	159	25.5	7.5	12.2
27	649	272	188	13.8	20.7	416	222	61.5	11.2	17.7	295	169	28.2	8.1	13.2
30	693	285	212	14.3	21.8	443	231	67.8	11.9	19.0	314	178	31.0	8.6	14.0
33	733	295	236	14.7	22.7	468	239	74.0	12.4	20.1	332	186	33.7	9.1	14.8
36	770	304	260	15.1	23.6	491	245	80.0	12.9	21.1	349	193	36.4	9.5	15.5
39	802	311	283	15.4	24.3	511	251	86.1	13.3	22.0	365	200	39.0	10.0	16.2
42	831	318	307	15.6	24.9	530	255	92.0	13.7	22.9	380	205	41.7	10.4	16.8
45	858	323	331	15.8	25.4	547	258	97.8	14.0	23.7	394	210	44.3	10.7	17.3
48	882	327	355	15.9	25.8	563	261	103.6	14.3	24.4	407	215	46.9	11.1	17.8
51	577	264	109.2	14.5	25.1	419	218	49.5	11.4	18.3

¹ From birth.

TABLE 14
THE PERCENTAGE COMPOSITION OF MISSOURI STEERS AT DIFFERENT AGES

Age ¹ mos.	Group I					Group II					Group III				
	Water	Fat	Nitro- gen	Ash	Diff. ²	Water	Fat	Nitro- gen	Ash	Diff. ²	Water	Fat	Nitro- gen	Ash	Diff. ²
6	50.3	12.5	2.27	3.24	19.8	51.4	10.6	2.44	3.98	18.8	57.8	7.3	2.60	3.98	14.7
9	50.2	17.6	2.48	3.44	13.3	56.0	11.5	2.64	4.08	11.9	59.2	7.8	2.66	4.20	12.2
12	48.2	20.3	2.46	3.43	12.7	57.6	12.1	2.71	4.13	9.3	59.7	8.1	2.69	4.32	11.1
15	46.7	22.4	2.40	3.38	12.5	57.5	12.7	2.74	4.17	8.5	59.6	8.4	2.71	4.39	10.7
18	45.3	24.0	2.33	3.33	12.8	56.7	13.2	2.75	4.20	8.7	59.0	8.7	2.71	4.41	11.0
21	44.1	25.9	2.26	3.28	12.6	55.5	13.7	2.73	4.22	9.5	58.7	9.0	2.73	4.45	10.8
24	43.1	27.3	2.19	3.23	12.7	54.5	14.2	2.72	4.24	10.1	58.0	9.3	2.73	4.46	11.1
27	41.9	29.0	2.12	3.19	12.7	53.4	14.8	2.70	4.26	10.6	57.3	9.6	2.73	4.46	11.5
30	41.1	30.6	2.06	3.14	12.3	52.1	15.3	2.68	4.28	11.6	56.7	9.9	2.74	4.46	11.8
33	40.2	32.2	2.01	3.10	11.9	51.1	15.8	2.66	4.29	12.2	56.0	10.1	2.73	4.45	12.4
36	39.5	33.8	1.96	3.06	11.4	49.9	16.3	2.63	4.30	13.1	55.3	10.4	2.73	4.44	12.8
39	38.8	35.3	1.92	3.02	10.9	49.1	16.8	2.61	4.31	13.5	54.8	10.7	2.73	4.43	13.0
42	38.3	36.9	1.87	2.99	10.1	48.1	17.4	2.58	4.32	14.1	53.9	11.0	2.72	4.41	13.7
45	37.7	38.6	1.84	2.96	9.2	47.2	17.9	2.56	4.33	14.6	53.3	11.2	2.72	4.40	14.1
48	37.1	40.2	1.80	2.93	8.6	46.4	18.4	2.54	4.33	15.0	52.8	11.5	2.72	4.38	14.3
51	45.8	18.9	2.52	4.34	15.3	52.0	11.8	2.72	4.37	14.8

¹ From birth. ² After converting nitrogen to conventional protein by the use of the usual factor, 6.25.

Since the weight of a steer is a better indication of its stage of growth than is the age, the same sort of data may be obtained for equal intervals of weight rather than of time. From Equations (2), (3), and (4) the average age of each group of steers at weights of 100, 200, 300, etc., pounds in weight may be computed, and the composition of the steers at these ages determined as were the values in Table 13. The percentage composition, derived directly from such estimates, for the steers at equal intervals of weight has been summarized in Table 15.

The rate of deposition of nutrients and the percentage composition of gains at different ages and weights.—The ultimate purpose of this mathematical analysis of the Missouri data on steers is to secure satisfactory estimates of the rate of deposition of each of the body constituents at different times and at different weights. This information may be readily obtained from equations obtained by differentiating Equations (9) to (20) inclusive, in accordance with the general Equation (5). The linear equation used in fitting the age-fat content data of Group I (see footnote to page 19) gives a constant, upon differentiation, of 7.932 kgs. of fat per month. For this group, therefore, the rate of deposition of fat was constant.¹

Obtained in this manner, the rate of gain per day by the steers of each of the three groups in each of the constituents will be found, for equal intervals of time in Table 16, and for equal intervals of weight in Table 17. The energy increments in these tables are obtained by assuming a gross energy value for protein ($N \times 6.25$) of 5.7 cal. per gram, and for fat a value of 9.5 cal. per gram.

¹The differential equations obtained, from which the rate of deposition of the constituent in question per month ($\frac{dW}{dt}$) at any time t may be calculated are as follows:

Water content:

$$\text{Group I} \quad \frac{dW}{dt} = 37.36e^{-.0581t}$$

$$\text{Group II} \quad \frac{dW}{dt} = 35.00e^{-.0641t}$$

$$\text{Group III} \quad \frac{dW}{dt} = 13.06e^{-.0291t}$$

Nitrogen content:

$$\text{Group I} \quad \frac{dW}{dt} = 2.809e^{-.0728t}$$

$$\text{Group II} \quad \frac{dW}{dt} = 1.149e^{-.0449t}$$

$$\text{Group III} \quad \frac{dW}{dt} = 0.448e^{-.0216t}$$

Fat content:

$$\frac{dW}{dt} = 7.932$$

$$\frac{dW}{dt} = 2.511e^{-.000481t}$$

$$\frac{dW}{dt} = 1.013e^{-.00281t}$$

Ash content:

$$\frac{dW}{dt} = 2.568e^{-.05107t}$$

$$\frac{dW}{dt} = 1.196e^{-.0288t}$$

$$\frac{dW}{dt} = 0.840e^{-.0286t}$$

TABLE 15
THE PERCENTAGE COMPOSITION OF MISSOURI STEERS AT DIFFERENT WEIGHTS

Wt. lbs.	Group I					Group II					Group III				
	Water	Fat	Nitro- gen	Ash	Diff. ¹	Water	Fat	Nitro- gen	Ash	Diff. ¹	Water	Fat	Nitro- gen	Ash	Diff. ¹
300	47.8	7.9	1.91	2.90	29.5	50.7	10.6	2.41	3.97	19.6	58.8	7.7	2.65	4.15	12.8
400	50.3	13.2	2.31	3.28	18.8	55.8	11.3	2.62	4.08	12.4	60.2	8.2	2.70	4.35	10.4
500	51.1	16.4	2.48	3.44	13.6	57.3	11.9	2.70	4.12	9.8	59.5	8.7	2.71	4.42	10.5
600	50.0	18.2	2.49	3.45	12.8	57.7	12.6	2.74	4.17	8.4	58.1	9.3	2.73	4.46	11.0
700	48.7	19.6	2.47	3.43	12.9	56.6	13.2	2.75	4.20	8.8	56.6	9.9	2.73	4.45	12.0
800	47.9	21.0	2.45	3.41	12.4	55.4	13.9	2.74	4.24	9.4	54.8	10.6	2.73	4.43	13.1
900	46.8	22.2	2.39	3.39	12.7	53.7	14.7	2.71	4.27	10.4	52.7	11.6	2.72	4.38	14.3
1000	45.8	23.3	2.36	3.65	12.9	51.8	15.5	2.67	4.28	11.7					
1100	45.1	24.6	2.31	3.32	12.6	49.7	16.6	2.63	4.31	13.0					
1200	44.1	25.9	2.26	3.28	12.6	47.4	17.9	2.57	4.34	14.3					
1300	43.2	27.1	2.20	3.24	12.8										
1400	42.3	28.5	2.14	3.19	12.6										
1500	41.3	30.1	2.08	3.16	12.4										
1600	40.4	31.8	2.02	3.11	12.1										
1700	39.4	33.9	1.96	3.06	11.4										
1800	38.5	36.0	1.90	3.01	10.6										
1900	37.5	38.7	1.83	2.95	9.5										

¹ After converting nitrogen to protein.

TABLE 16
DAILY GAINS IN BODY WEIGHT AT DIFFERENT AGES AND THE COMPOSITION OF THE GAINS. MISSOURI CATTLE

Age ¹ mos.	Group I					Group II					Group III									
	Gain per day in water gms.	Gain in fat gms.	Gain in nitrogen gms.	Gain in ash gms.	Gain in energy cals.	Gain per day in water gms.	Gain in fat gms.	Gain in nitrogen gms.	Gain in ash gms.	Gain in energy cals.	Gain per day in water gms.	Gain in fat gms.	Gain in nitrogen gms.	Gain in ash gms.	Gain in energy cals.					
6	953	509	204	30.5	39.0	3594	6	590	435	77.8	19.2	25.7	1423	6	359	238	32.3	10.23	18.0	671
9	975	428	"	24.5	33.4	3380	9	540	359	76.7	16.8	23.6	1327	9	335	212	32.1	9.50	16.5	643
12	880	359	"	19.7	28.7	3209	12	494	296	75.6	14.7	21.6	1241	12	313	189	31.8	8.83	15.2	616
15	794	302	"	15.9	24.6	3074	15	450	244	74.5	12.8	19.8	1164	15	293	168	31.5	8.20	13.9	591
18	712	251	"	12.8	21.1	2964	18	411	201	73.4	11.2	18.2	1096	18	273	149	31.2	7.63	12.8	568
21	640	213	"	10.3	18.1	2875	21	376	166	72.4	9.80	16.7	1037	21	255	133	31.0	7.07	11.7	547
24	581	179	"	8.23	15.5	2801	24	343	137	71.3	8.57	15.4	982	24	239	118	30.7	6.57	10.7	526
27	522	150	"	6.63	13.3	2744	27	313	113	70.3	7.50	14.1	935	27	223	105	30.5	6.10	9.87	507
30	472	126	"	5.33	11.4	2698	30	286	93.3	69.3	6.53	12.9	890	30	208	93.3	30.2	5.67	9.03	489
33	424	106	"	4.23	9.83	2659	33	263	77.0	68.3	5.73	11.9	853	33	195	83.0	29.9	5.27	8.30	472
36	382	89	"	3.43	8.43	2630	36	239	63.3	67.3	5.00	10.9	817	36	182	73.7	29.7	4.90	7.63	456
39	344	75	"	2.77	7.23	2607	39	218	52.3	66.4	4.37	10.0	787	39	171	65.7	29.4	4.53	7.00	440
42	310	63	"	2.22	6.20	2587	42	199	43.3	65.4	3.83	9.17	757	42	159	58.3	29.2	4.23	6.43	428
45	280	53	"	1.79	5.33	2572	45	182	35.7	64.5	3.33	8.40	732	45	148	52.0	28.9	3.93	5.90	415
48	252	44.3	"	1.44	4.57	2559	48	166	29.4	63.6	2.92	7.73	708	48	138	46.3	28.7	3.63	5.40	402
51	...	37.3	"	1.16	3.90	2549	51	152	24.3	62.6	2.55	7.10	686	51	129	41.0	28.5	3.38	4.97	391

¹ From birth.

TABLE 17
DAILY GAINS IN BODY WEIGHT AT DIFFERENT BODY WEIGHTS AND THE COMPOSITION OF THE GAINS, MISSOURI CATTLE

Wt. lbs.	Group I					Group II					Group III										
	Age ¹ mos.	Gain per day gms.	Gain in water fat gms.	Gain in nitro- gen gms.	Gain in ash gms.	Gain in en- ergy cals.	Age ¹ mos.	Gain per day gms.	Gain in water fat gms.	Gain in nitro- gen gms.	Gain in ash gms.	Gain in en- ergy cals.	Age ¹ mos.	Gain per day gms.	Gain in water fat gms.	Gain in nitro- gen gms.	Gain in ash gms.	Gain in en- ergy cals.			
300	4.6	848	551	264	33.7	41.8	3708	5.8	594	441	77.9	19.3	25.8	1427	7.9	343	221	32.2	9.77	17.0	654
400	6.3	971	501	"	29.9	38.4	3572	8.4	549	372	76.9	17.2	23.9	1343	12.6	309	184	31.7	8.70	14.9	611
500	8.0	1012	454	"	26.4	35.2	3448	11.3	503	310	75.8	15.1	22.1	1258	17.8	274	150	31.3	7.67	12.8	570
600	9.5	957	415	"	23.6	32.6	3348	14.4	458	253	74.7	13.2	20.2	1180	23.7	240	120	30.7	6.63	10.8	528
700	11.1	907	378	"	21.0	30.0	3256	17.9	412	202	73.4	11.3	18.3	1099	30.5	206	91.7	30.2	5.60	9.1	486
800	12.9	853	341	"	18.5	27.4	3167	21.8	367	158	72.1	9.47	16.3	1022	38.5	171	67.0	29.5	4.60	7.10	444
900	14.7	798	307	"	16.2	25.0	3085	26.2	321	119	70.6	7.77	14.4	948	48.4	137	45.7	28.7	3.60	5.33	401
1000	16.7	748	274	"	14.0	22.6	3006	31.3	275	85.7	68.9	6.17	12.4	875							
1100	18.8	694	243	"	12.1	20.3	2939	37.3	229	58.3	66.9	4.70	10.5	803							
1200	21.0	644	213	"	10.2	18.1	2871	44.7	183	36.3	64.6	3.40	8.47	735							
1300	23.5	590	185	"	8.57	16.0	2813														
1400	26.2	540	158	"	7.03	13.9	2758														
1500	29.1	485	133	"	5.67	12.2	2710														
1600	32.4	433	110	"	4.47	10.1	2667														
1700	36.1	376	88.3	"	3.40	8.37	2629														
1800	40.4	328	69.0	"	2.49	6.73	2597														
1900	45.4	275	51.7	"	1.73	5.20	2570														

¹ From birth.

The percentage composition of the gains is given for equal intervals of age, in Table 18, and for equal intervals of body weight in Table 19. The per cent "difference" in these tables and the absolute "difference" in Tables 14 and 15 are accounted for by the "fill" of the cattle except for errors in analysis or in mathematical estimation. Negative "differences" in relation to gains may be interpreted as due to a decreasing fill.

The significance of group differences in terms of growth and fattening.—The essential results of this investigation in so far as the question of food requirements is concerned are contained in Tables 16 and 17, i. e., the daily increments in nitrogen, ash and energy. These increments must come from the day's food and represent those portions of the food that are being used productively. For all three groups of steers, the daily increments in these constituents decrease progressively after six months of age, except the fat increment for Group I, which remains constant.

Striking differences exist among the three groups of cattle in respect to the amount of nutrients added to the body daily during growth and fattening. These differences are greatest with respect to fat and energy, and least with respect to water, nitrogen, and ash. It is important to evaluate more precisely the significance of these group differences in daily tissue increment occasioned by the imposed differences in the plane of nutrition.

According to the plan of the experiment, Group II represents more nearly a normal growth of Hereford-Shorthorn steers, while Group I represents an additional and considerable fattening and Group III a retarded growth. In accordance with this plan, Group II eventually attained a fat content of about 20 per cent, Group I a fat content of about 40 per cent, and Group III a fat content of about 12 per cent. It would be interesting to compare the composition changes of these steers with those of dairy heifers during normal growth, but this is impossible to do directly in the absence of chemical analyses of the carcasses of dairy heifers of different ages.

In the absence of a direct method of comparison, an indirect one must be resorted to. Brody and Ragsdale⁽⁴⁴⁾ have suggested a height-weight relation as indicative of the state of nutrition of dairy heifers differing in age. They point out that although the weight of growing cattle is primarily dependent upon the plane of nutrition, the height at withers is a remarkably good index of the growth attained and is not readily affected by fattening. If weight is plotted against height, the curve obtained for Jersey heifers follows very closely the curve obtained for Holstein heifers, although a considerable age difference exists with

TABLE 18
THE PERCENTAGE COMPOSITION OF GAINS AT DIFFERENT AGES, MISSOURI CATTLE

Group I							Group II							Group III						
Age ¹ mos.	Fat	Nitro- gen	Ash	Energy per lb.	Pct. diff. ²		Age ¹ mos.	Fat	Nitro- gen	Ash	Energy per lb.	Pct. diff. ²		Age ¹ mos.	Fat	Nitro- gen	Ash	Energy per lb.	Pct. diff. ²	
6	53.4	27.7	3.20	4.09	1711	- 5.2	6	73.7	13.2	3.25	4.36	1095	- 11.6	6	66.3	9.0	2.85	5.01	848	1.9
9	43.9	27.1	2.51	3.43	1572	9.9	9	66.5	14.2	3.11	4.37	1115	- 4.5	9	63.3	9.6	2.84	4.93	870	4.5
12	40.8	30.0	2.24	3.26	1654	11.9	12	59.9	15.3	2.98	4.37	1139	1.8	12	60.4	10.2	2.82	4.86	893	6.9
15	38.0	33.2	2.00	3.10	1757	13.2	15	54.2	16.6	2.84	4.40	1172	7.0	15	57.3	10.8	2.80	4.74	916	9.7
18	35.7	37.1	1.80	2.96	1888	13.0	18	48.9	17.9	2.73	4.43	1208	11.7	18	54.6	11.4	2.79	4.69	944	11.9
21	33.3	41.2	1.61	2.83	2039	12.6	21	44.1	19.3	2.61	4.44	1252	15.9	21	52.2	12.2	2.77	4.59	972	13.7
24	30.8	45.4	1.42	2.67	2188	12.2	24	39.9	20.8	2.50	4.49	1299	19.2	24	49.4	12.8	2.75	4.48	1000	16.1
27	28.7	50.6	1.27	2.55	2386	10.3	27	36.1	22.5	2.40	4.50	1353	21.9	27	47.1	13.7	2.74	4.43	1033	17.7
30	26.7	55.9	1.13	2.42	2594	7.9	30	32.6	24.2	2.28	4.51	1410	24.4	30	44.9	14.5	2.73	4.34	1065	19.2
33	25.0	62.3	1.00	2.32	2844	4.2	33	29.3	25.9	2.18	4.52	1473	26.7	33	42.6	15.3	2.70	4.26	1100	20.9
36	23.3	69.1	0.90	2.21	3124	0.2	36	26.5	28.2	2.09	4.56	1553	27.6	36	40.5	16.3	2.69	4.19	1137	22.2
39	21.8	76.7	0.80	2.10	3435	- 5.6	39	24.0	30.5	2.00	4.59	1640	28.4	39	38.4	17.2	2.67	4.09	1164	23.7
42	20.3	85.2	0.72	2.00	3782	- 12.0	42	21.8	32.9	1.92	4.61	1724	28.7	42	36.7	18.4	2.66	4.05	1223	24.3
45	18.9	94.3	0.64	1.90	4169	- 19.1	45	19.6	35.4	1.83	4.62	1825	29.0	45	35.1	19.5	2.66	3.99	1269	24.8
							48	17.7	38.3	1.76	4.66	1929	28.3	48	33.6	20.8	2.63	3.91	1318	25.3
							51	16.0	41.2	1.68	4.67	2048	27.6	51	31.8	22.1	2.62	3.85	1372	25.9

¹ From birth.
² Counting the nitrogen as protein (N x 6.25).

TABLE 19
THE PERCENTAGE COMPOSITION OF GAINS AT DIFFERENT BODY WEIGHTS, MISSOURI CATTLE

Body wt.	Group I					Group II					Group III							
	Water	Fat	Nitro- gen	Ash	Energy per lb.	Pct. diff. ¹	Water	Fat	Nitro- gen	Ash	Energy per lb.	Pct. diff. ¹	Water	Fat	Nitro- gen	Ash	Energy per lb.	Pct. diff. ¹
300	65.0	31.1	3.97	4.93	1983	-25.8	74.2	13.1	3.25	4.34	1089	-11.9	64.4	9.4	2.85	4.96	864	3.4
400	51.6	27.2	3.08	3.95	1669	-	67.8	14.0	3.13	4.35	1110	-5.7	59.5	10.3	2.82	4.82	897	7.8
500	44.9	26.1	2.61	3.48	1546	9.2	61.6	15.1	3.00	4.39	1133	0.2	54.7	11.4	2.80	4.67	942	11.7
600	43.4	27.6	2.47	3.41	1587	10.2	55.2	16.3	2.88	4.41	1168	6.1	50.0	12.8	2.76	4.50	998	15.5
700	41.7	29.1	2.32	3.31	1628	11.4	49.0	17.8	2.74	4.44	1209	11.7	44.5	14.7	2.72	4.43	1070	19.4
800	40.0	30.9	2.17	3.21	1685	12.3	43.0	19.7	2.58	4.44	1265	16.8	39.2	17.3	2.69	4.15	1175	22.6
900	38.5	33.1	2.03	3.13	1753	12.6	37.1	22.0	2.42	4.49	1341	21.3	33.4	21.0	2.63	3.89	1323	25.3
1000	36.6	35.3	1.87	3.02	1822	13.4	31.2	25.1	2.24	4.51	1444	25.2						
1100	35.0	38.0	1.74	2.93	1921	13.2	25.5	29.2	2.05	4.59	1590	27.9						
1200	33.1	41.0	1.58	2.81	2022	13.2	19.8	35.3	1.86	4.63	1819	28.7						
1300	31.4	44.7	1.45	2.71	2164	12.1												
1400	29.3	48.9	1.30	2.58	2318	11.1												
1500	27.4	54.4	1.17	2.52	2533	8.4												
1600	25.4	61.0	1.03	2.33	2796	4.9												
1700	23.5	70.2	0.90	2.23	3171	-1.5												
1800	21.0	80.5	0.76	2.05	3592	-8.2												
1900	18.8	96.0	0.63	1.89	4234	-20.6												

¹ Counting the nitrogen as protein (N x 6.25).

respect to the weight-height relation. Thus, a 10-month-old Holstein heifer possesses the same weight-height relation as a 15-month-old Jersey.

The steers of Groups I, II and III, although differing markedly in their age-weight relationships, were strikingly similar in their age-height relationships, indicating that skeletal growth, at least, was very little affected by the plane of nutrition. Thus, at 4 years of age, the average height at withers for all groups was very close to 56 inches, although the average weights were, in order, 1945, 1238, and 897 pounds.

If the height-weight relation of these beef steers is compared with that of the dairy heifers cited by Brody and Ragsdale, it becomes evident that a better agreement exists between the Group II steers and the dairy heifers than between either the Group I or the Group III steers and the dairy heifers. Thus, at a height of 40 inches, the dairy cattle weighed about 365 pounds, while the steers of Group I weighed about 415 pounds, those of Group II about 415 pounds, and those of Group III about 320 pounds. At a height of 45 inches, the dairy cattle averaged about 550 pounds in weight, while the steers of Group I averaged about 710 pounds, those of Group II about 595 pounds, and those of Group III about 450 pounds. For a height of 50 inches, the dairy cattle weighed 855 pounds, the Group I steers 1070 pounds, the Group II steers 810 pounds, and the Group III steers 605 pounds. For greater heights, the Holstein heifers weighed increasingly more than the Group II steers, though far less than the Group I steers. From this comparison, it appears evident that the nutritive condition of the steers of Group II was kept approximately the same as that of growing dairy heifers of equal height, except at the greater heights (above 50 inches) where the condition of the dairy cattle (Holstein only) was superior to that of these steers.

This constitutes confirmatory evidence that the body changes occurring in the steers of Group II, approximate what might be considered normal growth changes. If this is true, then the changes occurring in Group III represent subnormal growth changes and need not be considered further in this discussion of the protein requirements of growth and fattening. It is a matter of interest, however, to investigate the difference in nitrogen content and in the rate of nitrogen deposition between Group II and Group I, as revealed in Tables 13 and 16. The steers of Group I contained on an average a larger amount of nitrogen at all ages, and for the first two years averaged a greater rate of nitrogen deposition. Are these differences due only to a greater rate of growth in Group I than in Group II, or are they due in part at least to the greater rate of fatten-

ing? If the latter is true, then an examination of the nitrogen content of the fatty tissues in the two groups of steers should reveal a corresponding difference.

Does fattening involve nitrogen deposition?—Although all of the fatty tissues from the carcasses of these steers were not analyzed separately, separate analyses are reported for the offal fat, the kidney fat and the fat from the round, loin and rib, except, apparently, when there was no considerable amount of fat on the rib cut. In Table 20, the weights of nitrogen in the analyzed fat samples are summarized and totaled separately for the steers of each group. The steers whose results are collected on the same line of this table were of approximately the same age when slaughtered.

The total nitrogen content of the adipose tissues of the steers of Group I was always considerably greater than that of the steers of Group II of approximately the same age, the same relation existing between the steers of Group II and those of Group III. Evidently the deposition of fat in adipose tissue involves cellular proliferation (or cellular protoplasmic enlargement), and therefore fattening possesses its own nitrogen requirement, distinct from that of growth. This difference in adipose tissue nitrogen may account largely for the difference in nitrogen content of Group I and Group II steers of like age, and for the more rapid deposition of nitrogen in the Group I steers during the first two years, although the differences in Table 20 are far too small to account for the differences in Table 13. However, a greater nitrogen content of the other adipose tissues, such as the subcutaneous, intermuscular, and marrow connective tissues, in the steers of Group I over that of the steers of Group II also must have occurred.

A comparison of the nitrogen content of the lean samples for the steers of Groups I and II slaughtered at ages of two years or more is of interest in this connection. The nitrogen in the lean of the round for the five oldest steers in Group II averaged 94 per cent of the nitrogen in the same samples for the five oldest steers of Group I. For the lean of the loin and the rib, however, this percentage was 78 and 83, respectively. But the lean of the latter cuts was considerably fatter than the lean of the round, and hence the greater nitrogen content for the Group I steers over that for the Group II steers in these cases may have been due to a considerable extent to the increased content of adipose tissue cells.

Further light may be thrown upon this question by computing the percentage composition of the added empty weight of Group I over

TABLE 20
WEIGHTS OF NITROGEN IN FAT SAMPLES OF MISSOURI STEERS, ALL WEIGHTS IN GRAMS

Steer	Group I				Group II				Group III												
	Offal	Kidney	Round	Looin	Rib	Total	Offal	Kidney	Round	Looin	Rib	Total	Offal	Kidney	Round	Looin	Rib	Total			
556	13.5	2.5	15.1	11.5	...	43	554	7.7	1.7	13.9	6.0	...	29	555	8.0	1.6	9.8	6.0	...	25	
557	29.4	11.9	25.8	32.6	15.5	115	552	16.5	2.7	19.2	12.3	...	51	548	12.4	3.9	14.2	7.6	...	38	
547	23.5	5.1	28.9	39.2	15.9	113	550	15.3	3.4	13.6	19.6	...	52	558	11.5	2.1	14.9	9.3	...	38	
505	43.5	13.6	27.0	40.4	17.0	141	538	18.8	2.1	29.1	23.1	6.2	79	540	12.6	7.9	11.0	22.1	...	51	
541	32.3	12.2	38.0	63.1	26.3	172	503	38.5	7.2	41.2	44.7	12.4	144								
532	57.6	17.6	67.5	100.8	52.8	296															
504	83.9	21.5	89.0	97.6	56.4	351	523	39.2	14.5	72.0	55.0	23.0	204	531	18.0	11.5	22.7	31.4	...	84	
515	84.0	17.7	149.4	157.9	64.2	475	507	46.6	12.4	58.8	84.2	23.0	225	525	63.8	5.9	31.4	41.3	10.7	153	
527	88.8	35.5	161.6	188.2	98.8	573	526	47.4	11.0	83.8	98.9	43.2	284	524	38.1	3.4	56.5	30.2	...	128	
513	115.6	22.6	176.0	182.7	96.3	593	502	53.2	12.3	71.6	95.7	49.5	282	509	53.0	5.0	82.8	78.2	20.9	240	
501	79.2	37.1	148.6	79.9	113.6	458	512	64.4	8.7	76.0	99.5	45.3	294	500	54.6	10.0	78.5	40.8	23.3	207	

Group II at different ages, from the data in Table 13. The results of this calculation are given in Table 21.

Although at the younger ages the composition of the added tissues in the Group I steers over the Group II steers probably indicates a greater attained growth, at 36 months of age and after, the results are not incompatible with the belief that much the greater part of this added tissue is adipose tissue.

It may be concluded, therefore, that the steers of Group I probably grew at a somewhat faster rate than the steers of Group II for the first two or two and a half years, but that their greater content of nitrogen at all ages and their greater rate of nitrogen retention for the first two years was to a considerable extent accounted for by the greater protoplasmic content of their adipose tissues accompanying fattening.

TABLE 21
THE PERCENTAGE COMPOSITION OF THE AVERAGE DIFFERENCE IN
EMPTY WEIGHT BETWEEN THE STEERS OF GROUP I AND
THOSE OF GROUP II AT DIFFERENT AGES

Age mos.	Water pct.	Fat pct.	Nitrogen pct.	Ash pct.
12	33.2	49.7	2.4	2.2
24	26.8	62.3	1.5	1.7
36	23.1	70.6	0.85	0.96
48	20.1	76.4	0.49	0.43

The histogenesis of adipose tissue.—The histological work of Bell⁽⁴⁵⁾, carried out in close connection with the Missouri experiments on growing and fattening steers, indicates clearly that the formation of adipose tissue involves both cellular proliferation and cellular enlargement. In the formation of intramuscular as well as subcutaneous adipose tissue, fat cells are formed around the blood vessels, the process extending out on the smaller vessels as the animal fattens. Apparently the fat passes out of the blood stream and is taken up by the adjacent cells. Acting directly upon the relatively undifferentiated connective tissue cells, the fat (in some soluble form) causes them to pass into the preadipose and later into the adipose condition. Thus, according to Bell, "the blood vessel is the center around which the fat lobule develops. Whether in a mass of preadipose tissue, or in ordinary connective tissue, the first fat cells appear immediately around the blood vessels. The lobules thus established increase in size to a large extent by the addition of cells adjacent to the periphery. The increase in the number of fat cells is, however, to a considerable extent due to the division of fat-free cells inside the lobule." The occurrence of cellular proliferation was directly demonstrated by the finding of mitotic figures and of old and young cells.

Furthermore, "in almost any selection of adipose tissue a few nuclei may be seen crowded in the angles between the fat cells. The protoplasm around these nuclei is so small in amount that it can hardly be demonstrated. From a study of fattening animals I am convinced that these interstitial cells may form many new fat cells when the animal fattens. It is generally believed that a cell does not divide after any considerable amount of fat has been deposited in it." Further studies of these interstitial granules of muscle and their relation to the nutrition of the animal were reported by Bell in later papers⁽⁴⁶⁾.

Armsby's prediction equation of the rate of gain of protein at different ages.—In 1908 Armsby⁽⁴⁷⁾ computed the daily gains in protein of calves, lambs, and a number of pigs of varying ages from available slaughter data, and observed that when these gains were expressed per 1000 pounds live weight they exhibited similar variation with age, expressible by the very simple hyperbolic equation

$$g = \frac{135}{a + 20} \quad (21)$$

in which g is the gain of protein per day per 1000 pounds live weight and a is the age of the animal in days. A general similarity among different species of animals in the rate of gain of protein during growth would be remarkable, and it becomes a matter of great interest to compare the gains in nitrogen of the Missouri steers in Groups I and II at different ages with the prediction obtained from Armsby's equation. Such a comparison, at the ages at which the body weights are 300, 400, etc., pounds, is afforded by the computations contained in Table 22.

The Armsby equation gives a very poor prediction of the nitrogen gains by the Group I steers, greatly underestimating the early gains, and, to an even greater extent, over-estimating the later gains. Probably the Armsby equation will not apply to animals carrying as much fat as the steers of this group.

For the steers of Group II, a much better fit was secured between the rates of nitrogen retention computed from the Missouri data and the rates predicted by the Armsby equation for animals of their weight and age, though the agreement is not particularly good. Again the Armsby equation underestimates the early gains in spite of the fact that the equation used above to describe the relation between age and nitrogen content (13), from which the rate of nitrogen retention has been computed, underestimated the nitrogen content of the steers less than 6 months of age (see Table 10). Also, with Group II, as with Group I, the later daily gains of nitrogen are greatly over-estimated by the Armsby equation, which would lead one to expect a much more protracted gain

of nitrogen for these animals than actually occurred. In this case, however, the animals were not over-fat, so that their nitrogen gains should not stand in an abnormally low ratio to their body weights.

The explanation of the discrepancy becomes evident when Armsby's original publication is consulted. It is evident from the curve drawn to illustrate the closeness of fit of the equation to the data, but more so when estimates made from the equation are compared with the data from which it was obtained, that the agreement is not good for animals of 450 days of age or older, and in particular that for these later ages

TABLE 22

THE ESTIMATED DAILY GAINS OF NITROGEN BY THE MISSOURI STEERS OF GROUPS I AND II AT DIFFERENT BODY WEIGHTS, COMPARED WITH ARMSBY'S PREDICTED GAINS

Body weight lbs.	Group I				Group II			
	Age days	Daily nitrogen retention		Age days	Daily nitrogen retention			
		Computed from data gms.	Predicted by Armsby gms.		Computed from data gms.	Predicted by Armsby gms.		
300	139	33.7	18.5	173	19.3	15.2		
400	188	29.9	18.8	253	17.2	14.4		
500	240	26.4	18.9	339	15.1	13.7		
600	286	23.6	19.2	433	13.2	13.0		
700	334	21.0	19.4	538	11.3	12.3		
800	386	18.5	19.3	655	9.47	11.6		
900	441	16.2	19.1	787	7.77	10.9		
1000	500	14.0	18.9	940	6.17	10.2		
1100	563	12.1	18.5	1120	4.70	9.5		
1200	631	10.2	18.1	1342	3.40	8.7		
1300	704	8.57	17.6					
1400	785	7.03	17.1					
1500	873	5.67	16.5					
1600	972	4.47	15.8					
1700	1084	3.40	15.1					
1800	1212	2.49	14.3					
1900	1363	1.73	13.5					

the equation would lead one to expect rates of gain of protein (nitrogen) two or three times as rapid as actually occurred.

It seems improbable that any such generalized relation between gain in protein and age of animal as that assumed by Armsby should exist. It is true that inequalities of weight are taken care of after a fashion by expressing the daily gain in protein per unit of weight, but inequalities in the growing period of different animals or in the characteristics of the growth curve are not removed. The equation appears to assume that the growing period of all farm animals is the same, and that at the same age the growth processes are equally intense and equally near

to completion. But Brody⁽⁴⁸⁾ has shown that it takes dairy cows from 81 to 93 months from conception to attain to 98 per cent of their mature weight, Duroc-Jersey sows 67 months, and Suffolk ewes only 26 months. For the Missouri Hereford-Shorthorn steers of Group II, it may be shown from equation (3) that approximately 138 months from conception would be required for the attainment of 98 per cent of the estimated mature weight of 1600 pounds. Any attempt to formulate a generalized equation of the relation between the daily gain of protein per unit of weight and the age of animals must evidently take account of such specific differences in the time relations of growth. The use of *equivalent ages* in Brody's sense, rather than absolute ages, may ultimately prove successful in such an attempt.

The Minnesota investigations on fattening steers.—Besides the Missouri investigation of the changes in weight and composition of beef steers during growth and fattening, there is available for this study the extensive investigation of Haecker of the Minnesota Agricultural Experiment Station⁽⁴⁹⁾. This study involved a total of 63 steers, slaughtered at approximately 100 pound intervals up to a weight of 1500 pounds. From one to five animals were analyzed at each 100 pound weight. No uniform method of feeding was employed, however: different groups of animals were fed in the different years of the experiment on different rations and no attempt was made to control the ratio of roughage to grain. It appears from the following quotation that a very indefinite control of the food consumption was practised:

During all the feeding operations the intent was to provide the steers with sufficient feed to satisfy the appetite, but not to provide more nutriment than would be utilized. Each steer received as much hay and silage as he would eat, and the amount of grain required was determined by the feeder. The chief reliance in estimating the amount of grain needed was the odor given off from the feces. Eating the ration quickly, restlessness, and looking for more indicated a need of more feed; while slow feeding, failure to clean up the feed box, and sluggishness indicated that less feed would suffice.

The result of this ill-defined and unequal method of feeding the various steers is reflected in the data secured. The age-weight relation, not given in the Minnesota bulletin but secured from Missouri Research Bulletin 62, is quite abnormal in shape. There is no slowing up of the rate of weight increase and all attempts to fit the data by the Brody growth curve, which has been applied so successfully to a large number of animal species, were unavailing. Starting out at a growth rate slightly less than that of the Missouri steers of Group II, after 12 months of feeding they increased in weight at an increasingly faster rate, so that they attained an average weight in 28 months that the Missouri steers attained only after 44 months of feeding.

Comparing the percentage composition of the Minnesota steers at different weights (Table xxiv of Minn. Bull. 193) with the composition of the Missouri steers of Group II (Table 15 of this paper), the percentage of fat is quite similar up to 700 pounds in weight, but beyond this point the Minnesota steers fattened much more rapidly. Thus at 900 pounds the Minnesota cattle contained over 20 per cent of fat, more than the Missouri cattle contained at 1200 pounds.

The rapid fattening of the Minnesota cattle in the latter half of the weight range appears to account for the abnormal shape of the age-weight curve and indicates that a much heavier plane of feeding was in vogue at that time. The data thus are not homogeneous. The composition of the cattle at the higher weights is not comparable with that at the lower rates, since it is the result of a different system of feeding, and the heterogeneity thus prevailing precludes an exact interpretation of the experiment as a whole, and rules out the possibility of obtaining such a simple and satisfactory mathematical description as the Missouri data have yielded.

As far as the specific question of nitrogen deposition is concerned, the Minnesota data do not include direct protein determinations ($N \times 6.25$), the reported protein values being obtained by difference.¹

Estimated daily nitrogen requirements for different breeds of cattle.— It appears, therefore, that the Missouri data are the only data available from which the nitrogen requirements of growing cattle may be estimated. The daily nitrogen retention figures for the steers of Group II at different weights, given in Table 17, may be considered as measures of the nitrogen requirements of Hereford-Shorthorn unsexed male cattle. Is there any reasonable method by which similar estimates may be made for other breeds of cattle?

Upon certain approximate assumptions it appears possible to make such estimates for those breeds of cattle for which satisfactory growth data are available. For certain breeds of dairy cattle, Eckles and his associates have secured fairly satisfactory information concerning the age-weight relation, although the numbers of animals involved, particularly at the later ages, are not sufficiently large to give the averages any degree of finality. However, the data are not markedly irregular and have been shown by Brody⁽⁴¹⁾ to be satisfactorily describable by his growth

¹In thus failing to use the Minnesota data for the purpose of studying the growth of steers, there is no intention to detract from the value of this work for other purposes. If the method of feeding these steers conforms to accepted practices, the results secured may be of great practical value.

curve in the area of decreasing growth rate. According to Brody, the following equations describe the age-weight relations of these cattle:

$$\text{Holstein-Friesian, females, } W = 1215 - 1775e^{-.0460t} \quad (22)$$

$$\text{Ayrshire, females, } W = 1014 - 1468e^{-.0500t} \quad (23)$$

$$\text{Jersey, females, } W = 926 - 1499e^{-.0540t} \quad (24)$$

In all of these equations W is the weight of the heifer in pounds at age t , expressed in months from conception. The mature weights of the cows are estimated at 1215, 1014, and 926 pounds, respectively, and the percentage monthly declines in the velocity of growth with age are, in order, 4.5, 5.0, and 5.4.

If it may be assumed that dairy heifers retain daily per 1000 pounds live weight as much nitrogen for growth as Hereford-Shorthorn steers of "equivalent ages," then the nitrogen requirements of these cattle may be readily estimated.

According to Brody⁽⁴⁸⁾, the equivalence of age among different species of animals may be computed from the value k in equation (1) describing growth in weight beyond the point of inflection of the growth curve. This constant k measures the fractional decline in the weight increment per unit of time. The greater the fractional decline, k , the more rapidly will the limiting or mature weight, A , be approached. "Indeed," according to Brody, "the rapidity of approach to the mature weight, A , is directly proportional to the numerical value of k , . . . and the relative duration of the periods of growth of two animals is, therefore, inversely proportional to the numerical values of their k 's. These facts give us a basis for computing the equivalence of growth age in different animals."

For example, the numerical value of k for the Hereford-Shorthorn steer is .0303 (Equation (3)) and that found by Brody for Jersey heifers is .0540. Therefore, one month for the Jersey heifer is equivalent, during the phase of growth following the point of inflection of the growth curve, to $\frac{.0540}{.0303} = 1.78$ months for the Hereford-Shorthorn steer. As Brody points out, this relation may or may not hold true for the phase of growth preceding the point of inflection, characterized by a positive acceleration of growth.

Thus, neither conception nor birth can be taken as points of reference in computing age equivalence according to this method. Instead, Brody takes the age when the curve of Equation (1) cuts the age axis, corresponding to zero body weight. This age is termed t^* and may be readily obtained for any equation of this type by putting $W=0$ and solving for t .

For the Jersey heifer, $t^* = 8.9$ months and for the Hereford-Shorthorn steer $t^* = 8.3$ months. Hence, a heifer, at 20 months after conception, is equivalent in age to a steer of a conceptional age of $[(20 - 8.9) \times 1.78] + 8.3 = 28.1$ months.

Computing equivalent ages by this method, and assuming that the nitrogen retention of calves per day per unit of weight is the same at equivalent ages, the data given in Table 23 are obtained, relating to regular intervals of body weight. The Hereford-Shorthorn data are taken from Table 17. The calculation of the data for the other breeds may be best explained by a specific illustration.

TABLE 23

THE ESTIMATED DAILY NITROGEN RETENTION OF CATTLE OF DIFFERENT BREEDS AT DIFFERENT BODY WEIGHTS

Body weight lbs.	Hereford-Shorthorn		Holstein-Friesian		Ayrshire		Jersey	
	Age ¹ mos.	Daily N retention gms.	Age ¹ mos.	Daily N retention gms.	Age ¹ mos.	Daily N retention gms.	Age ¹ mos.	Daily N retention gms.
200	3.3	21.7	2.7	15.9	2.4	21.3	2.2	20.0
300	5.8	19.3	5.0	13.3	5.0	14.5	4.6	13.7
400	8.4	17.2	7.5	11.2	8.0	10.5	7.4	10.1
500	11.3	15.1	10.4	9.12	11.6	7.68	10.7	7.46
600	14.4	13.2	13.6	7.43	15.9	5.40	15.0	5.07
700	17.9	11.3	17.5	5.65	21.5	3.47	20.9	3.06
800	21.8	9.47	22.2	4.11	29.1	1.95	30.3	1.40
900	26.2	7.77	28.2	2.73	41.7	0.76	55.5	0.18
1000	31.3	6.17	36.5	1.54	83.7	0.02		
1100	37.3	4.70	50.1	0.62				
1200	44.7	3.40	94.4	0.03				

¹ From birth.

A 600-pound Holstein-Friesian heifer, according to Eckles' data, would possess an average age of 13.6 months (from birth). Using Brody's method as just explained, such a calf is equivalent in age to a Hereford-Shorthorn steer of 21.2 months from birth or 30.6 months from conception. From the differential equation giving the rate of nitrogen deposition of the Group II steers at any age (see footnote to page 30), it may be computed that a calf at 30.6 months (from conception) will retain daily 9.72 grams of nitrogen. But such a calf weighs 785 pounds (computed from Equation (3)). Hence, on the assumption that calves of equivalent age will retain equal amounts of nitrogen per unit of body weight, the daily nitrogen retention of the 600 pound Holstein-Friesian heifer would be $9.72 \times \frac{600}{785} = 7.43$ gms., the value given in Table 23.

The daily amounts of nitrogen retained by calves of the four breeds are offered as measures of the nitrogen requirements for growth. They are, of course, only tentative in nature, being subject to revision as more data accumulate. The nitrogen retention of young calves in particular needs further study, and the estimated values of Table 23 for 200 and to a less extent for 300 pound calves cannot be considered accurate or even satisfactory. The growth curve at the younger ages and in particular the change in composition of young calves needs further investigation. It may further be questioned whether data on steer calves should be applied to heifer calves. It is known that heifer calves fatten more rapidly, especially at the younger ages, than do steer calves. This difference undoubtedly vitiates to some extent the method of calculation employed in estimating the values given in Table 23. It appears that this effect will lead to an over-estimate of the nitrogen requirement of the heifer calves.

The questions here raised call for further study, but until more data become available the estimates presented may be used tentatively, with no probability of great error, in computing the nitrogen (protein) requirements of cattle for growth.

THE PROTEIN REQUIREMENT FOR FATTENING

Armsby has concluded^(2, p. 355) from a study of available slaughter and respiration experiments on cattle that the laying on of fat, although accomplished largely by an increase in the fat content of existing cells, also involves cellular proliferation. Hence, the fattening even of mature animals increases the protein requirement of the animal. The Missouri data confirm this conclusion in a very decisive fashion.

It is a difficult matter, however, to assess quantitatively the increased nitrogenous needs of fattening. The composition of adipose tissue affords an uncertain basis for estimation, since it varies with age and with anatomical location. A study of the Missouri data shows that the younger the animal, the less fat and the greater protein its adipose tissues contain, and that at all ages the kidney and offal fatty tissue are higher in fat and lower in protein than the loin, rib, and particularly the round fatty tissue. These differences are very largely explained by the fatty saturation of such tissues, since on the fat-free, or "protoplasmic" basis, the different adipose tissues are not greatly different in composition.

No rational method of separating the requirements of growth from those of fattening suggests itself, since no qualitative differences appear to exist between the two processes. Normal growth does not occur without a considerable deposition of fat, and fattening is just as surely accompanied by a deposition of protein. For steers growing and fattening

simultaneously, the results from the Missouri cattle of Group I (Tables 16 and 17) would be more applicable than those for Group II, involving what may be considered as growth alone. The differences in the rate of nitrogen retention between these two groups of cattle appear to be due to a considerable extent to the difference in the rate of fattening.

For the fattening of mature cattle, use may be made of the average values computed by Armsby (2, p. 354) of the composition of the organic matter gained. Protein appears to make up about one-eighth of the organic matter deposited in the tissues of mature fattening cattle. If the moisture content of this increase is taken roughly as 25 per cent, then each pound of gain contains 0.096 pound of protein or a little less than 7 grams of nitrogen. These figures represent, therefore, the protein or nitrogen requirement for each pound of increase in the fattening of mature animals. It is interesting to note that the Group I steers at 2 years of age, and at an average weight of 1320 pounds, were retaining 6.4 gms. of nitrogen per pound gain in weight (Table 16). However, much of this nitrogen increase, as well as that represented in Armsby's calculations, represents probably a growth requirement, since feeder cattle are not mature, in any strict sense of the term.

THE PROTEIN REQUIREMENT FOR PREGNANCY

The requirement for nitrogen or protein peculiar to the gestating female is measured by the rate of nitrogen deposition in the developing embryo or fetus and in the maternal nourishing and protecting tissues and fluids. Intra-uterine growth thus presents an experimental problem similar in all respects to that of extra-uterine growth.

Unfortunately, the published data on intra-uterine growth in the bovine species are few, and appear to be limited to the weights and analyses of three Jersey fetuses at the Missouri Agricultural Experiment Station (50). However, in this work the maternal tissues and fluids apparently¹ were not included in the chemical samples. Eckles has shown (51) that at full term the placenta and amniotic fluid contain 18 per cent as much protein as the fetus (Holstein); at younger ages the proportion may be much greater. The neglect of these tissues would thus introduce a considerable error in estimating the daily protein requirements of pregnancy.

Illinois investigations on the bovine fetus.—During the last year, Professor W. W. Yapp of the Department of Dairy Husbandry, University of Illinois, has secured some valuable data on the composition

¹The description of the preparation of the fetuses for analysis given in the bulletin is not sufficiently complete to warrant a definite statement on this point.

of 9 fetuses, of different ages, with their membranes. The amniotic fluid was collected, but its nitrogen content was not determined. Through the courtesy of Professor Yapp, these unpublished data have been made available for this study of the protein requirements for intra-uterine growth.

TABLE 24

AGE AND BREEDING OF BOVINE FETUSES ANALYZED BY W. W. YAPP

Fetus No.	Age from date of breeding days	Dam	Sire
718	91	F ₂ Holstein-Guernsey cross.....	Pure-bred Guernsey bull
2	95	Grade Holstein	" " Holstein "
26	95	Holstein-Guernsey back-cross to Holstein sire.	" " Guernsey "
21	104	Holstein-Guernsey back-cross to Holstein sire.	" " " "
27	123	Holstein-Guernsey back-cross to Holstein sire.	" " " "
1	162	Holstein-Guernsey back-cross to Holstein sire.	" " " "
644	162	F ₁ Holstein-Guernsey cross.....	" " " "
11	167	Grade Holstein	" " Holstein "
19	186	Grade Holstein	" " " "

TABLE 25

THE PROTEIN CONTENT OF BOVINE FETUSES OF DIFFERENT AGES, FROM DATA SECURED BY W. W. YAPP

Fetus No.	Age days	Weight of fetus and membrane gms.	Protein content pct.	Protein content gms.
718	91	363	5.85	21
2	95	489	5.25	26
26	95	666	6.16	41
21	104	513	5.59	29
27	123	1122	6.84	77
1	162	6179	9.20	568
644	162	4838	9.22	446
11	167	5531	9.49	525
19	186	13002	11.08	1441

The fetuses were obtained from grade Holstein and Holstein-Guernsey cows slaughtered at varying periods after being bred either to a pure-bred Holstein bull, in the case of the Holstein cows, or to a pure-bred Guernsey bull, in the case of the cross-bred cows. The description of the fetuses is contained in Table 24 and data relating to their nitrogen content will be found in Table 25. Fetuses Nos. 2, 11 and 19 were sired by the same bull, as were the remaining six.

At these early ages, it seems justifiable to treat these data as homogeneous, in spite of the fact that some of the fetuses were largely of Holstein blood, while others were largely of Guernsey blood. In the same series may be included Eckles' analysis of a full-term Holstein fetus, weighing 95 pounds. With its placenta this fetus contained 9162 gms. of protein. Since the average weight of Holstein calves at birth is 89 pounds, instead of 95 pounds, the protein content of the full-term Holstein fetus with its membranes may be roughly estimated at

$$\frac{89}{95} \times 9162 = 8585 \text{ grams.}$$

A mathematical analysis of the data.—The data at hand evidently give only a fragmentary picture of the intra-uterine growth in protein of the bovine fetus. A mathematical description of the data by a continuous function is essential to any effective use of them. The growth in weight of the chick embryo has been closely described by Murray⁽⁵²⁾ by means of the simple exponential (or parabolic) equation.

$$W = kt^n \quad (25)$$

in which W is the weight of embryo, and t is the incubation age. In adapting this equation to the intra-uterine growth of the mouse embryo, MacDowell and Allen⁽⁵³⁾ found it necessary to change the significance of t from conception age to something less, specifically 7 days less. This reduced age they call the "embryo age" on the following basis:

"Since the first stages of development of a mammal consist of the formation of the pro-embryo, a considerable period elapses before the first organization of the embryo proper. This is the justification for assuming embryo age to be less than conception age. In the mouse the first differentiation of the embryo proper (primitive streak) is not found before the end of the first week. Thus the embryological evidence bears out the purely graphical result obtained by shifting the age ($t-7$) until the embryo weights fit a logarithmic straight line."

Thus, the mouse embryo at seven days after conception and the chick embryo at the beginning of incubation are in practically the same stage of development. Evidently the shifting of the embryo age seven days ahead of the conception age will also include whatever error is made in measuring conception age from the time of copulation with the male.

In accordance with the experience of MacDowell and Allen, the nitrogen-growth data of bovine fetuses were not very closely described by an equation of type (25), but if t is taken as one month less than the age counted from the date of breeding, a fairly satisfactory fit is obtained, the equation becoming

$$W = 0.143(t-1)^{4.29} \quad (26)$$

W being expressed in grams, and t, the age from breeding, in months. The agreement between observations and corresponding values derived from this equation is given in Table 26.

Having obtained a continuous mathematical function descriptive of the relation between the age and the nitrogen content of the fetus, in so far as the limited amount of available data will permit, it is possible to derive a second mathematical function or equation from which the rate of nitrogen deposition, $\frac{dW}{dt}$, may be computed for any age. The differentiation of Equation (26) will give

$$\frac{dW}{dt} = 0.611(t-1)^{3.29} \tag{27}$$

from which, by substituting any desired value for t, the monthly rate of nitrogen retention in pregnancy at the particular time chosen may be

TABLE 26
THE NITROGEN-AGE RELATION OF BOVINE FETUSES, BY OBSERVATION AND BY CALCULATION FROM EQUATION (26)

Fetus No.	Age in mos.	Nitrogen content		Fetus No.	Age in mos.	Nitrogen content	
		Observed gms.	Calculated gms.			Observed gms.	Calculated gms.
718	3.0	3.4	2.8	644	5.4	71	82
2	3.2	4.2	4.2	1	5.4	91	82
26	3.2	6.6	4.2	11	5.6	84	99
21	3.5	4.6	7.2	19	6.2	231	167
27	4.1	12.3	18.2	Mo.	9.4	1373	1305

obtained. In Table 27, this has been done for successive months from 1 to 9, the results being divided by 30 to give the daily retentions of nitrogen in grams.

A tentative estimate of the protein requirements of pregnancy.—In this table an attempt has been made to adapt these values to different breeds of cattle. The values themselves are considered as applying to the Holstein breed, since the fetuses themselves were largely of Holstein breeding and since the value at full-term was taken from a Holstein fetus. The gestation periods of different breeds of cattle are the same, but their birth weights vary considerably. It seems reasonable to assume tentatively, therefore, that the rate of nitrogen retention at any age will vary among the different breeds of cattle in proportion to their birth weights. The values given in Table 27 for the Jersey, Ayrshire, Guernsey and Dairy Shorthorn have been computed on this basis from the average birth weights given by Eckles⁽⁵⁴⁾, i. e., 89 pounds for Holstein

calves, 55 pounds for Jersey calves, 71 pounds for Guernsey calves, 72 pounds for Ayrshire calves, and 73 pounds for Dairy Shorthorn calves.¹ Sex differences have not been considered here, since the percentage error in the estimate is probably greater than the percentage difference in growth rate between the sexes.

It is evident from these estimates that the nitrogen requirement of pregnancy is inconsiderable for the first four or five months, but that it rapidly increases from then on, until in the last two months it is equivalent to a deposition of 0.17 to 0.26 pound of protein daily for the Holstein cow.

TABLE 27

THE ESTIMATED DAILY RETENTION OF NITROGEN IN THE GROWTH OF THE BOVINE FETUS AND MEMBRANES¹

Age in mos.	Daily retention of nitrogen		
	Holstein breed gms.	Jersey breed gms.	Guernsey, Ayrshire and Dairy Shorthorn breeds gms.
2	0.02	0.01	0.02
3	0.20	0.12	0.16
4	0.75	0.47	0.61
5	1.9	1.2	1.6
6	4.0	2.5	3.3
7	7.3	4.5	5.9
8	12.2	7.5	9.9
9	19	12	16

¹ Exclusive of the nitrogen retained in the amniotic fluid. At full term, Eckles estimates (51) that the amniotic fluid contains 1.07 pounds of protein, or 77.7 gms. of nitrogen. In so far as this is of fetal urinary origin, it possesses no significance with reference to nitrogen requirements during intra-uterine growth.

In a calf weighing 75 pounds at birth, Popov (55) found 6 kgms. of protein. Assuming that this was all laid down in the last 100 days of gestation and that the digestible protein possessed a biological value of 70, Popov computes that the pregnant cow would require an average of 85 gms. of digestible protein daily for the formation of her fetus. This is equivalent to a daily requirement of 9.6 gms. of digestible nitrogen with a biological value of 100. This estimate is comparable with the values of Table 27 for the 7th, 8th and 9th months for the Guernsey, Ayrshire and Dairy Shorthorn breeds.

THE PROTEIN REQUIREMENT FOR MILK PRODUCTION

According to the general scheme of expressing the protein requirements of animals pursued in this paper, the protein requirement for milk pro-

¹The latter three breeds have been grouped together for an average birth weight of 72 pounds.

duction is measured by the protein content of the milk produced. This fact was first recognized by Haecker, who says in the bulletin describing his fundamental investigations in milk production (56):

During the time when the feeding experiments in milk-production in review were in progress, it occurred to the writer that in order to determine the actual net nutrients required to produce a given animal product, the composition of the product should be known, as well as the composition and the available nutrients in the food which is to be fed for its production, so that the nutrients in the ration might be provided in the proportions needed by the animal. Before a builder bids on a contract, he determines the quantity needed of each of the materials that are to appear in the structure. Without such specifications he would not know how much of each of the different materials would have to be provided.

The fat-to-protein ratio in milk.—It is well known that cows' milk is subject to wide variations in composition, depending upon the breed and individuality of the animal, the stage of lactation, the plane of nutrition, the character of the feed consumed, and possibly other factors. The fat content of milk appears to be the most readily affected by these factors, the sugar and ash content the least. The commercial importance of the fatty constituents of milk and the relative ease of their quantitative determination *in toto* are responsible for the fact that different milk samples are distinguished and graded in accordance with their content of fat. Therefore, the most profitable and practically significant method of studying the protein content of milk is to consider the fat-to-protein ratio, with the hope of finding some formula from which the protein content of milk may be satisfactorily predicted if the fat content is known.

The protein of milk appears to be more closely correlated with the fat content than any other constituent. Gaines(57) has determined the correlation existing between the fat content and the content of protein, sugar and ash from Haecker's(56) published analyses of mixed milk. The correlation coefficient for fat and protein was $+0.812 \pm 0.010$, for fat and sugar, $+0.263 \pm 0.027$, and for fat and ash, $+0.232 \pm 0.027$. Overman and Sanmann(58), working with a smaller set of data, report a correlation coefficient of $+0.729 \pm 0.017$ for fat and protein, and one of $+0.184 \pm 0.045$ for fat and lactose.

The prospect would seem favorable, therefore, for devising a prediction formula for protein from fat. In 1899, Timpe(59) derived the relation $p=2+0.35 f$ between the protein and fat contents of milk from analyses of milk from 21 cows of various breeds. Van Slyke(60) in 1908 proposed the relation $p=1.6+0.4 f$ from a large, but unspecified, number of analyses of herd milk and milk from individual cows of dif-

ferent breeds. Again, Andersen and Langmack⁽⁶¹⁾ in 1923 published the results of a study of 1080 analyses of the milk of Red Danish cows, from which they derived the equation $p=1.597+0.446f$. From his study of the 543 analyses of milk published by Haecker, Gaines⁽⁵⁷⁾ arrived at the equation $p=1.46+0.40f$.

These proposed formulæ are not satisfactorily concordant. It is particularly significant that Haecker's data on the composition of milk, upon which the Haecker, Savage, Eckles, and Armsby feeding standards for milk production are directly or indirectly based, indicate a much lower protein content for milk of any grade than any of the formulæ derived from other sets of analyses. This discrepancy is too large to be accounted for by the use of a different protein factor applied to the total nitrogen analyses. The factor used by Haecker is not reported in his publication.¹

Illinois investigations on the composition of cows' milk.—A splendid opportunity to throw further much needed light on the question of the relation of the protein to the fat content of cows' milk was afforded by the courtesy of Dr. O. R. Overman, of the Department of Dairy Husbandry of the University of Illinois in permitting the use for this purpose of about 2000 analyses (largely unpublished)² performed in his laboratory during the last six years upon samples of milk of known antecedents. In this extensive work, 3-day composite samples of milk were analyzed every 5 weeks from each of 133 cows in the University herds, through 1 to 3 complete lactation periods. Analyses were thus secured upon 1002 samples of milk from 67 Guernsey-Holstein cross-bred cows, 208 samples from 14 pure bred Ayrshire cows, 268 samples from 19 pure bred Holstein cows, 200 samples from 15 pure bred Jersey cows, and 321 samples from 18 pure bred Guernsey cows. The results with reference to fat and protein ($N \times 6.38$) have been averaged in groups covering a range in fat content of 0.5 per cent, with the results given in Table 28.

The correlation between the fat and protein percentages in these series of analyses is not as high as that noted above. Overman has obtained correlation coefficients of 0.635 for the Guernsey-Holstein analyses, 0.727 for the Holstein analyses, 0.679 for the Guernsey analyses, 0.588 for the Ayrshire analyses, and 0.500 for the Jersey analyses.

¹ Although samples of milk from 53 cows are represented in Haecker's analyses, it is significant that about 39 per cent of the samples (210 in number) were from 17 per cent (9 in number) of the cows, while 57 per cent (309 samples) were taken from 28 per cent (15 in number) of the cows. These relations were called to the attention of the author by Dr. O. R. Overman.

² The data will be published in full, with a statistical analysis, as a bulletin from the Illinois Agricultural Experiment Station.

TABLE 28
THE AVERAGE PROTEIN CONTENT OF MILK OF DIFFERENT GRADES AND FROM DIFFERENT BREEDS OF COWS, ACCORDING TO UNPUBLISHED ANALYSES OF O. R. OVERMAN

Range of fat pct. (inclusive)	Guernsey-Holstein			Holstein			Guernsey			Ayrshire			Jersey		
	Samples	Aver. fat pct.	Aver. protein pct.	Samples	Aver. fat pct.	Aver. protein pct.	Samples	Aver. fat pct.	Aver. protein pct.	Samples	Aver. fat pct.	Aver. protein pct.	Samples	Aver. fat pct.	Aver. protein pct.
2.26-2.75	1	2.72	3.27	6	2.69	2.90
2.76-3.25	28	3.09	3.10	81	3.05	3.12	7	3.09	3.24
3.26-3.75	142	3.56	3.45	106	3.48	3.35	2	3.68	4.06	40	3.55	3.32	5	3.58	3.80
3.76-4.25	283	4.00	3.61	46	3.93	3.58	22	4.04	3.45	74	4.01	3.53	18	4.02	3.52
4.26-4.75	292	4.48	3.85	20	4.45	4.06	71	4.55	3.70	64	4.46	3.69	40	4.53	3.63
4.76-5.25	156	4.98	4.10	3	5.16	4.59	98	5.02	3.95	14	4.94	3.84	47	5.01	3.78
5.26-5.75	74	5.44	4.38	5	5.46	5.17	59	5.48	4.14	9	5.41	4.08	44	5.45	3.96
5.76-6.25	17	5.93	4.71	1	6.00	4.88	40	5.95	4.38	31	4.96	4.26
6.26-6.75	8	6.45	4.59	21	6.44	4.57	9	6.51	3.97
6.76-7.25	5	6.89	4.94	2	6.79	4.23
7.26-7.75	1	7.50	5.54	3	7.56	4.76	2	7.46	4.07
7.76-8.25
8.26-8.75	2	8.36	4.92
Average	1002 ¹	4.24	3.65	268 ¹	3.55	3.42	321 ¹	5.03	3.89	208 ¹	4.03	3.51	200 ¹	5.05	3.79

¹ These figures are totals, not averages.

Concerning the homogeneity of these sets of data, it appears from the distributions given in Table 28 that no distinct discrepancies exist among the first four sets of data. The Jersey data, however, indicate a rather distinctly lower protein content for milk containing more than 3.50 per cent of fat. Since the greater discrepancies occur in those groups possessing the smaller numbers of samples, it appears that no serious error would be committed by pooling all of the analyses together. This has been done in Table 29, and the average percentages in each array have been corrected to the nearest even 0.5 per cent of fat on the assumption of an equal ratio of protein to fat. For example, in the array of analyses from 3.26 to 3.75 per cent of fat inclusive, the actual averages of the 295

TABLE 29

A COMPARISON OF HAECKER'S AND OVERMAN'S AVERAGES FOR THE PROTEIN CONTENTS OF MILK OF DIFFERENT FAT CONTENT AND OF PREDICTED PROTEIN PERCENTAGES DERIVED FROM THE EQUATION $P = 2.0 + 0.4 F$

Per cent fat	Per cent protein according to				Mathematical prediction
	Haecker		Overman		
	No. of analyses	Protein	No. of analyses	Protein	
2.50	..	2.55 ¹	7	2.74	3.00
3.00	47	2.68	116	3.06	3.20
3.50	55	2.81	295	3.37	3.40
4.00	57	3.08	443	3.58	3.60
4.50	116	3.27	486	3.81	3.80
5.00	103	3.45	318	4.00	4.00
5.50	89	3.65	191	4.25	4.20
6.00	39	3.82	89	4.45	4.40
6.50	24	4.02	38	4.46	4.60
7.00	13	4.22	7	4.84	4.80

¹ According to Haecker: "There were so few samples containing less than 2.5 per cent of fat, and so many more testing from 2.5 to 2.75, that no satisfactory average could be obtained for milk testing 2.5 per cent butter fat, so the averages were computed from the ratio of variation of milk testing from 3 to 3.5 per cent fat."

analyses are 4.53 per cent of fat and 3.40 per cent of protein. The per cent protein corresponding to 4.50 of fat is computed from the proportion

$$\frac{4.50}{4.53} = \frac{x}{3.40}$$

For comparison, Haecker's analyses¹ are also given in Table 29, and in the last column of the table will be found the results of predicting the protein content of milk from the fat content by the equation $p = 2.0 + 0.4 f$.

¹ It is a peculiar fact that, in the appendix of Haecker's bulletin containing presumably the original 543 analyses of milk, the average percentages of fat in each of the 9 classes are equal exactly to the mid-values of the class. If these were random selections of samples, such an ideal result would be statistically impossible with the small numbers of samples analyzed in each group. The obvious conclusion is that some selection of data has occurred, or that the analyses have been modified in some unrevealed way, in order to secure these ideal averages.

Overman's analyses for protein evidently are considerably higher than Haecker's for milk of all grades,¹ and for milk containing 3.50 per cent or more of fat, they are remarkably closely predictable by the simple linear equation above given. For lower grades of milk, the equation evidently over-estimates the protein content of milk from the fat content.

A method of estimating the protein requirements for milk production.—In view of the systematic method by which Overman's samples were taken, assuring an equal representation of milk from all stages of lactation, and in view of their number and the number of cows and of breeds represented, it appears justifiable to use the Overman data as the basis of estimates of the protein requirements of cows for milk production. Since thus far protein requirements have been expressed in terms of nitrogen, and since in practice a pound of milk is a convenient unit, the prediction equation above given has been changed to

$$N = 1.45 + 0.29 f \quad (28)$$

in which *N* is the nitrogen in grams per pound of milk, and *f* is the percentage of fat in the milk. This equation is applicable only to milks containing 3.50 or more per cent of fat. For milks of lower grade, the nitrogen content may be computed roughly from the Overman averages given in Table 29.

THE PROTEIN REQUIREMENT FOR MUSCULAR ACTIVITY

The great variation in the muscular activity of farm animals, depending mainly upon their temperament and upon the nature of their confinement, offers a serious obstacle to the estimation of their requirements for those nutrients necessarily consumed in muscular metabolism. It is a matter of considerable importance, therefore, to determine whether protein necessarily serves as a source of muscular work, either directly as the dietary amino acids coming to the muscles from the intestinal tract, or indirectly as the result of an increased catabolism of the muscle tissue itself.

Does the muscle cell wear out?—Since the days of Liebig, the relation of muscular contraction to protein metabolism has been shown to be of less and less importance. The classic experiments of Fick and Wislicenus, which have been abundantly confirmed, showed that muscular work could be performed at the expense of carbohydrates and fats, and the trend of modern investigation into the chemical and calorimetric

¹Perkins (Ohio Mo. Bull., 1: 304 (1916)) has also published a summary of a series of milk analyses (807), in which the protein to fat ratio is considerably higher than that of Haecker's analyses.

phases of muscular contraction⁽⁶²⁾ is toward the view that carbohydrate is the immediate source of muscular energy. The entire series of events occurring during and subsequent to a muscular contraction can be described, apparently, without reference to protein, amino acids, or the nitrogenous extractives, with the exception of creatine, which seems not to be destroyed in the process⁽⁶³⁾. Furthermore, the so-called fatigue products of muscles, in so far as available information indicates, are neither nitrogenous in character nor related to protein metabolism⁽⁶⁴⁾. It is true that investigations are on record indicating changes in the content of purines⁽⁶⁵⁾ and of creatine⁽⁶⁶⁾ in muscle as the result of activity, but the relation of these changes to the activity itself is obscure. The relation is probably indirect, and the function of the non-protein nitrogenous constituents of muscle relative to contraction is quite possibly regulatory only. Functioning in this way it is conceivable that they may not be consumable during muscular activity.

To many investigators it has seemed almost axiomatic that muscle tissue must undergo disintegration as the result of contractile activity. And when experimental investigation has failed to indicate any such disintegration, or at least any considerable disintegration, it has been considered necessary to assume that most of the nitrogen thus degraded escapes excretion in the urine by some process of reutilization in the tissues⁽⁶⁷⁾, a hypothesis apparently beyond the scope of experimental enquiry.

It seems that this view of the necessary wastage of muscle tissue during activity has resulted from the analogy so often drawn between the mechanical motor and the animal motor. In the early history of physiology this analogy has served an admirable purpose, and in pedagogy it is still extremely useful. But, like many other analogies, it is only partially true, and if pushed too far it will confuse rather than enlighten. It seems apparent that one phase of motor activity to which the analogy has an extremely doubtful application, if it applies at all, is the wearability of the motor. The mechanical motor undoubtedly wears out at a rate that bears a close relation to the amount or the intensity of the work performed. But this wearing out is due mainly to the friction of moving parts on bearings, that is, to a factor that has no *known* counterpart at least, in the animal body. Nevertheless, the opinion prevails in many quarters and is frequently expressed in print, that the animal motor also must wear out, and since the substance of the animal motor is largely protein, the conclusion has seemed reasonable, if not inevitable, that the protein catabolism must be increased as the result of muscular work. Rubner's term for the maintenance requirement of protein, the "wear-

and-tear quota," obviously is based upon this analogy of the mechanical motor and obviously implies that the catabolism of tissue protein will increase with increased motor activity. The term is an unfortunate one, particularly since it has attained a wide currency.

Reviews of the literature—a critical comparison.—Since as early as 1855, the influence of work on protein metabolism has been a favorite subject of inquiry and a considerable number of experiments have been reported in the literature. However, the results secured have been variable and at the present writing opinion is divided as to whether work increases the catabolism of tissue proteins or is without appreciable effect. From time to time these experiments have been reviewed, but it appears to be difficult to reconcile them with any definite conclusion. In 1909 they were reviewed by Tigerstedt⁽⁶⁸⁾, who was not convinced that there is an increase of nitrogen metabolism resulting from muscular work. On the other hand, they were reviewed by Magnus-Levy⁽⁶⁹⁾ at about the same time (1907) and the conclusion was reached that it is probable that such an effect occurs. In 1917 Lusk⁽⁷⁰⁾ concluded from a study of the same literature not only that "muscular work does not increase protein metabolism," but also that "the character of the protein metabolism is unchanged by muscular activity." However, in 1925 Cathcart⁽⁷¹⁾ again reviewed the literature, which, in conjunction with recent experiments of his own, induced him to state that, in spite of this very definite conclusion of Lusk, "the accumulated evidence seems to me to point in no unmistakable fashion to the opposite conclusion, that muscle activity does increase, if often only in small degree, the metabolism of protein," although "there is no possible ground for the view that protein is *the* source of muscular energy." But the former statement may be more nearly true than Cathcart himself suspects.

It would be far beyond the scope of this paper to inquire into the many reasons why critics weigh essentially the same evidence with such discordant results. It appears to be due in part to different criteria as to what constitutes competent experimental evidence. Thus, Atwater and Sherman⁽⁷²⁾ insist that the diet during the working period should be increased in its energy content to allow for the increased energy requirement; otherwise, an increase in the protein catabolism may mean simply that tissue protein is being destroyed as a source of muscular energy, rather than as the result of an inevitable wear on the muscular tissues. Cathcart, however, is not so discriminating, and does not disregard or even discount experiments in which no such assurance is given. Again, the results of some experiments are considered *in toto* by some, and only in part by others. Shaffer's⁽⁷³⁾ well-known experiment offers a case in point.

Considering the average urinary excretions of rest and work periods, Shaffer and Lusk both conclude that work has had no effect on the nitrogenous metabolism. Catheart, however, notes a marked increase in urinary nitrogen on the fourth work day, and, without reference to the rest periods at all, implies that this is a result of the work performed. Catheart's recent experiments⁽⁷⁴⁾, although very carefully controlled and undivided in their obvious interpretation, seem no more convincing than the much earlier experiments of Wait⁽⁷⁵⁾ at the University of Tennessee, which yielded entirely negative results. These experiments of Wait are not considered in Catheart's review.

A definition of the problem and its bearing on experimental methods.—The maze of conflicting experimental results relative to the effect of work on protein metabolism suggests that a precise definition of the real problem, followed by a consideration of the proper experimental conditions to impose, might be of value in deciding which of the recorded experiments are capable of the most exact interpretation. Since there is now no question of the availability of protein as a source of energy for muscular work, the real problem becomes that of determining whether or not there is an *inevitable* disintegration of muscle protein (or of other nitrogenous compounds) as a result of muscular activity.

This definition of the problem imposes certain necessary conditions upon any experimental method of attack. In the first place, there should be an adequate intake of energy during the working periods, otherwise an increase in urinary nitrogen may mean a destruction of tissue protein merely to serve the unnecessary rôle of a source of energy. If no evidence is obtained of an increase in tissue destruction as the result of work, this in itself would seem to be satisfactory evidence that the energy intake was adequate. On the contrary, if the experimental results indicate an increased destruction of tissue, the investigator himself must assume the burden of the proof that the energy intake was adequate, and hence that the increased tissue catabolism was an inevitable consequence of muscular activity. Thus, the significance of negative results in the investigation of this problem would seem to be much more easily established than the significance of positive results.

The only known method of investigating the extent of the destruction of tissue protein under any experimental conditions is to determine the nitrogen content of the urine on a nitrogen-free diet of adequate energy value, and even under these conditions the contribution of the non-protein nitrogenous constituents to the urinary nitrogen cannot be determined. It would seem to be impossible, therefore, to rule out the rôle of the latter constituents in the phenomena under investigation. How-

ever, it is equally clear that if protein is included in the experimental diet, at least in quantities above the absolute minimum required to replace endogenous losses of nitrogen, the significance of the urinary nitrogen is difficult if not impossible to establish definitely. Under such conditions the urine contains the products of an exogenous protein metabolism, the intensity of which for a given protein intake will vary with the intensity of the prevailing anabolic processes and with the extent to which the protein is utilized in anabolism. Hence, even though the protein intake is kept constant in rest and work periods, the output of urinary nitrogen might be affected if muscular activity alters either the intensity of the anabolic processes or the extent to which the dietary protein is utilized in anabolism. It is not inconceivable that an increase in muscular activity may do both, and hence a slight increase in the day's urinary nitrogen in work as compared with rest periods may bear no relation to the intensity of the minimum endogenous metabolism.

On the other hand, if the energy intake is adequate, an increased catabolism of muscle due to increased activity may be entirely obscured if the protein intake exceeds the requirements, since dietary amino acids may be diverted from catabolic to anabolic processes with no effect on the output of urinary nitrogen.

Returning now to past experiments on the effect of work on protein metabolism, as reviewed for example by Cathcart, the increases in the excretion of urinary nitrogen as a result of work, so frequently noted, are in most cases not accompanied by a demonstration that the intake of energy was adequate; in fact, in a considerable number of cases a suspicion that the contrary was true is almost inevitable. Furthermore, in the large majority of experiments, the protein intake was much above the minimum requirement, and in some cases was excessive. The precise interpretation of the positive results obtained with such diets seems impossible. They may have resulted from a depression of the anabolic processes involving dietary protein, or from a depression of its biological utilization. Although it is true that work tends to favor muscular hypertrophy, it seems more probable that this increase in anabolism is an after effect of work rather than a contemporary effect, since the immediate result of muscular contraction is undoubtedly catabolic. Even in the adult, some tissues are continually growing. If this growth is inhibited during muscular activity on a protein-containing dietary, the urinary nitrogen will be increased by a corresponding amount. The effect of muscular activity on the utilization of absorbed protein in metabolism is entirely unknown, but it is not inconceivable that a depression of utilization, with a corresponding increase in urinary nitrogen (or sulfur) may

result. It might even be considered a matter for surprise if the great acceleration in the catabolic processes of the body during muscular work did not conscript some of the dietary amino acids that otherwise would be used for the growth of hair or other tissues, or for the replenishment of the digestive glands, or for the replacement of the nitrogenous losses resulting from the minimum endogenous catabolisms. Entirely aside from such admittedly hypothetical considerations as these, it would seem a futile undertaking to detect the effect of muscular work upon the rate of tissue catabolism that is measured normally, in the human subject, by the excretion of 1.5 to 3 grams of nitrogen in the urine daily, by imposing upon this an exogenous protein catabolism yielding 5 or 10 times as much urinary nitrogen.

Such considerations as these lead inevitably to the conclusion that the characteristic effect of work on tissue catabolism should be investigated under conditions such that the exogenous catabolism of nitrogen is entirely eliminated or is reduced to an insignificant minimum. This can be accomplished by the feeding of a very low-nitrogen diet, or, if possible, a nitrogen-free diet. Furthermore, the experiment proper should be preceded by a feeding period of sufficient length to remove the "deposit protein" from the tissues, since this stored nitrogen will tend to vitiate the results of the experiment in the same way as would dietary nitrogen.

On protein-containing but creatine-free dietaries, the excretion of creatinine nitrogen is generally considered to be proportional to the total endogenous nitrogen. Hence the effect of work upon the creatinine excretion is taken to indicate the effect upon muscle endogenous catabolism. Although the reasoning upon which this conclusion is based is not fortified throughout by adequate experimental data, it may be considered justifiable if not entirely convincing.

The crucial experiments.—Of all the experiments that have been reviewed so thoroughly from time to time, there appear to be only two in which the diet was approximately nitrogen-free and at the same time was approximately adequate in energy value, and in which the period of nitrogen-free feeding was sufficiently extended so that the urinary nitrogen had approximated the endogenous level. These are the experiments of Thomas⁽⁷⁶⁾ reported in 1910, and those of Kocher⁽²⁷⁾ reported in 1914.

Thomas reduced his output of urinary nitrogen to approximately the endogenous level by subsisting on a diet of pure sugar. Following a fore-period of four days at this level, was a work period of three days during which work on an ergostat was performed amounting to 120,000 kilogram-meters. The experiment was concluded by an after-period of three days

on the same diet. The daily excretions of urinary nitrogen were as follows: fore-period, 3.05, 2.73, 3.22, and 2.85 gms.; work-period, 2.47, 2.90, and 2.97 gms.; and after-period, 2.71, 2.22, and 2.31 gms. Judged by a comparison of the average excretions only the work had no effect on the excretion of urinary nitrogen, the averages being, respectively, 2.96, 2.78, and 2.41 gms. If it is considered that in the latter period only was the endogenous level of tissue catabolism reached, Thomas concludes that the work might have effected a slight increase in muscle catabolism. However, if the endogenous catabolism were not attained until the last few days of the experiment, the increased excretion of nitrogen in the work period above this level would appear to be due more probably to the catabolism of "deposit protein" than to an effect of work. This experiment of Thomas, therefore, does not support the conclusion that muscular work increases the endogenous muscular catabolism. It is to be noted that definite proof of the adequacy of the diet in energy value during the work period, by a comparison of intake with outgo of energy, is not required when the urinary nitrogen shows no increase in this period.

Kocher's experiment involved two subjects and a study of the nitrogenous constituents in the urine. The diet used contained a minimal amount of nitrogen (1.01 gms. per day) derived entirely from cream and contained over 5000 cal. daily. In each case there was but one work day, during which the subject walked 60 kilometers (about 37.8 miles) in 10 hours. With both subjects the total urinary nitrogen and the creatinine nitrogen increased on the day of work, and the increased excretion continued for the next day or two. The effect is much more noticeable in the first subject than in the second, a fact possibly correlated with the much lower creatinine coefficient of the first subject. Unfortunately there is not sufficient assurance that the calorie intake of the work days was adequate. On the basis of average values for the energy requirements at rest and during horizontal walking, Kocher estimates that the energy intake of Subject R. A. K. was about 4.0 per cent in excess of the requirements of the day of work; with Subject J. G. F. the estimated excess was 4.6 per cent. However, the estimates of energy requirements might conceivably be in error by much more than this. Hence, the experiments do not demonstrate that an increase in endogenous catabolism is an inevitable consequence of muscular work; in other words, that there is an inevitable wear on the muscle machine.

In further experiments on the same subjects, using a high-protein high-fat diet, the urinary nitrogen showed no increase as a result of work when the calorie intake was high, but a distinct increase when the calorie intake was deliberately lowered so as to be obviously insufficient.

With the other subject, on a high-protein diet, even when the calorie intake was obviously insufficient, the urinary nitrogen did not increase as a result of work. Of more significance than the total urinary nitrogen on a high-protein diet, are the values for the creatinine excretion. These appeared to be unaffected by work, except for a slight increase on the day of work in one of the experiments in which the energy intake was deliberately made insufficient.

The experiments of Cathcart and Burnett⁽⁷⁴⁾ also indicate a slight effect of work upon the creatinine excretion. The diets used in the different series of experiments varied in nitrogen content, but were all estimated to contain 2900 cal. Their specific dynamic effect probably approximated 200 cal. In each series the diet was constant in the pre-work periods of 4 days, the work periods of 6 days, and the post-work periods of 4 days. The work was performed for one hour daily on a hand-lever ergometer and was equivalent to 25,000 kgm. meters (equivalent to 58.6 cal.). Depending upon whether the efficiency is taken at 20 or 25 per cent, the heat output due to this quantity of work may be taken as 295 or 236 cal. The subject weighed 79 kgms., and, with a surface area of 1.99 square meters, would have a basal requirement of 1920 cal. If the estimate of the energy intake is accurate, and the other activities of the working days did not exceed 500 cal., the energy intake may be considered as adequate. During the twelve months of experimental feeding the subject gained 1.6 kgms., but in seven of the eleven working periods, a slight loss in weight occurred. The adequacy of the energy intake for the entire experiment cannot be doubted, but it is unfortunately not equally clear that the diet was adequate during the working days. Although it is difficult to interpret the figures for total urinary nitrogen and sulfur with reference to an effect of work, for reasons fully explained above, the slight increases in creatinine nitrogen during the work periods were sufficiently consistent to indicate a direct or indirect effect of work upon the endogenous catabolism of muscle.

The experiments of Kocher and of Cathcart and Burnett¹ thus indicate an increased excretion of creatinine simultaneous with the performance of muscular work, but other investigators^(73, 77) have obtained no such increases. If the creatinine excretion is followed at intervals as short as two hours, the output during a short period of work has been found by Shulz⁽⁷⁸⁾ to be invariably increased, often to a large extent; however,

¹ A more recent experiment by R. C. Garry (*J. Physiol.*, 72: 364 (1926-27)) possesses much the same significance as that of Cathcart and Burnett for work on an ergometer, but no clear effect of static effort on the excretion of total nitrogen or of creatinine was reported.

in some later period of the day, generally the following period, the creatinine output is with equal regularity decreased, so that the total day's output of creatinine is not appreciably affected by the work performed. Hartmann⁽⁷⁹⁾ found much the same fluctuations in the uric acid and phosphoric acid content of 2-hour urine samples, and suggests that such variations are not due to variations in the production of these compounds, but in the excretory activity of the kidneys as a result of muscular work.¹ That the excretory activity of the kidney is affected by muscular work has been clearly shown by Wilson and associates, by Dobreff, and by Asher and Weber⁽⁸⁰⁾.

Impressed by the scarcity of experimental investigations planned to determine directly the effect of work on tissue catabolism, and by the discordance among the results obtained, Mitchell and Kruger⁽⁸¹⁾ have recently performed a number of experiments on rats in which the effect of work on the excretion of endogenous nitrogen in the urine was determined. The results of 19 experiments clearly indicate that muscular effort, either static or motive, may be performed with no appreciable increase in the excretion of total endogenous nitrogen or of creatinine in the urine, and hence presumably with no appreciable increase in the catabolism of muscle tissue. This was true whether the diet was predominantly carbohydrate in character, or whether it was predominantly fat and contained only traces of carbohydrate. These experiments on rats, as well as the experiment of Thomas on himself, point to the conclusion that an increased catabolism of muscle tissue is not an *inevitable* consequence of increased muscular activity.² It is equally clear, however, that if the diet does not provide sufficient non-nitrogenous nutrients to supply the working muscle with energy, the muscle tissue itself may be sacrificed for this purpose.

Whether an accelerated breakdown of muscle tissue results from any other contingency during contraction cannot definitely be decided from available information, although several possibilities suggest themselves. Intense muscular work, in which the rate of consumption of non-nitrogenous nutrients by the muscles greatly exceeds the rate of replenish-

¹ This explanation is supported by the experimental findings of Rakestraw (J. Biol. Chem., 47: 565 (1921)), as well as of Levine, Gordon and Derick (J. Amer. Med. Assoc., 82: 778 (1924)), who have noted consistent increases in the uric acid concentration of the blood during work, possibly due to diminished kidney function.

² In work experiments on dogs, Chambers and Milhorat (J. Biol. Chem., 77: 603 (1928)) have more recently shown that the urinary output of nitrogen in fasting was greatly increased by work, but that this increase could be entirely obviated by the administration of carbohydrates.

ment by the blood, may ultimately be shown to stimulate muscle breakdown. Possibly, also, if the general body temperature, or the temperature of the working muscles, increases above a certain point, the endogenous catabolic processes, whatever they may be, are speeded up, with a resulting increase in the output of endogenous metabolites in the urine. Experiments by Myers and Volovic and others⁽⁸²⁾ and by Linser and Schmid⁽⁸³⁾ show that increases in body temperature artificially induced in healthy subjects may increase the endogenous catabolism.

The interesting results of Lee and Tashiro⁽⁸⁴⁾ indicating an increased production of ammonia in excised muscles during contraction may have a bearing on the general problem of the effect of work on the endogenous catabolism. On the other hand, this production of ammonia may be due to the abnormal condition of the excised muscle, or to the lack of readily available sources of energy, or to anoxemia⁽⁸⁵⁾.

The protein requirement of work may be considered practically non-existent.—Until evidence to the contrary is forthcoming, it appears justifiable to assume, however, that ordinary work carried out under favorable conditions does not accelerate the breakdown of muscle substance, and that, even with working animals, the slight increase that might occur would be more than covered by the increased protein intake necessarily accompanying the increased intake of total food. Hence, the balancing of the rations of farm animals with respect to protein need not involve any consideration of the amount of muscular activity that the animal will undertake voluntarily, or that would be imposed upon it under the usual conditions.

ESTIMATING THE PROTEIN REQUIREMENTS OF CATTLE UNDER ANY SET OF CONDITIONS

The problem factored.—The problem of the nutrient requirements of animals is solved satisfactorily only when it is factored into its ultimate and independent terms. The amount of protein required to nourish a pregnant heifer in milk cannot be applied directly to another heifer differing either in size, stage of gestation, or rate of milk production, nor can a satisfactory correction be made for such differences unless the requirement for each independent function is known and some information is at hand relative to the manner of its change with size, time, or intensity of functioning. It has been the purpose of this paper to attempt such factoring of the protein requirements of cattle in so far as available experimental data will permit.

The use of the information thus obtained may be readily illustrated and an approximate method of putting the results in a form for practical

application to feeding problems may be devised, but the tentative nature of the data must be constantly kept in mind and the existence of certain interrelations between animal functions occurring simultaneously must be pointed out, even though nothing quantitative can be said about them at present. The need for further information on many points throughout this scheme of measuring the protein requirements of cattle suggests continually a number of profitable lines of reasearch.

Using the factors in any particular case.—An 800 pound Holstein heifer requires for maintenance an amount of nitrogen equivalent to 0.030 gm. per kilogram of weight per day (see page 11), or 1.36 gms. per 100 pounds, or a total of 10.88 gms. For growth it would require daily 4.11 gms. of nitrogen (Table 23), making a total requirement of 14.99 gms. of nitrogen, or 0.206 pound of protein ($N \times 6.25$). If the heifer is in calf and is at the end of her sixth month of gestation, her daily nitrogen requirement should be increased by 4.0 gms. daily (Table 27), and if she is in addition producing 15 pounds of 4 per cent milk,¹ a further considerable quota of 39.1 gms. of nitrogen must be allowed daily (Equation 28), thus raising her total nitrogen requirement to 58.1 gms. of nitrogen, or 0.80 pound of protein ($N \times 6.25$).

The composite nitrogen (or protein) requirements obtained in this way for any combination of conditions represent the amounts of nitrogen that are expended by the animal and used by the animal in the elaboration of new tissue or of milk. The amounts of digestible dietary nitrogen needed to cover these requirements must allow in addition for the wastage of dietary nitrogen in metabolism.

The biological value of a given source of nitrogen measures the minimum wastage of absorbed nitrogen in tissue syntheses. With information concerning the biological values of the digestible nitrogen of different feeds and rations, it would be possible to convert the nitrogen requirements of the animal, compounded in the fashion just illustrated, into requirements for digestible nitrogen or protein. Thus, a requirement of 0.8 pound of protein, would, for a source of nitrogen possessing a biological value of 50 at approximately the level of protein feeding appropriate for the specified conditions, call for 1.6 pounds of digestible protein. It should of course be realized that the biological value of a given source of nitrogen *may* vary, depending upon the purpose for which it is to be used in the animal body.

The biological value of feed protein for ruminants.—In a tentative way, can it be safely assumed that the biological value of ordinary sources of nitrogen in cattle rations is 50 or more? If so, the values for the nitrogen

¹ Admittedly an extreme, though not an impossible, illustration.

requirements of cattle derived above can be roughly used in estimating the requirements in terms of digestible protein. Laboratory animals have given biological values for feed proteins of 60 or more, provided the protein is not fed at a level higher than 10 per cent (^{86, 18}). Will ruminants give values of approximately the same order of magnitude?

Hart, Humphrey and Morrison (¹⁸) have reported nitrogen metabolism studies on four growing calves consuming rations containing approximately 10 per cent of protein, either entirely from the corn plant or largely from the alfalfa plant. The data obtained appear to be suitable for estimating roughly the biological values of the dietary nitrogen upon the basis of certain assumptions concerning the magnitude of the endog-

TABLE 30

ESTIMATED BIOLOGICAL VALUES OF THE NITROGEN OF ALFALFA AND CORN RATIONS FROM DATA OBTAINED ON GROWING CALVES BY HART, HUMPHREY AND MORRISON

Animal No.	Ration	Body wt. kgms.	Daily N intake gms.	Daily fecal N gms.	Daily urine N gms.	Daily metabolic ¹ N gms.	Daily endogenous N ² gms.	Biological value ³ pct.
1	Alfalfa	258	65.8	29.3	22.8	19.0	7.7	73
	Corn	235	59.9	27.3	24.5	16.0	7.1	64
2	Alfalfa	202	58.0	26.0	25.4	16.7	6.1	60
	Corn	230	66.4	33.2	26.5	18.0	6.9	62
3	Alfalfa	159	49.0	19.9	15.0	14.3	4.8	76
	Corn	179	60.8	24.5	21.6	18.0	5.4	70
4	Alfalfa	197	65.9	25.0	21.4	19.3	5.9	74
	Corn	179	59.0	21.3	16.3	17.5	5.4	80

¹ Assumed to equal 0.5 gm. per 100 gms. dry matter consumed. All rations were assumed to contain 90 pct. dry matter.

² Assumed to equal 0.030 gm. per kgm. body weight.

³ Biological value = $100 \times \frac{N \text{ intake} - (\text{Fecal N} - \text{met. N}) - (\text{Urine N} - \text{end. N})}{N \text{ intake} - (\text{Fecal N} - \text{met. N})}$

enous nitrogen in the urine and the metabolic nitrogen in the feces. The former is taken to be 0.030 gm. of nitrogen per kilogram of body weight, and the latter 0.5 gm. of nitrogen per 100 gms. of dry matter consumed (^{1, p. 28}). The calculations will be found in Table 30.

The biological values average 71 for the alfalfa rations and 69 for the corn rations. These values are in good agreement with what would be expected from laboratory animals, and may be taken to justify the tentative use of the value 50 in converting protein requirements in terms of animal expenditures and storages to protein requirements in terms of digestible protein. Probably this value includes a considerable margin of safety, particularly with reference to the use of dietary protein in milk production. Future experimental work, it is hoped, will serve to differentiate the different farm feeds and rations with respect to the biological values of their protein constituents.

Tentative estimates of the total requirements of calves for digestible protein of definite biological value, compared with Armsby's standards.—It will be of interest to compare estimates of the digestible protein requirements of calves of different weights and breeds, as thus obtained from the data developed in this report, with the digestible protein requirements of the Armsby feeding standard, which in turn were taken from Kellner's standard. Such a comparison is made in Table 31.

It should be emphasized that the estimates in Table 31 based upon the information developed in this report (see Table 23, and page 11) are

TABLE 31

THE REQUIREMENTS OF DIGESTIBLE PROTEIN BY GROWING CALVES ACCORDING TO THE DATA DEVELOPED IN THIS REPORT, AS COMPARED WITH THE ARMSBY STANDARDS

Body weight lbs.	Hereford-Shorthorn calves: Daily requirement of digestible protein		Dairy calves: Daily requirement of digestible protein			
	Armsby's standard ¹ lbs.	The data of this report ² lbs.	Armsby's standard ¹ lbs.	The data of this report ²		
				Holstein lbs.	Ayrshire lbs.	Jersey lbs.
200	0.90	0.67	0.60	0.51	0.66	0.63
300	1.07	0.65	0.72	0.48	0.51	0.49
400	1.20	0.62	0.80	0.46	0.44	0.43
500	1.32	0.60	0.83	0.44	0.40	0.39
600	1.40	0.59	0.85	0.43	0.37	0.36
700	1.40	0.57	0.85	0.42	0.36	0.35
800	1.37	0.56	0.85	0.41	0.35	0.34
900	1.30	0.55	...	0.41	0.36	0.34
1000	1.30	0.54	...	0.42	0.38	...
1100	...	0.54	...	0.43
1200	...	0.54	...	0.45

¹ In terms of true protein.

² On the assumption of a biological value of 50 for the dietary protein. Since the biological value is determined from the utilization of the total nitrogen of the ration, the estimated requirements obtained by the use of biological values refer to crude protein.

estimates of protein requirements for rates of growth equal to those exhibited by the Missouri Hereford-Shorthorn calves of Group II, and by Eckles' various groups of dairy heifer calves. If these calves did not grow at normal rates, then the estimates of protein requirements are correspondingly too low.

It will be noted that the estimates given are much lower than the Armsby standards, generally 50 per cent or more lower.¹ Can such low estimates of the protein requirements of calves be reconciled with the general trend of modern investigations, or must they be considered the

¹ The difference is really greater than appears from the figures themselves, since the Kellner-Armsby standards refer to digestible true protein, but the estimates from this report refer to digestible crude protein ($N \times 6.25$).

result of inaccuracies in the information upon which they are based, or of an incomplete summation of the independent factors involved?

In his discussion of the protein requirements of farm animals, Armsby himself (2, p. 412-414) has referred to a number of investigations, including some of his own, that appear to show that animals can support what seems a normal rate of growth upon a supply of protein little greater than the maintenance requirement plus the amount actually stored. In fact, in embodying the Kellner protein standards in his own feeding standards for animals, Armsby says:

On the whole, one can hardly fail of the impression that the requirements for protein as such in growth have been over-estimated and that the organism may utilize its protein supply more economically than the current feeding standards would indicate; in other words, that the actual protein supply may be made considerably smaller than has been supposed before it becomes a limiting factor in growth. Until this impression is confirmed by more extensive investigation, however, it appears the safer course to adhere provisionally to the accepted standards. . . .

A comparison of the tentative estimates in Table 31 with the results of the Armsby cooperative experiments.—On the initiative of Armsby, a series of cooperative investigations on the protein requirements for the growth of cattle was undertaken by eight agricultural experiment stations in this country under the auspices of the National Research Council (87). The plan of these investigations called for the testing of two rations, identical in respect to the source of protein, but differing in the content of this nutrient by reason of a variable inclusion of starch. The low-protein ration was to contain 20 to 35 per cent more digestible true protein than an estimate of the minimum requirements obtained by adding a maintenance requirement of 0.5 pound daily per 1000 pounds live weight and an estimate of the daily protein retention per 1000 pounds from Armsby's equation given above (p. 41, Equation 21). The high-protein ration was to conform to Haecker's standards, and contained from 50 to almost 100 per cent more protein than the low-protein ration. The calves were to be fed in pairs, selected for similarity of age, sex, weight, and breeding, both to receive the same intake of net energy, one from the low-protein ration and one from the high-protein ration. The growth of the calves was to be followed by nitrogen balance studies and by determinations of the increase in body weight and body measurements.

For one reason or another, it was found impossible to conform to the plan as outlined, mainly because the digestible nutrient and net energy contents of the rations fed were found to be considerably lower than were expected from average analyses and digestibilities, and because the high-protein rations were generally more acceptable to the calves and were

more readily consumed than the low-protein rations. The results of the experiments were not considered to be sufficiently significant and concordant to warrant positive statements concerning either the adequacy of the low-protein ration for a normal growth rate, or the superiority of the high-protein over the low-protein ration in growth-promoting value.

In the metabolism experiments that were undertaken upon a minority of the calves, the high-protein calves in general seemed to be storing nitrogen at a considerably greater rate than the low-protein calves, though the significance of the nitrogen balances obtained is seriously questioned by the investigators themselves, either because the collection periods or the periods of preliminary feeding were too short, or because the balances were quite inconsistent with the live weight increases.

On the basis of gains in live weight, differences between high-protein and low-protein calves were not consistent, and in a large proportion of comparisons the low-protein calf gained faster than its mate on the high-protein ration.

However, significant comparisons cannot readily be made between high-protein and low-protein calves, since, either intentionally or inadvertently, the net energy intakes of the paired calves were not equalized, the result being that differences in growth cannot be interpreted with reference to differences in protein intake only. It was very clearly shown in these investigations that the planning of experimental rations and the control of the consumption of food by experimental animals cannot safely be based upon the use of average analyses of feeds and average digestion coefficients.

The performance of the low-protein calves is of particular interest to this discussion, since their intake of crude protein was of the same order of magnitude in most cases as the estimates of this report summarized in Table 31. The data on these calves pertinent to the question at issue are given in Table 32.¹ The computed normal growth of the calves was obtained from the growth equations given above on the basis of the initial weight of the calf, rather than the initial age. Values for true protein have been converted into values for crude protein on the basis of the findings in the digestion trials relative to the ratio between digestible true protein and digestible crude protein.

The markedly subnormal growth of the first four Massachusetts calves, receiving slightly less than the estimated requirements of protein is not surprising in view of the large underestimation of the energy intake.

¹Two of the low-protein calves in the North Dakota experiments are not included in this table, since their intake of digestible crude protein was considerably greater than the requirements as estimated in this report.

The high-protein calves in this experiment also gained at a subnormal rate. The subnormal growth of the two Virginia steer calves, of two of the Pennsylvania calves, and of the one Maryland calf is readily explainable on the basis of the low intake of protein, markedly lower than the estimated requirements. The following seven calves made approximately normal or supernormal gains in weight on amounts of protein but little above the estimated requirements, in some cases in spite of an energy intake much less than the intended: Massachusetts Nos. 18, 20, and 22,¹ Virginia Nos. 3 and 4, Pennsylvania No. 974, and North Dakota No. 3A. It is true that Massachusetts calf No. 24 and Pennsylvania calf No. 1032 made subnormal gains on amounts of protein almost exactly equal to the estimated requirements, but on the other hand, the Ohio calf grew at a rate much faster than normal on an intake of digestible crude protein that must have been considerably less than the estimated requirements. No calf in the entire series of studies receiving more protein than the estimated requirements grew at a subnormal rate.

On an average of 18 digestion trials, the actual intake of digestible crude protein by the low protein calves was found to be only 68 per cent of that expected from average analyses and average coefficients of digestibility, a fact readily explainable by the inclusion of large amounts of starch in their rations.² The starch thus added would increase the excretion of metabolic nitrogen in the feces without increasing the nitrogen intake. Hence, the estimated intakes of digestible crude protein in the Ohio, North Dakota, South Dakota, Maryland, and Nebraska experiments are probably much greater than the actual.

From this study it seems fair to conclude that calves can grow at a rate as great as that expected from available data on normal growth with intakes of digestible crude protein but little if any greater than the estimates developed in this report. In fact, with more liberal energy intakes, it may be possible that such growth could be attained upon even smaller intakes of protein than these.

A comparison of the tentative estimates of protein requirements in Table 31 with the Missouri data on dairy calves.—Another extensive investigation of the protein requirements of growing calves was conducted at the Missouri Agricultural Experiment Station from 1913 to 1921 by Swett, Eckles, and Ragsdale⁽⁸⁸⁾. A total of 34 Holstein and Jersey

¹ In the second Massachusetts experiment no consistent difference was noted between the gains of the high-protein calves and those of the low-protein calves.

² The low-protein ration in the North Dakota experiments was not composited according to the plan of these studies and contained no starch. Hence, the estimated crude protein intake is not subject to this considerable error.

TABLE 32

RECORDS OF CALVES ON LOW-PROTEIN RATIONS FROM THE COOPERATIVE EXPERIMENTS SPONSORED BY THE NATIONAL RESEARCH COUNCIL

Station	Calf No.	Breed	Sex	Initial weight lbs.	Feeding period days	Average daily gain		Daily intake of digestible crude protein			Net energy consumed in per cent of estimated requirements pct.
						Observed lbs.	Computed from growth equation of breed lbs.	Estimated from ration lbs.	Observed in digestion trial lbs.	Min. requirement to this report lbs.	
Massachusetts	270	Ayrshire	Bull	202	180	0.76	1.17	0.41	0.33	0.56	66
	4	Holstein	Heifer	286	180	0.97	1.24	0.52	0.43	0.46	66
	6	Holstein	Steer	264	180	0.90	1.27	0.49	0.40	0.47	66
	8	Holstein	Steer	267	180	0.98	1.27	0.50	0.41	0.47	66
	18	Holstein	Heifer	302	180	1.21	1.22	0.74	0.55	0.46	69
	20	Holstein	Steer	288	180	1.26	1.24	0.74	0.55	0.46	69
	22	Holstein	Steer	292	180	1.15	1.24	0.68	0.49	0.46	69
	24	Holstein	Heifer	300	180	0.94	1.23	0.60	0.45	0.46	69
Ohio	Shorthorn	328	77	1.83	1.24 ¹	0.65	...	0.62	..
Virginia	Holstein	Steer	230	165	0.30	1.33	0.42	0.27	0.50	88
	74	Holstein	Steer	240	165	0.33	1.32	0.44	0.29	0.50	88
	3	Holstein	Bull	487	78	1.10	1.05	1.05	0.57	0.44	79
	4	Holstein	Bull	470	78	1.41	1.08	1.04	0.56	0.44	79
Pennsylvania	Holstein	Heifer	526	133	1.08	0.95	...	0.57	0.48	..
	982	Holstein	Bull	470	132	1.62	1.04	...	0.86	0.43	..
	1013	Holstein	Heifer	257	243	0.37	1.23	0.17	...	0.50	..
	1017	Jersey	Bull	203	243	0.24	1.05	0.15	...	0.54	..
	1032	Holstein	Heifer	461	150	0.84	1.03	0.68	0.43	0.44	83
North Dakota	...	Shorthorn	Steer	648	64	1.28	0.92 ¹	0.62	...	0.57	..
	3A	Shorthorn	Steer	703	64	0.97	0.86 ¹	0.75	...	0.57	..
	5C	Shorthorn	Bull	339	408	1.12	1.00	0.79	...	0.43	..
South Dakota	...	Holstein	Bull	468	408	1.29	0.85	1.02	...	0.42	..
	309	Holstein	Bull	468	408	1.29	0.85	1.02	...	0.42	..
Maryland	Guernsey-Jersey	Bull	153	138	0.69	1.23 ²	0.38	...	0.63	..
Nebraska	Jersey	Heifer	550	266	0.76	0.54	1.01	...	0.36	..
	2	Jersey	Heifer	536	266	1.01	0.56	0.90	...	0.35	..
	4	Jersey	Heifer	429	407	1.05	0.63	0.75	...	0.36	..
	6	Jersey	Heifer	429	407	1.05	0.63	0.75	...	0.36	..
	256	Holstein	Heifer	532	252	1.25	0.87	0.95	...	0.42	..

¹ Computed from the growth equation of the Hereford-Shorthorn steers of Group II in the Missouri investigation.

² Computed from the growth equation of Jersey heifers.

heifers was under experimental feeding for periods ranging from 90 to 840 days. Different levels of protein feeding were tested on these animals in periods ranging in length from 30 to 750 days. The level of protein intake was varied by varying the proportions of timothy and alfalfa hay, and of corn, oats, bran, cottonseed meal, dried skim milk, starch and sugar in the rations. The lower protein rations thus contained more timothy hay, corn, oats, starch, and sugar than did the higher protein rations. The number of different protein levels tested on each animal as well as the order in which they were tested was irregular for most of the animals. The feeds were analyzed during the course of the experiments, but average digestion coefficients were used in computing the intakes of digestible crude protein. The energy intakes were computed by the use of Armsby's tables. The measurements of growth included body weights and body dimensions.

The results are summarized according to a number of different plans, and upon these summaries the final conclusions are based. It was found that dairy heifers could make normal growth, in accordance with Eckles' standards, upon amounts of protein far smaller (56 to 77 per cent for Holstein and Jerseys, respectively) than those called for by the Armsby standards; in fact, with an increase in energy intake, normal growth was attained on still smaller intakes of protein. However, an increase in protein intake above that requisite for normal growth increased the rate above the expected.

It is also concluded that the Holstein heifers were able to make distinctly better growth with reference to their normal, on lower levels of protein, than were the Jersey heifers. However, this unexplainable distinction between the breeds is not clearly shown in all of the summary tables (Nos. 42, 43 and 44).

The lowest *estimated* levels of protein intake in these Missouri experiments were equal to or lower than the intended low protein intakes in the Armsby cooperative experiments, and on the average were about 40 per cent higher than the estimated requirements as given in Table 31 of this report. However, the *actual* intake of digestible crude protein in the low-protein periods of the Missouri studies probably approximated closely in the average to the estimated requirements in Table 31 in the same manner and for the same reasons as obtained in the Armsby cooperative experiments. The use of average digestion coefficients for protein with rations containing considerable amounts of starch and sugar will always overestimate the content of digestible crude protein, and for large amounts of these carbohydrate materials, such as must have been used in the low-protein Missouri rations, the overestimation might well amount to 40 per cent or more.

Since normal growth was never obtained on these low intakes of protein, the Missouri data afford no support for the estimates of the protein requirements of growing calves developed in this paper. It may be permissible, however, to go behind the summary tables and to examine the original data and the observations upon individual calves so lavishly recorded. A great number of disturbing factors immediately become evident. Besides the probable progressively increasing overestimation of the digestible protein intakes from the higher to the lower protein levels, and the changes in the source of protein, quite possibly further handicapping the low-protein rations, there are indications, mentioned by the authors themselves, that the low-protein rations were inadequate in vitamins or minerals. These indications are based not only upon a study of the rations themselves, but upon the pathological symptoms (stiffness, irregular appetite, ill-defined sickness, blindness) not infrequently observed in the calves while subsisting upon them. These periods of sickness and refusal of feed apparently have not been eliminated from the experimental data relating to the low-protein periods.

The change from one ration to another was made abruptly and the data obtained during the periods of adjustment to the new ration are included in the summaries. The change from a prolonged régime on an inadequate diet that has induced a stunted and unthrifty condition, to an adequate ration, will tend to occasion a rapid resumption of growth that, for a time at least, will exaggerate the nutritive value of the second ration. It would appear that such transitional periods should not be considered in the comparative evaluation of experimental rations.

Most of the calves in the Missouri experiments were bred during the period of experimental observation and calved shortly after the termination of the experiment. The disturbances in body weight thus produced must have been considerable and their effects in all cases will complicate the interpretation of the weight data secured for the last few periods.

These irregularities and disturbances in the Missouri experiments may be fairly considered as detracting from the significance of the summarized data upon which the conclusions are based. A number of them will operate definitely in detracting from the apparent value of the low-protein rations, and all of them will interfere with effective comparisons between rations. The conclusions apparently based so securely upon the various summaries of data, must be discounted accordingly, so that the disagreement between these summaries and the estimates of protein requirements given in Table 31 may be more apparent than real.

Uncertainties in the use of factored requirements.—In conclusion, it should be pointed out that there are uncertainties in the method of

compositing protein requirements from the factors studied separately in this report. For example, the occurrence of pregnancy in a growing heifer may retard growth somewhat, while lactation is known to do so. Hence, the protein requirement of a pregnant lactating heifer would presumably be somewhat less than that indicated by the sum of the separate requirements. On the other hand, the protein requirements for pregnancy considered above have not included any estimates for the hypertrophy of the uterus, no basis for such estimates being available. This error, therefore, would tend to compensate for any depression of growth caused by pregnancy. The occasional occurrence of twin births in cattle⁽⁸⁹⁾ is another special problem not touched upon by this report.

The effect of pregnancy upon the nitrogen metabolism of the mother has been the subject of a number of researches, and the results obtained have lent support to two theories. One theory pictures pregnancy as a distinct sacrifice of the mother to the perpetuation of the species. This theory is based upon a number of studies on dogs and rabbits, which have been ably reviewed by Murlin⁽⁸⁹⁾, whose own data upon dogs are taken to support the theory. According to this theory, the total nitrogen stored by the maternal organism during pregnancy is not sufficient to form the fetus and its membranes; thus, the maternal tissues themselves are sacrificed. As a result, during the first half of the period of pregnancy a negative nitrogen balance persists, especially during the third and fourth week in the dog; not until the last half of pregnancy is a positive balance attained. It may be said, however, that not all of the observations upon dogs agree in indicating a negative balance as a characteristic of the first half of gestation, and that it is difficult to believe that the mere transference of protein from the maternal to the embryonic organism by whatever means before the complete establishment of the placenta should occasion such marked and persistent losses of nitrogen in the urine, particularly in view of the infinitesimal size of the embryo at this time, and of the absence of other signs of a marked disturbance of the maternal metabolism, particularly the basal metabolism.

In direct opposition to the view that pregnancy is destructive of the maternal organism, is the view developed from a number of somewhat fragmentary observations on the nitrogen metabolism of human pregnancy, according to which the nitrogen stored in pregnancy is in great excess of the needs of the fetus for its own growth and for the growth of its protective and nourishing membranes. A nitrogen reserve is thus built up to carry the mother over the puerperium and the initial period of lactation, removing the necessity for a sacrifice of the maternal tissues. This view has been discussed and subscribed to by Wilson⁽⁹⁰⁾ and has received support from his experimental observations.

In attempting to reconcile these two views, Harding in his recent article on metabolism in pregnancy⁽⁹¹⁾ believes that the former applies to the lower animals and the latter only to the human organisms. However, it appears that much more investigational work should be done before this compromise position can be considered with complacence.

Apparently the only evidence of this nature bearing on bovine pregnancy has been contributed by Crowther and Woodman⁽⁹²⁾, who determined the nitrogen balance of a pregnant cow (D) and of a dry cow not in calf (C) over long periods of time. The data bearing on the question at issue may be briefly referred to. Cow D was bred on September 6, after which the collection of excreta was unfortunately suspended until November 8. On resumption of the metabolism experiment, the cow was found to be in slight negative nitrogen balance which persisted for three weeks, at which time a positive balance was established and maintained until parturition. Before being bred, Cow D had shown throughout a uniformly higher positive nitrogen balance than Cow C, but when the experiment was thus resumed, the reverse relation was observed. The authors, therefore, conclude that the cow, like the dog and the rabbit, suffers a disturbance in its nitrogen metabolism in the first stage of pregnancy, and this conclusion is accepted by Harding in the review of the subject previously cited.

However, another conclusion from this extensive study is that "even after nitrogen equilibrium is established and a relatively constant nitrogen consumption is maintained, there may arise from time to time considerable deviations from equilibrium either in the positive or negative direction. It would appear therefore that for reliable work of this character long experimental periods are essential." Such an apparently fortuitous disturbance in the nitrogen equilibrium of Cow C occurred about December 1 and a considerable negative nitrogen balance persisted for five weeks on an intake previously adequate for nitrogen storage. The point may therefore be raised that the similar disturbance of Cow D, to which such great import is attached, may be of this fortuitous nature and thus may bear no relation to conception or pregnancy.

The investigations thus briefly reviewed evidently have an important bearing upon the problem of the protein requirement for pregnancy in cattle, since if the condition of pregnancy disturbs the nitrogen economy of the maternal organism, either by inducing excessive tissue catabolism or a storage of nitrogen greatly in excess of the needs of the fetus, the rate of nitrogen retention by the fetus and its membranes would not represent the total nitrogen requirements of pregnancy, but would be something less than these. The subject is of great physiological interest as well as of practical importance.

Another opportunity for future research relates to the nitrogen metabolism of the cow after parturition throughout her puerperium. Crowther and Woodman have shown that their one subject was in a condition of negative nitrogen balance for several weeks after parturition. Might not the extent of this excess nitrogen catabolism measure the involution of the uterus⁽⁹³⁾ and hence the nitrogen needed for the hypertrophy of this organ during pregnancy? Obviously the initiation of lactation during this period will complicate the study of this important question.

*Is the optimum requirement of protein different from the minimum?—*The significance of "deposit protein" in animal nutrition also has a bearing on the method of computing protein requirements that has been advocated and exemplified in this report. When the nitrogen intake of an animal is suddenly raised, a temporary increase in the nitrogen balance may occur extending over one, two or three days; if it be suddenly decreased, the reverse temporary disturbance in nitrogen balance occurs. The phenomenon is explained by assuming that there is a variable and labile form of protein in the body, different from that of the organized tissues, and probably retained in the cellular fluids. This circulating reservoir of protein rises and falls with the level of protein feeding. However, this explanation is not based upon the results of direct experimental inquiry.

It is interesting to speculate whether the amount of deposit protein in the tissue bears any relation to the well-being of the animal or to its physiological efficiency. If it does, then an animal should thrive better when nourished on amounts of protein greater than its requirements, as judged by a thorough study of all possible ways in which protein is utilized. This conception implies that there is an optimum intake of protein greater than the minimum, which in turn implies that the mere presence of an excess of protein (or amino acids) in the body fluids exerts a favorable physiological effect. The apparent existence of an optimum requirement cannot be postulated until it has been shown that all of the requirements for protein have been included in the minimum requirement with which the assumed optimum has been compared.

The theory of optimum nutrition has been applied particularly to the dairy cow. Although early investigations in this country and in Europe may be cited in support of the view that an excess of protein above the requirements of maintenance and the production of milk stimulates milk secretion, even up to very high levels of protein intake, and although current practice in feeding cows on test is based upon the correctness of this assumption, more recent investigations have not afforded any considerable support for it. Besides establishing the fact that the protein

requirements for milk production are much lower than the current feeding standards would indicate, the experiments of Haecker⁽⁵⁶⁾, Hills and associates⁽⁹⁴⁾, Ellett, Holdaway, and Harris⁽⁹⁵⁾, Fries, Braman, and Kriss⁽⁹⁶⁾, Perkins⁽⁴⁾, and particularly Buschmann⁽⁵⁾ indicate that little if any increase in milk production may result from increasing the protein intake above the minimum requirements. Neven's⁽⁹⁷⁾ self-feeding experiments on dairy cows also offer no support—in fact quite the contrary—for the belief that nitrogenous concentrates are stimulants to the mammary glands.¹ It is probably still premature to deduce any final conclusions on this point, since there is great need for a careful definition of "minimum" and "optimum" and for crucial, carefully planned and carefully controlled investigations concerned specifically with this question. It is equally true, however, that the theory of optimum protein nutrition, exceeding the minimum, is difficult to explain on physiological grounds, and has suffered rather than benefited by the later experimental work in milk production.

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