OCTOBER, 1936 RESEARCH BULLETIN 246

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION

F. B. MUMFORD, *Director*

The Utilization of Energy at Different Levels of Protein Intake

S. R. JOHNSON, A. G. HOGAN, AND U. S. ASHWORTH

(Publication Authorized September 24, 1936)

COLUMBIA, MISSOURI

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

Agricultural Experiment Station

STATION STAFF, OCTOBER, 1936

FREDERICK A. MIDDLEBUSH, Ph.D., President

F. B. MUMFORD, M.S., D. Agr., Director — S. B. SHIRKY, A.M., Asst. to Director

MISS ELLA PAHMEIER, Secretary

AGRICULTURAL CHEMISTRY
A. G. HOGAN, Ph.D.
L. D. HAIGH, Ph.D.
E. W. COWAN, A.M.
LUTHER R. RICHARDSON, Ph.D.
S. R. JOHNSON, Ph.D.
VIRGIL HERRING, B.S.

AGRICULTURAL ECONOMICS
O. R. JOHNSON, A.M.
BEN H. FRAME, A.M.
†C. H. HAMMAR, Ph.D.
HERMAN HAAG, Ph.D.
ELLSWORTH SPRINGER, B.S.
DARRYL FRANCIS, B.S.

AGRICULTURAL ENGINEERING
J. C. WOOLEY, M.S.
MACK M. JONES, M.S.
LLOYD HIGHTOWER, B.S.
PAUL DOLL, B.S.

ANIMAL HUSBANDRY
E. A. TROWBRIDGE, B.S. in Agr.
L. A. WEAVER, B.S. in Agr.
A. G. HOGAN, Ph.D.
F. B. MUMFORD, M.S., D. Agr.
F. F. McKENZIE, Ph.D.*
J. E. COMFORT, A.M.*
H. C. MOFFETT, A.M.
VICTOR BERLINER, M.S.
C. S. WILLIAMS, B.S.
VIRGENE WARBRITTON, Ph.D.

BOTANY AND PATHOLOGY
W. J. ROBBINS, Ph.D.
C. M. TUCKER, Ph.D.
C. G. SCHMITT, A.B.

DAIRY HUSBANDRY
A. C. RAGSDALE, M.S.
WM. H. E. REID, A.M.
SAMUEL BRODY, Ph.D.
C. W. TURNER, Ph.D.
H. A. HERMAN, A.M.
E. R. GARRISON, A.M.
WARREN C. HALL, A.M.
E. P. REINEKE, B.S.
E. T. GOMEZ, A.M.
C. W. McINTYRE, M.S.
LLOYD E. WASHBURN, M.S.
RALPH P. REECE, M.S.
R. C. CUNNINGHAM, B.S. in E.E.
W. R. GRAHAM, Ph.D.

ENTOMOLOGY
LEONARD HASEMAN, Ph.D.
T. E. BIRKETT, A.M.
H. L. KOCH, B.S.
LEE JENKINS, B.S.
C. H. BALDWIN, B.S.

*In cooperative service with the U. S. Department of Agriculture.

FIELD CROPS
W. C. ETHERIDGE, Ph.D.
C. A. HELM, A.M.*
L. J. STADLER, Ph.D.*
B. M. KING, A.M.*
E. MARION BROWN, A.M.*
G. F. SPRAGUE, Ph.D.*
J. M. POEHLMAN, A.M.*
MISS CLARA FUHR, M.S.*

HOME ECONOMICS
MABEL CAMPBELL, A.M.
JESSIE ALICE CLINE, A.M.
ADELLA EPPLE GINTER, A.M.
BERTHA BISBEY, Ph.D.
JESSIE V. COLES, Ph.D.
EMMA G. HOLMES, B.S.
LOUISE WOODRUFF, B.S.
INEZ EKBLAD, B.S.
ADELIA WEIS, A.M.
ELIZABETH DYER, A.M.

HORTICULTURE
T. J. TALBERT, A.M.
A. E. MURNEEK, Ph.D.
H. G. SWARTWOUT, A.M.
GEO. CARL VINSON, Ph.D.
H. F. MAJOR, B.S.
R. A. SCHROEDER, B.S. in Agr.
GEORGE E. SMITH, B.S. in Agr.
AUBREY D. HIBBARD, M.A.

POULTRY HUSBANDRY
H. L. KEMPSTER, M.S.
E. M. FUNK, A.M.

RURAL SOCIOLOGY
E. L. MORGAN, Ph.D.
MELVIN W. SNEED, B.S. in B.A.
P. V. BENNER, B.S.
A. W. NEBEL, A.M.

SOILS
M. F. MILLER, M.S.A.
H. H. KRUSEKOPF, A.M.
W. A. ALBRECHT, Ph.D.
HANS JENNY, Ph.D.
L. D. BAVER, Ph.D.

VETERINARY SCIENCE
A. J. DURANT, A.M., D.V.M.
J. W. CONNAWAY, D.V.M., M.D.
CECIL ELDER, A.M., D.V.M.
O. S. CRISLER, D.V.M.
ANDREW UREN, A.M., D.V.M.
HAROLD C. McDOUGLE, A.M.
P. L. PIERCY, D.V.M.

OTHER OFFICERS
R. B. PRICE, B.L., Treasurer
LESLIE COWAN, B.S., Sec'y of University
A. A. JEFFREY, A.B., Agricultural Editor
L. R. GRINSTEAD, B.J., Ass't. Agr. Editor
J. F. BARHAM, Photographer
LEON WAUGHTAL, Assistant Photographer
JANE FRODSHAM, Librarian

†On leave of absence.

TABLE OF CONTENTS

ABSTRACT

Growing rats were pair-fed diets that contained different amounts of protein, one adequate and one inadequate for normal growth. The utilization of energy by these animals has been studied by examination of: The food consumed, the excreta, the heat production, and the body gains.

Growth was more rapid on the reasonably high protein diets than on like diets inadequate in protein content. These differences were not due to differences in energy lost in the excreta, in the total heat lost, or in the energy gains, but were due to differences in the kind of nutrients stored. The animals on the high protein diets stored more water, protein, and ash than their pair-mates, and thus stored less energy per unit gain, while those on the low protein diets stored more fat than their pair-mates, and thus stored more energy per unit gain. The net utilization of energy for body gain by all animals was the same.

The paired feeding technique has been discussed in the light of these findings.

The Utilization of Energy at Different Levels of Protein Intake

S. R. JOHNSON*, A. G. HOGAN, AND U. S. ASHWORTH

HISTORICAL

Hogan and Pilcher (1933) observed that rats with a liberal intake of protein may grow more rapidly than those on an inadequate level, even though they consume the same amount of energy. One might suppose that the more adequate ration will always support the more rapid rate of growth, but Hogan and Pilcher obtained evidence that this was not the case. Rats that received an inadequate supply of the vitamin B complex grew at the same rate as those that received a more generous allowance. According to their point of view the second comparison indicated that the utilization of energy had not been affected by differences in adequacy of the vitamin B supply. The interpretation of the first comparison, however, was much more equivocal. The difference in growth rate may have been due to an unlike utilization of total energy; on the other hand all animals may have retained the same amounts of energy, but in different forms. One gram of fat has approximately the same energy value as 2 grams of protein. In addition these 2 grams of protein would be accompanied by about 6 grams of water. It would be theoretically possible then for one animal to gain 8 times as much as another, in weight, although their gains in energy were the same. In order to decide betwen these two alternative explanations it is necessary to make a painstakingly complete determination of the distribution of the food energy. Such a determination would show whether there are differences in the utilization of energy, and that was the objective of the investigation to be described.

In addition to its theoretical interest this problem is of considerable practical importance, notably in the method of conducting certain types of nutrition studies. The majority of investigators commonly provide their experimental animals with all the food they will consume, and this is designated as the *ad libitum* method of feeding. Others restrict the animals to be compared to the same energy intake. The advantages of this method have been presented by Mitchell (1927, 1930) who designates it as the paired-feeding method. More recently Forbes and others have made effective use of this same procedure.

*Submitted by S. R. Johnson in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of the University of Missouri, 1935.

Paper 124 in the Herman Frasch Foundation Series

No one doubts that this method is essential for the solution of some problems, but its general applicability has not been conceded in all quarters. It is desirable, therefore, to determine what are the limitations, if any, of the paired-feeding method.

The Paired-Feeding Technique in Nutrition Studies

The paired-feeding method was first introduced by Armsby in 1921 in studies of the protein requirement of growing calves. A schedule of protein and energy intake per 1000 pounds live weight was drawn up, so that the only variable was the amount of protein consumed. The animals were divided in pairs, one on a low and the other on an optimum protein intake. Balance trials showed that the animals on the low protein rations retained less nitrogen than the others and presumably for this reason they grew at a slower rate. The growth records, however, were not sufficiently uniform to support any definite conclusion, probably because the cooperators failed to adhere to the schedule.

The first published paired-feeding experiment was performed by Gulick (1922, 1924). The purpose was to study the resting energy metabolism of rats suffering from a vitamin B deficiency, as compared with normal animals. A small quantity of yeast was supplied daily to one rat of a pair, and the food consumption of this animal was restricted to the amount consumed by its pair-mate which received no supplement. No difference in the metabolic rates of these two animals was found, and their body weights remained practically constant.

Mitchell and Carman (1926b) used the paired-feeding method as described by Gulick and also a controlled feeding method in which each of two animals of a pair was fed amounts of the diet proportional to its surface area. In 1927 Mitchell advocated the paired feeding method unconditionally in studies of dietary adequacy and adopted the method in his subsequent work. He and his collaborators uniformly find that, for all essential dietary factors investigated, an adequate diet supports a more rapid rate of growth than does one that is inadequate.

Many other laboratories have tried the paired-feeding method in recent years, but some were not able to use it successfully in certain types of work. However, in studies of the nitrogenous (protein) constituents, the results have been consistent, as the more adequate ration has uniformly sustained a more rapid rate of growth. It is a common experience though that the *ad libitum* method gives greater differences, and some of the foremost investigators in the field prefer this method. It is probably unnecessary to cite, as an example, the many notable advances Rose and associates have made in our knowledge of essential amino acids, by the use of the *ad libitum* method.

Jackson (1929) has compared the paired and the *ad libitum* methods of feeding in amino acid studies. He showed that according to the *ad libitum* method tryptophane could be replaced by indol pyruvic acid but not by certain other indol derivatives. When the paired-feeding method was used he observed that the rat which received tryptophane or its replaceable indol derivative grew for a few days, but after gaining 15 to 20 grams only maintained its weight or even lost a little, in close agreement with the deficiently fed animal. The paired-feeding method did not, therefore, give as clear-cut an answer to the problem as did the *ad libitum* method. Jackson suggests that greater differences might be obtained if the basal diet contained sufficient tryptophane to allow a moderate rate of growth rather than maintenance only, but "Even if that were the case, a considerable portion of the method's sensitiveness would be lost in having to distinguish between two rates of growth rather than between growth and maintenance."

That greater differences in growth rates are secured when the pace-setter in such a protein or amino acid experiment consumes sufficient food to allow some growth, was shown by Jackson and Block (1932). The inadequacy studied was in cystine, but the basal diet contained enough to allow some growth. Addition of cystine to the diet allowed greater differences in final weights than were obtained in the earlier experiment in which tryptophane was the variable.

On the other hand, Osborne and Mendel (1915a, 1918) find that in protein studies the *ad libitum* method of feeding may yield variable results. One method of attack used by these investigators was to compare low levels of different proteins in promoting growth and still lower levels in maintaining weight. Differences in the daily protein consumption, and in growth as a result of differences in food consumption, would occur among animals on the same ration. However, by keeping food consumption records these authors observed (1915b) that 9 per cent of lactalbumin is superior to the same percentage of casein or edestin even if the food intakes are similar. This is the first experimental evidence that growth is affected directly by the amount and quality of protein as well as through the effect of this constituent on food consumption. The next year these authors offered further evidence of this nature, and presented a controlled feeding method basicly the same as the paired-feeding method. A schedule of food intake a little below the normal growth requirement was drawn up and strictly adhered to. The only variables were the quantity and kind of protein supplied. Some of their results are reproduced in Table 1.

TABLE 1.—GROWTH OF ALBINO RATS AS AFFECTED BY DIETARY PROTEIN.

Protein	Percentage Protein in diet	Food Intake grams	Length of Trial days	Total Gain grams
Lactalbumin	14.8	438	77	122
Lactalbumin	8.0	438	77	77
Edestin	8.0	438	77	50
Casein	8.0	438	77	71
Casein plus cystine	8.0	438	77	95
Casein	10.8	438	77	85
Casein	16.2	438	77	105

Examination of the table shows that different proteins, even though the same amounts are consumed, may support different rates of growth; also, when casein was supplemented with cystine, or when the percentages of lactalbumin or casein were increased, the rate of gain was accelerated.

Although the above results seem beyond criticism Osborne and Mendel preferred to allow a definite amount of food per unit live weight as growth proceeded, so as to allow for the more rapidly increasing maintenance requirement of the faster growing animal. From the practical standpoint it appears that even this method does not demonstrate the full advantage of feeding the more adequate kind and amount of protein, because it limits the amount that will be consumed of the better ration, and so partly conceals one of the reasons for its superiority.

Several other protein studies by various methods of controlled feeding were reported by Osborne and Mendel, among which was one in 1918 which showed that the proteins of corn or of oats are inferior to those of rice or of barley. In this report the authors emphasize the importance of keeping the calorific value of the various diets uniform.

Mitchell and collaborators (Mitchell, 1924; Mitchell and Kick, 1927) applied the paired feeding method to a study of the biological value of protein, and the relative and supplementary values of various proteins were satisfactorily established.

Mitchell and Beadles (1930), using the paired-feeding method, found the proteins of dried skimmilk, whole milk, peas, and potatoes to be deficient in cystine. Swift et al. (1934) affirmed, by the use of the same technique, that the proteins of dried skimmilk are deficient in cystine. Mitchell and Smuts (1932) demonstrated that the proteins of lean beef and of soybeans are deficient in cystine. The addition of this amino acid definitely improved growth, even though the other constituents of the diet were consumed in equal amounts. Haag (1931), by the same technique, found that cystine definitely improves a ration that contains alfalfa leaves as the source of protein. Jackson

and Block (1932) used a cystine-low diet of whole milk powder 15 per cent, and gelatin 2 per cent, to study the availability of methionine for growth. Rats grow very slowly on this diet unless it is supplemented. Methionine improved the diet as judged either by the controlled or the *ad libitum* method of feeding, though the differences by the *ad libitum* method were much more distinct than by the paired method. Weichselbaum, Weichselbaum, and Steward (1932) reported the same results. These authors fed daily the same amount of food to all animals, and when cystine or methionine was added to the Sherman-Merrill cystine-low diet the gains were significantly greater than those of the controls.

The experience of Hogan and Pilcher (1933) with rations varying from 8 to 33 per cent of protein was cited in the beginning. In this work both paired and *ad libitum* feeding were practiced. While both methods showed that the high protein rations uniformally supported more rapid growth than the low protein rations, the *ad libitum* method gave more distinct differences in growth than did the paired method of feeding.

Forbes et al (1935) found, by the use of the paired-feeding method, that increasing the percentage of casein in the diet of rats up to 20 per cent resulted in significantly greater growth, while a further increase to 25 per cent did not materially improve the ration. Energy determinations were made on the feed, visible excreta, and carcasses. The animals on the higher levels of protein gained more energy and body protein, the latter generally accounting for all the difference in energy gains.

We must conclude then that the paired-feeding method may be used successfully in studies of the protein factor. There is less certainty, however, as to the suitability of the method for vitamin studies. There have been numerous failures to demonstrate differences in vitamin content by the use of this method of feeding, even when there was no doubt that they existed. On the other hand, when the *ad libitum* method has been used the differences have been quite apparent, by the criteria of growth rate, and physiological and pathological observations. For example no one would dispute that the purified diet designated by Gulick (1922, 1924) as inadequate in vitamin B, is markedly improved by the addition of yeast, though the weight records of the pair-fed rats in this experiment did not indicate that the diet was improved by such an addition. When the supplemented diet was allowed *ad libitum* the animals tripled in weight in 45 days, and when the supplement was withheld no growth was obtained. Kon (1929) reported similar results. The negative results of Hogan and Pilcher (1933)

were cited in the beginning. McClure, Voris, and Forbes (1934) reported that there was no significant difference between the growth rates of animals on a low level of vitamin B and pair-mates on a somewhat higher level. These authors examined the food utilization for body gain, and found that the adequately fed animals stored more energy than their pairmates. However, if these differences are calculated as fractions of the energy consumed they are so small (1.1 per cent) that it is not impossible for them to be within the limits of experimental error. Such an experiment is complicated, and an error in equalizing food intakes, collecting and preparing samples, or choosing suitable control animals for analysis at the beginning of the trials could easily account for the differences found. Such small differences, accompanied by equal rates of growth, offer rather substantial proof that they lack practical importance, and cast doubt on their theoretical significance. The studies of Palmer and Kennedy (1930, 1931) with growing rats, using both the *ad libitum* and the paired-feeding technique, have led them to the conclusion that differences in vitamin content of various foods cannot be consistently demonstrated by the paired-feeding method. They conclude that the marked effect of certain vitamin rich supplements in promoting growth when the diet is allowed *ad libitum* is due entirely to appetite stimulation, i. e., increased food consumption. Johnson and Palmer (1934) noted a considerable increase in growth in *ad libitum* feeding of rats when liver was included in a so-called complete diet of rats. Food consumption records showed that part of the differences were due to the appetite stimulating property of the liver supplements and paired-feeding experiments showed that this property was solely responsible for the differences observed, since no differences were obtained when food intake was equalized. Seegars and Smith (1932) came to the opposite conclusion, but Johnson and Palmer point out that the considerable quantity of nutriment in the added liver evidently was not accounted for. Johnson and Palmer's observation upon rats have been confirmed by Dunlop (1935) for swine. When small quantities of liver were added to a so-called complete ration for swine growth improved, but only in the *ad libitum* type of feeding. No benefit resulted from the liver additions in the paired-feeding trials.

Rose, Stucky, and Mendel (1929-30) and Drury, Harris, and Maudsley (1930) find that the weight curves of mature rats are remarkably similar when they are pair-fed on diets that are adequate and inadequate in vitamin B. Kon and Drummond (1927) find the same to be true for pigeons. Record, Bethke, and Wilder (1934) find no increase in food utilization when vitamin supplements were added

to certain diets of chicks, though the improvement in the diets was distinctly indicated by a marked reduction in incidence of leg weakness. Hoet (1923) believes that appetite of pigeons on polyneuritic diets can be measured equally well by the weight curve or by the measured food intake. Mendel (1923) mentions the agreement between weight curves and food intake of rats on B-deficient diets, and Cowgill (1921) showed the same to be true for dogs. Cowgill (1934) contends that the appetite is a dependable measure of the adequacy of vitamin B for all species studied.

In contrast to the above results Mitchell (1930-31, 1933) obtained positive differences in growth of rats in a paired-feeding experiment, which were attributed to differences in the vitamin B content of the cereal grains used. Significant differences were not always evident from inspection, but statistical analysis showed them to be of significance. Graham and Griffith (1933 a, b) also report small positive differences, explained by differences in the vitamin B content of the diets, though in most cases there were also differences in food consumption which complicated the results.

Braman, Black, Kahlenberg, Voris, Swift, and Forbes (1935) studied growth and energy utilization in vitamin G deficiency, using the paired-feeding technique. Larger gains in weight and energy, which were statistically significant, were registered by the animals that received the vitamin supplement. These differences may not be entirely conclusive, however, in view of the difficulties in equalizing food intakes when one diet is so deficient as to be consistently refused. Some of the average data for the 12 pairs of animals are as follows:

length of feeding period, weeks	14
weekly energy intake, G-supplemented series, calories	121.5
weekly energy intake, G-deficient series, calories	120.2
weekly difference in intake due to the vitamin carrier, calories	1.3
difference in gain per week, grams	1.1
difference in gain per week, calories	2.9

The difference in intake due to the energy value of the vitamin supplement is 45 per cent of the difference in gains, and no doubt this extra energy is used very economically at such a low level of intake. The remaining difference, 1.6 calories, is only 1.3 per cent of the total energy intake, which justifies some doubt as to its biological significance.

The most consistent and marked differences obtained in such studies are those of Sure and collaborators (1928, 1932, 1933). The differences obtained by these workers were sometimes on the order of 1 gram per day for long periods. The adequately fed animals were described as growing steadily on an intake restricted to that on which their deficiently fed pair-mates grew not at all, or even lost weight, and eventually died. It is difficult to explain the discrepancy between these results and the negative results frequently reported elsewhere, for example those of McClure et al. (1934) who measured the food intake with sufficient accuracy to obtain reasonably acceptable nitrogen balances. Negative results cannot be explained by difficulties in the feeding technique, but this explanation is suggested immediately when positive results are obtained.

In the experiment reported in the following pages an explanation is presented for the difference in growth rates obtained in a paired-feeding trial with the protein factor as the variable. The utilization of nutrients and of energy has been followed by determinations of the respiratory exchange, analysis of the food, the visible excreta, and the bodies of the experimental animals. Adequate and inadequate levels of protein were chosen for comparison, since the literature shows that this is the most reliable method of obtaining definite differences in gains in a paired-feeding experiment. The question of energy utilization in vitamin deficiencies is one of minor importance until it is satisfactorily shown that distinct differences in growth rates do occur.

Since this work was begun studies by Lee and Schaffer (1934) upon the utilization of energy, by pair-fed animals with and without injections of anterior pituitary extract, have appeared. The more efficient energy utilization was effected by the slower growing animal of the pair, demonstrating clearly that wide differences in growth rate may be brought about simply by some factor which has a favorable effect on protein storage, thus causing gains of high protein and water and low fat and energy content.

EXPERIMENTAL

The experimental animals were male albino rats, with an initial age of 18 to 23 days, and with initial weights of about 30 grams. These animals are particularly suitable for this work because of their small size and their rapid and economical increase in weight. Uniform litters of 6 were chosen when possible; three were offered the high and three the low protein ration for a preliminary period of 3 days, during which each received the same amount of food. The animals were paired, and

at the end of the preliminary feeding period each pair, one animal on the low and one on the high protein diet, were treated as follows:

Pair (a): These were slaughtered, full weights obtained, the contents of the digestive tracts removed and empty weights were obtained. The carcasses were dried, first with 95 per cent and absolute alcohol and then in the vacuum oven, and ground for analysis.

Pair (b): Digestion animals. These were housed in round hardware cloth cages with raised ½ in. mesh screen floors, and were treated in other respects as nearly as possible in the same manner as pair (c), that is the same amount of food, fed at the same time, the same environmental temperature and light, and the same weighing and handling manipulations. An acid-hardened filter paper was kept under the raised floor and the feces and urine were collected separately, daily. The feces were stored in a vacuum desiccator, the urine was washed out of the paper with hot N-free distilled water and stored in bottles in a dark cool place, being preserved with copper sulphate from the filter paper and with mercuric chloride which was added later. The same filter paper was used throughout a trial.

Pair (c): Haldane animals. These were placed in airtight metal chambers connected with a Haldane (1892) open circuit gas absorption train, and the respiratory exchange was measured continuously except for a 10 minute period daily required to change the chambers. The chamber and absorption train are shown in Figures 1 and 2. Duplicate chambers and trains were made ready each day to facilitate rapid change. The 10 minute interval occasioned no special excitement, and in reality the animals were out of the chambers for a maximum of 2 minutes daily. The chamber with equipment weighed 3000-3500 grams and was weighed to 10 milligrams. The carbon-dioxide absorption tubes, consisting of shell soda followed by magnesium perchlorate ("Dehydrite") weighed 150-180 grams each and were weighed to 1 milligram, or about 0.02 per cent of the total daily elimination of CO_2. At one time standard acid and a combustion tube followed by a CO_2 absorber were placed at the end of the train, to determine the loss of nitrogen and carbon by way of the air current, but none was found. Check tubes were used at all necessary points. It was calculated that the maximum errors could not exceed 0.1, 0.5, and 0.6 per cent of the CO_2 elimination, O_2 consumption, and R. Q., respectively. The heat production was calculated from the nitrogen elimination, the CO_2 production, and the non-protein R. Q., a correction being made for the 10 minute daily interval described above. The visible excreta were handled in the same manner as that from the (b) pair on digestion trial.

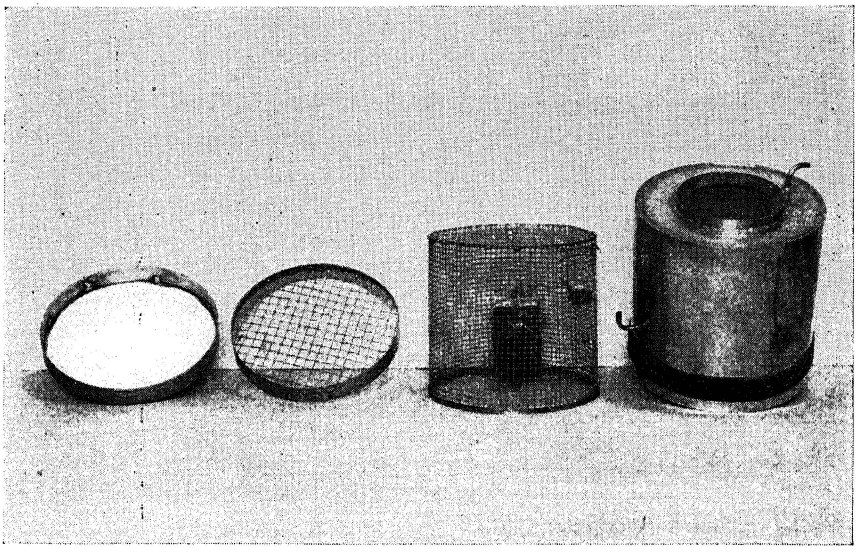

Fig. 1.—The Haldane Chamber.

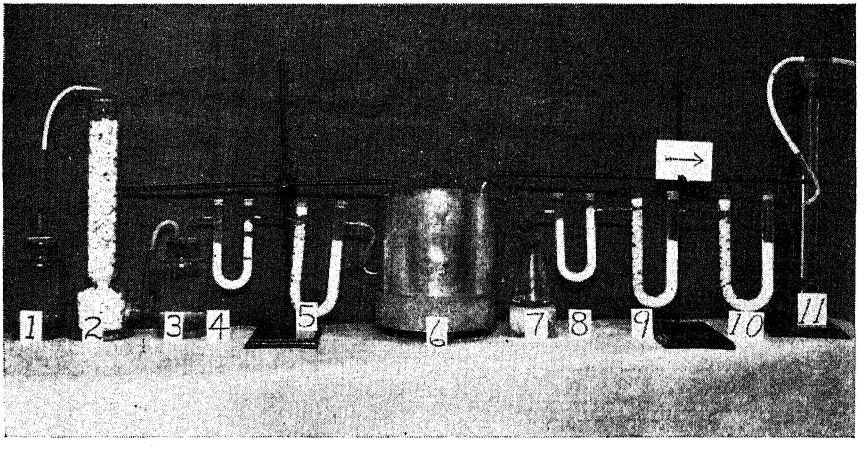

Fig. 2.—The Haldane Apparatus. 1, 3, 7, sulphuric acid bottles, moisture absorbers. 2, shell NaOH, CO_2 absorber. 4, 8, "Dehydrite" moisture check tubes. 5, 9, 10, shell NaOH and "Dehydrite," CO_2 absorbers. 6, animal chamber. 11, water column, negative pressure equalizer.

At the end of each trial all animals were slaughtered and the carcasses prepared for analysis as described above for the initial carcasses.

TABLE 2.—COMPOSITION OF RATIONS.

Ration No.	1864	1865	1874	1875	1890	1891	1920	1921
Corn starch	71	56	66	53	70	68	64	46
Casein (Purified)	10	25	12	25	8	10	6	24
Bone ash			3	3	3	3	3	3
Yeast							8	8

Each ration contained in addition 12.5 per cent lard, 2.5 per cent cod liver oil, and 4 per cent Osborne and Mendel (1919) salt mixture.

Rations. The composition of the rations used is given in Table 2.

In Series 1, 2, 3, and 4 casein furnished the entire protein, and the vitamin B complex was fed separately as a 50-50 mixture (on a dry weight basis) of a liver extract and tikitiki. In the last four series Rations 1920 and 1921 were used exclusively. In these rations yeast supplied additional protein and completed the vitamin requirements. Food consumption and rate of growth with these latter rations were much more satisfactory than with the first six. On rations 1864 and 1865 the feces were often not well formed, too moist, and occasionally moderate diarrhoea developed. Incorporation of 3 per cent of bone ash remedied this difficulty, and resulted in well formed feces which dried quickly and ground easily.

In the first trials the method of feeding was to limit the HP (high protein) animals to that quantity consumed *ad libitum* by the LP (low protein) animals. It was found, however, that the animal which had food left in its box at the end of 24 hours was also the one most inclined to scatter it, in spite of all precautions to prevent spilling. In such a case all food particles were carefully brushed up from the filter paper, weighed, and refed to the same rat. By limiting the food to that amount consumed in the first part of the day scattering was usually prevented entirely, and consumption was very little less than with the old method. There were never any food refusals on the high protein diets and scattering rarely occurred. An animal allowed an inadequate amount of an adequate diet does not waste its food.

The type of food box described by Pilcher (1930) was used throughout. It consists of a specially constructed metal food box with a tunnel leading to the small opening through which the food is secured. Water was provided in a small inverted bottle.

There were 8 series in all, 7 of four animals each and 1 of 6 animals. Thus pairs (b) (digestion trials) comprised 18 animals and pairs (c) (Haldane trials) 16 animals, half of each on the low and the other half on the high protein diet. In addition there were 12 initial carcasses.

In the presentation of the results the animals are divided into 4 groups according to the completeness of the data. Groups 1 and 2 are composed of those animals on digestion trials, on which carbon balances and heat production were not determined directly. Groups 3 and

4 are composed of the animals kept in Haldane chambers throughout the experiment. Groups 2 and 4 (digestion and Haldane animals respectively) comprise the last four series. These were considerably more satisfactory than the first 4 in rate of growth and differences in growth, degree of wastage and refusal of food, completeness of balances, and general refinement of technique. Considerable confidence is placed in the data of these last four series, and particularly of those animals in Group 4, on which the most complete data were secured. However, the greatest care was practiced in all series to equalize the food intake and to record the actual consumption to the accuracy of 1 milligram. It will be seen that the important data for all groups are in general agreement.

Methods of Analysis

The feed was analyzed in the air-dry state. The carcass, feces, and excreta samples were kept in desiccators under vacuum and analyzed dry. The urine washings of the digestion trial animals were digested with acid and aliquots taken for analysis. The urines of the Haldane animals of the last four series were analyzed for nitrogen and carbon without concentration, after it was found from the first experiments that the samples could not be concentrated to dryness without some losses.

The official methods (1930) were used in the determination of nitrogen, fat, moisture, and ash. All samples were run in triplicate except for a few fecal samples of which very small quantities were available. No less than six determinations were made on each of at least two separate samples of each ration. The carbon determinations of the feeds, feces, and carcasses were made by the macro dry combustion method. The urine samples and the vitamin solution were analyzed by a modification of the micro wet combustion method (White and Holben, 1925, 1934), using chromic and sulphuric acids. The methods were repeatedly checked with pure materials of known composition. For the energy determinations the Emerson calorimeter was used, equipped with a well insulated adiabetic jacket (Daniels, 1916) so that the temperature in the water jacket could be regulated at will, and was kept within 0.1 degree of the bomb bath temperature. The equipment was standardized with benzoic acid obtained from the U. S. Bureau of Standards. Close agreement between duplicate samples was required, and frequently the agreement was within 0.1 per cent.

Factors Used in the Calculations

Some factors are necessary and others desirable for the various calculations that are permissible with the data collected. These factors were selected from the literature or calculated from the data obtainable. A small divergence from entire accuracy would not have an appreciable

effect on the results, though the values used were the best obtainable. These are given in Table 3.

TABLE 3.—FACTORS USED IN VARIOUS CALCULATIONS.

Factor for	Factor	Source
Gross energy of casein, cals.	5.85	Sherman (1932), p. 139
Gross energy of protein of R. 1920, cals	5.79	calculated
Gross energy of protein of R. 1921, cals.	5.83	calculated
Gross energy of fat, cals.	9.50	Armsby (1928), p. 228
Gross energy of carbohydrate, cals.	4.185	Armsby (1928), p. 228
Metabolizable energy of casein, cals.	4.58	calculated
Metab. energy of protein of R. 1920, cals.	4.51	calculated
Metab. energy of protein of R. 1921, cals.	4.56	calculated
Metab. energy of body protein, cals.	4.454	Armsby (1928), p. 641
Nitrogen in casein, per cent	15.67	calculated
Nitrogen in body protein, per cent	16.00	calculated
Nitrogen in protein of R. 1921, per cent	15.75	calculated
Nitrogen in protein of R. 1920, per cent	15.82	calculated
Carbon in body protein, per cent	52.5	Armsby (1928), p. 206
Carbon in body fat, per cent	76.5	Armsby (1928), p. 206
O_2 required per gm. casein oxidized, gms.	1.453	calculated
CO_2 produced per gm. casein oxidized, gms.	1.614	calculated
O_2 per gm. protein oxidized of R. 1920, gms.	1.410	calculated
CO_2 per gm. protein oxidized of R. 1920, gms.	1.565	calculated
O_2 per gm. protein oxidized of R. 1921, gms.	1.437	calculated
CO_2 per gm. protein oxidized of R. 1921, gms.	1.596	calculated

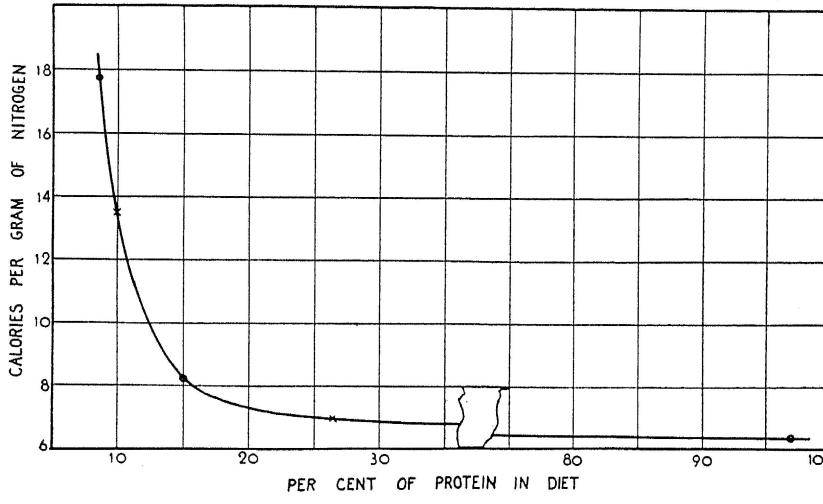

Fig. 3.—Energy Value of Urine.

Satisfactory energy determinations on the urine samples were not obtained, because of the poor oxidation of the dried, salt-rich samples. It was therefore necessary to rely on calculations based on the nitrogen content to determine the energy value of the urine. The appropriate factors were selected from a curve (Figure 3) drawn between points plotted from the following data:

TABLE 4.—THE ENERGY VALUE OF URINE OF RATS AS AFFECTED BY THE LEVEL OF DIETARY PROTEIN.

Citation	Protein level per cent	Calories per gram urinary nitrogen
Kriss and Miller (1934)	97.00	6.40
McClure et al. (1934)	15.00	8.20
Swift et al. (1934)	8.50	17.75
The values selected from this curve are:		
Mo. Ration No. 1920	9.96	13.50
Mo. Ration No. 1921	26.30	7.00

While this method is not strictly accurate, the urinary energy is a very small fraction of the total energy consumed, and a considerable error in this calculation does not materially affect the results.

RESULTS

Gains in Body Weight

In the case of each of the 17 pairs of rats used the animal on the high protein diet outgrew its pair-mate on the low protein diet. Figure 4 shows the growth curves by series. The animals in each series on the same diet are combined, so that each curve represents the average of two rats for the first 7 series, and three rats for Series 8.

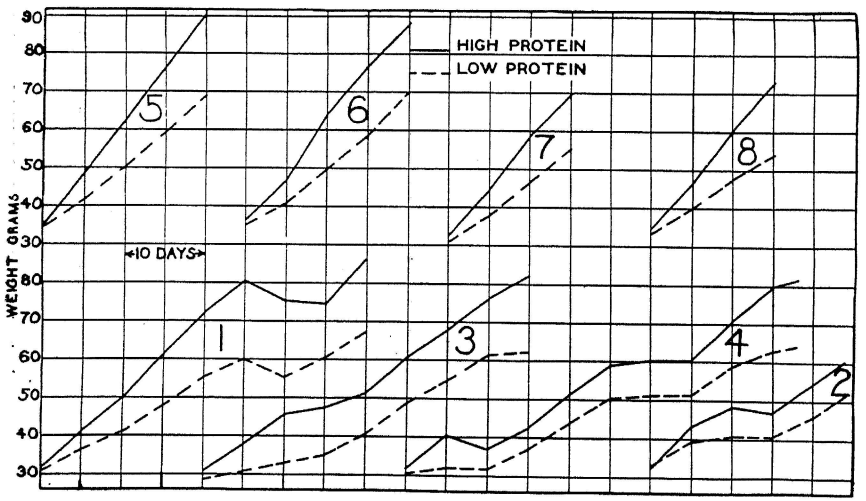

Fig. 4.—Growth Rates of Experimental Animals.

As mentioned earlier, a clear solution of the problem at hand is facilitated by rapid growth rates and considerable differences in gains per unit of energy consumed. These conditions are fulfilled in Series 5, 6, 7, and 8. The curves illustrate the fact that wider differences are

obtained when the animals grow rapidly than when they grow slowly. When difficulties were encountered in food refusals, as was often the case during the first four series, and the LP animals were not consuming enough to grow steadily or even to maintain weight, the HP animals were likewise unable to grow. When weight was being lost the HP animals frequently lost weight more rapidly than did their pair-mates. The energy intake was mounting without obtaining the necessary differences in gains. This experience is very similar to that of Jackson (1929) and Jackson and Block (1932), mentioned earlier. The relative differences in the present work, due to the food intake and the rate of growth of the controls are shown as follows:

TABLE 5.—EFFECT OF GROWTH RATE ON DIFFERENCE IN GAINS.

Series	Number of animals	Number of days	Average LP gain grams	Average HP gain grams	Avg. daily dif. in gain grams
1–2–3–4	16	38	31	46	0.4
5–6–7–8	18	17.2	27	45.5	1.1

The last column in Table 5 shows that the average daily difference in gain was almost three times as much in the last four series as in the first four. It is readily seen that the fraction of the total food used in maintenance must be much larger for the first four than for the last four series. The last series were short, growth rates were quite rapid, and the energy retained was a larger fraction of the energy consumed. For the sake of comparison the average dry matter intake required per gram difference in gain is given in Table 6.

TABLE 6.—RELATION OF FOOD CONSUMPTION TO DIFFERENCES IN GAINS.

Series	Number of Animals	D. M. Intake grams	Difference in gain grams	D. M. required per gram difference in gain, grams
1–2–3–4	16	123.0	15.3	8.04
5–6–7–8	18	78.7	18.5	4.26

Such large differences in rate of growth as that of the last four series of this study have not been reported elsewhere in the literature. Other workers may have obtained equally distinct differences in growth early in their feeding trials, but experiments were continued for longer periods of time, thus including the period of increasing energy cost for maintenance. In the last four series the animals were slaughtered at the time when the difference in gains per unit of food consumed began to decrease, so that energy utilization was examined only for the period of most economical growth.

TABLE 7.—AVERAGE BALANCES OF NITROGEN, CARBON, AND ENERGY.

Diet	No. of Animals	Nitrogen			Carbon			Energy		
		Consumed grams	Recovered grams	Difference per cent of intake	Consumed grams	Recovered grams	Difference per cent of intake	Consumed calories	Recovered calories	Difference per cent of intake
Group 1										
LP	4	2.125	2.080	−2.14	-----	-----	------	-----	-----	-----
HP	4	4.302	4.176	−2.92	-----	-----	------	-----	-----	-----
Group 2										
LP	5	1.301	1.316	1.18	-----	-----	----	-----	-----	---
HP	5	3.465	3.475	0.29	-----	-----	----	-----	-----	---
Group 3										
LP	4	-----	-----	----	58.08	56.59	−2.56	607.4	592.2	−2.5
HP	4	-----	-----	----	59.69	57.56	−3.58	629.0	607.3	−3.4
Group 4										
LP	4	1.371	1.399	2.02	38.01	37.78	−0.60	398.5	393.4	−1.3
HP	4	3.598	3.566	−0.88	39.65	39.42	−0.57	425.4	420.9	−1.1

Balances

The average balances, by groups, are shown in Table 7. As with the other data, the balances of Groups 2 and 4 are most satisfactory. These results are thought to be well within the limits of error involved in the necessary assumption of values for the initial stores of material. The nitrogen balances of Group 3 are omitted because they were not satisfactory, due to losses in an attempt to dry the excreta. In the later work liquid samples were analyzed in their original state, with the result that all food nitrogen and carbon were accounted for in Group 4. The individual balances for each animal are given in the appendix.

Digestibility

The average coefficients of digestibility are given in Table 8. Those of Group 1 are averaged separately, while those of Groups 2 and 4 are combined, since they received the same rations throughout. The feces and urine of the animals of Group 3 were not collected separately.

TABLE 8.—DIGESTIBILITY OF DRY MATTER, NUTRIENTS AND ENERGY.

Diet	Dry Matter per cent	Protein per cent	NFE per cent	Ether Extract per cent	Energy per cent
		Group 1, 4 pairs			
LP	94.03	86.96	98.24	98.38	96.32
HP	93.82	91.58	97.83	98.83	96.10
		Group 2 and 4, 9 pairs			
LP	92.22	86.49	97.31	98.19	95.24
HP	92.33	92.35	97.25	98.26	95.50
		Combined Average, 13 pairs			
LP	92.97	86.69	97.71	98.26	95.68
HP	92.94	92.08	97.51	98.48	95.74

There was no consistent difference in the digestibility of dry matter, confirming the previous work of Hogan and Pilcher (1933), and this was also true for Nitrogen-Free-Extract and Ether Extract. The average digestibility of the ash was 46.9 and 40.7 per cent for the HP and the LP animals respectively, the difference being due, no doubt, to greater reexcretion of ash by the slower growing LP rats.

The apparent digestibility of protein was 92.1 and 86.7 per cent for the HP and the LP animals, respectively. This difference is no doubt due to the fecal metabolic nitrogen.

TABLE 9.—DIGESTIBILITY OF CARBON BY GROUP 4 ANIMALS.

Series	LP Animal per cent	HP Animal per cent	Difference	Probable Error
5	95.96	96.02	0.06	
6	95.51	95.82	0.31	
7	95.45	96.07	0.62	
8	94.97	95.36	0.39	
Mean*	95.52	95.84	0.32	±0.08

*The weighted mean rather than the average of percentages is used. The average difference by the latter method is 0.35.

The digestibility of carbon was determined only for the animals of Group 4, and the results are shown in Table 9. While the difference is statistically significant, since it is four times its probable error, any real significance is doubtful.

The average digestibility of energy is almost identical for the HP and the LP animals, and this is not in accord with the conclusion of Forbes et al. (1935). The data of these authors showed that no significant effect on digestibility was secured by increasing a diet from 10 to 15 per cent protein, but further increases to 20 and 25 per cent protein effected an increase in digestibility of energy.

The average absorption of energy for those animals represented in the digestibility tables is 434.9 and 461.4 calories for the LP and the HP animals, respectively. These values were obtained by direct energy determinations of the feed and feces. The difference is chiefly due to the higher energy value of casein than the starch which it partly replaced in the HP diets. If the absorption of energy is calculated indirectly, using the appropriate heat factors for the nutrients contained in the feeds and feces, one obtains the values of 434.4 and 460.6 calories for the LP and the HP animals, respectively, illustrating the excellent agreement betwen the combustion values and the nutrient analyses and their heat factors.

Metabolizability

The energy values of the urine samples were calculated from the nitrogen content as previously explained. Although nearly twice as much energy per unit of nitrogen was allowed for the LP animals (13.5) as for the HP animals (7.0), the total urinary energy excreted is considerably higher on the diets containing 25 per cent protein than on those containing 10 per cent. The averages are 9.56, and 14.21 calories, and 0.71 and 2.03 grams of nitrogen, for the LP and the HP animals, respectively*. While exactness is not claimed for this calculation, any error involved must be a small percentage of the food energy as is shown in Table 10.

TABLE 10.—URINARY ENERGY IN TERMS OF TOTAL ENERGY CONSUMED.

Diet	LP	HP
Total Energy Consumed	490.51	516.54
Urinary Energy	9.56	14.21
Urinary Energy as percent of Food Energy	1.95	2.75

These calculations show that the total urinary energy is less than 3 per cent of the food energy. An error of 10 per cent in the estimates of urinary energy would therefore affect the energy balance by less than 0.3 per cent.

*In a private communication Dr. Forbes suggests that the energy value of the urines be calculated on the basis of the carbon content, using a constant 11.5 calories per gram of carbon. By this method the average urinary energy for the LP and the HP animals of Group 4 is 8.97 and 14.12 instead of 7.15 and 13.00 calories as determined by the method described in the text. The "carbon method" improves the energy balances in 6 of 8 cases and increases the energy value of the urines in 7 of 8 cases.

The urinary carbon was determined separately for the animals of Group 4, thus allowing the calculation of the C/N ratios. These are given in Table 11. The ratios are a little lower than those of Swift et al. (1934), but are in general agreement with them, and support the theme that the urinary energy is higher per unit of nitrogen on a low than on a high level of protein.

TABLE 11.—THE C : N RATIOS IN THE URINE OF RATS ON 10 AND 26 PER CENT PROTEIN DIETS.

LP 10 per cent protein		HP 26 per cent protein	
Animal No.	C/N Ratio	Animal No.	C/N Ratio
332	1.28	334	0.55
357	1.51	359	0.66
2	1.31	1	0.68
565	1.90	564	0.80
Average	1.50		0.67

By subtracting the urinary energy from the energy absorbed, the average metabolizable energy is found to be 460.7 and 480.9 for the 17 LP and the 17 HP animals, respectively. It will be seen that most of the above difference in metabolizable energy available is accounted for in the heat liberated, and did not lead to differences in energy storage. However, since there is some question as to the actual urinary energy the energy of the body gains is calculated as percentages of gross energy consumed, according to the method of Forbes and co-workers (1935).

Heat Production

Rubner (1894) showed that heat production may be accurately derived from the respiratory exchange. In experiments with 2 dogs covering a total of 45 days, the agreement between this method and the direct calorimeter measurement was 0.47 per cent. The heat production of the animals in the present experiment was derived in the same manner. This method is illustrated by Lusk (1928, p. 68).

Table 12 gives the heat production for all animals on which total respiratory exchange measurements were secured. It shows that the HP animals had a significantly higher heat production than the LP animals, according to the analysis of "Student" (1908). The difference of 14.3 calories is about 6 times its probable error, the odds being 223 :1 that the difference is not due to chance alone (Love, 1924). We are not prepared to emphasize this difference, however, since it is only about 3.5 per cent of the average heat production. The daily heat production records show the same relations that the totals do. For this reason, it was foreseen that the energy storage must be of the same magnitude

TABLE 12.—TOTAL HEAT PRODUCTION AS DETERMINED FROM THE RESPIRATORY EXCHANGE AND PROTEIN METABOLISM.

Low Protein			High Protein			Difference calories	Probable Error
Animal No.	Non-protein R. Q.	Heat Production calories	Animal No.	Non-protein R. Q.	Heat Production calories		
Group 3							
9461	.891	586.01	9466	.875	596.09	10.08	
9574	.865	276.33	9570	.856	267.39	− 8.94	
9845	.897	494.45	9846	.880	517.96	23.51	
9853	.890	580.71	9854	.872	599.61	18.90	
Average	.889	484.38		.873	495.26	10.88	
Group 4							
332	.943	322.05	334	.888	343.00	20.95	
357	.915	350.11	359	.879	373.12	23.01	
2	.932	225.10	1	.879	245.12	20.02	
565	.908	243.25	564	.867	249.91	6.66	
Average	.925	285.13		.879	302.79	17.66	
Grand Avg.	.902	384.75		.875	399.03	14.274	2.494

for both types of treatment. The daily R. Q.s showed that the HP animals must be storing less fat than the LP animals. While these results were unexpected they were borne out in detail by the body analyses at the end of the trials.

The data of Forbes et al. (1935) indicate that the heat production of animals on a low level of protein intake is higher than is that of those on a high protein level. However, the heat production was calculated as the difference in the energy accounted for in (feces + urine + body gain) and the feed energy. The unavoidable errors involved were not determined by energy balances, but were included in the heat produced. They give the data of two experiments, the second an almost exact duplicate of the first. In the first the average difference in the calculated heat production was 82.3 calories (lower for the HP animals) or 3.8 per cent of the food energy, while in the second experiment the difference was only 54.2 calories, or 2.5 per cent of the food energy. The decrease from the first result was 34 per cent, though only 4 per cent less food was fed in the second experiment. The cause of the increased heat production for the LP animals was assumed to be increased activity. Six-hour basal heat determinations were made upon all animals in the second experiment, showing that the basal heat for the 10 per cent protein group was 0.593 and for the 25 per cent protein group 0.6901 calories per hour. Assuming these values to be representative of the entire ten-week period, the average heat loss due to activity and S. D. A. may be calculated from their data, as follows:

Protein in diet, per cent	10	25
Total heat production, calories	1631.9	1577.7
Total basal heat, calories	996.2	1159.4
Remainder, left for activity and S. D. A.	635.7	418.3

While the total basal heat calculation may be somewhat high since the determinations were made somewhat past the middle of the 10 week experimental period, the calculations show that the basal heat was the largest fraction of the heat production, and were in an inverse order of magnitude from the total heat production. The LP animals have left over 150 per cent as much as the HP animals for activity and S. D. A. Earlier work (Kriss, Forbes, and Miller, 1934) from the same laboratory, however, shows that the HP animals should have a distinctly higher S. D. A., making it necessary for the LP animals to be at least more than twice as active as the HP animals to actually liberate the heat with which they were debited. In our experience casual observation disclosed no such effect of protein level on activity. In fact the HP animal was the most active of the pair at weighing-out time each day, apparently sensing that feeding-time approached. It was more hungry than its LP pair-mate at all times, and was constantly on the look out for more food.

Mitchell (1934) disagrees with Forbes as to the cause of the supposed increased heat production, believing that increased activity does not occur on the more inadequate diet in a paired feeding experiment, but offers the supposition that the presence of unbalanced nutrients in the tissues causes a higher heat production. However, the essential point is whether or not the total elimination of heat is greater on the more incomplete diet. This entire field of discussion centers around this point. In the present work therefore the entire heat production was observed by the most direct and accurate method available. The records were obtained under similar conditions for the individuals of each pair, and with all possible accuracy. The daily comparisons for each of the 8 pairs give a total of 222 daily comparisons and these are quite uniform throughout. From the first day with the animals at similar weights, to the last day with wide differences in weight, the HP animal as a rule showed a slightly higher CO_2 production and a definitely higher O_2 consumption that did its LP pair-mate. We conclude therefore that an inadequacy of protein does not increase total heat production above that of pair-mates receiving the same amount of a diet made adequate with respect to protein.

Although the respiratory exchange method is obviously the most reliable, the data collected permit the use of various other methods for the determination of heat production. The agreement among the more indirect methods depends upon the correctness of the various factors used and upon the accuracy of the collections and analyses. For this reason the agreement found among the various methods is best for those animals of Group 4, which gave the most accurate data in all

respects. The following methods of calculation were used, and the results are grouped for comparison in Table 13.

A. By the respiratory exchange and protein metabolism. This is applied to Groups 3 and 4.

B. By the nutrients adsorbed, the protein metabolism, and the carbon balance. This method is accurate only when the carbon and nitrogen balances are exceedingly accurate. (See Armsby, 1928, p. 241). This is applied to the animals of Group 4.

C. By the energy of the feed less that of the feces, urine, and body gain. This is the method used by Forbes and associates in recent studies of this nature (1935). It is applied to all 34 animals in these studies.

D. By the nutrients oxidized as the difference between the absorbed and the stored nutrients. The appropriate nutrient heat factors are then used. This method also is applied to all 34 animals.

The first two methods are applicable to the live animal, the last two necessitate slaughter. The third method, C, necessitates an energy balance, while the last, D, requires a nutrient balance.

TABLE 13.—TOTAL HEAT PRODUCTION ACCORDING TO FOUR METHODS OF CALCULATION.

Low Protein					High Protein				
Animal No.	A calories	B calories	C calories	D calories	Animal No.	A calories	B calories	C calories	D calories
				Group 1					
9460	------	------	593.99	597.67	9458	------	------	618.41	614.15
9572	------	------	262.37	265.84	9573	------	------	282.84	279.73
9847	------	------	511.76	517.05	9848	------	------	531.97	529.04
9858	------	------	609.02	610.98	9857	------	------	605.66	600.18
Average	------	------	494.29	497.89		------	------	509.72	505.78
				Group 2					
338	------	------	322.66	322.82	333	------	------	347.62	337.56
360	------	------	341.90	343.81	354	------	------	370.11	365.44
4	------	------	226.83	225.75	3	------	------	259.58	255.06
563	------	------	233.53	236.60	568	------	------	247.03	243.25
567	------	------	237.43	240.03	569	------	------	247.77	243.70
Average	------	------	272.47	273.80		------	------	294.42	289.00
				Group 3					
9461	586.01	------	608.54	612.64	9466	596.09	------	630.16	622.48
9574	276.33	------	281.87	283.95	9570	267.39	------	286.70	282.59
9845	494.45	------	527.33	534.61	9846	517.96	------	542.42	540.50
9853	580.71	------	613.34	613.81	9854	599.61	------	628.53	619.39
Average	484.38	------	507.77	511.25		495.26	------	521.95	516.24
				Group 4					
332	322.05	325.15	330.24	328.80	334	343.00	333.52	347.97	340.48
357	350.11	353.00	354.26	355.26	359	373.12	371.06	381.60	374.79
2	225.10	227.02	231.44	234.69	1	245.12	240.61	250.29	244.27
565	243.25	247.58	244.91	246.46	564	249.91	248.36	248.24	246.67
Average	285.13	288.19	290.21	291.30		302.79	298.39	307.03	301.55
Average of 8	384.75	------	398.99	401.28		399.03	------	414.49	408.90
Grand Average	------	------	384.20	386.52		------	------	401.58	396.43

The results by methods C and D (Table 13) agree closely for the LP animals, but the former method yields consistently higher results

than the latter for the HP animals. This is because the energy value of the urine per unit nitrogen decreases with increasing dietary protein, but method D does not consider this, since it assumes a constant value for the metabolizable energy of each nutrient at whatever level fed. Method A also gives a little higher values for the HP animals than does method B, for the reason just given.

As would be expected, the method of difference (C) gives higher results than the method using the respiratory exchange (A). Though the various methods give results that differ somewhat in detail, the variations are not great, and the relative magnitudes of the differences by any method are always similar, the HP animals showing greater heat production than the LP animals.

Body Gains

(a) **Composition of the Gains.**—Since the experimental animals were subjected to a preliminary period on the diet the animals to be slaughtered as sample bodies were treated likewise, but it was found that the composition of the bodies had been affected even in the short preliminary period, so that it was necessary to apply separate figures for the original composition of the HP and the LP animals. These differences are illustrated in Table 14, which gives the analysis of the bodies of the initial carcasses used for all animals fed on diets 1920 and 1921, i. e., Series 5, 6, 7, and 8. Except in the first comparison the paired animals were litter mates. Each animal was fed for a preliminary period of 3 days, each received 3, 4, and 4 grams of food for the 3 days, or 11 grams, all of which was completely consumed.

TABLE 14.—COMPOSITION OF INITIAL CARCASSES OF GROUP 2 AND 4.

LP				HP			
Animal No.	Nitrogen per cent	Carbon per cent	Energy cals. per gm.	Animal No.	Nitrogen per cent	Carbon per cent	Energy cals. per gm.
419	9.157	50.7	5.868	335	9.931	49.5	5.615
420	9.156	51.1	5.854	424	10.213	50.4	5.719
421	9.347	50.8	5.899	425	9.926	50.2	5.709
469	9.747	50.6	5.751	468	10.270	49.6	5.666
Average	9.352	50.8	5.843		10.085	49.9	5.677

The composition of the initial bodies is given in detail in the Appendix.

The above table shows that in every case the carcass of the HP animal contained a higher per cent of nitrogen, and a lower per cent of carbon and energy, than was found for the LP carcass. Furthermore, there was no overlapping, all the values being higher, or lower, for the HP animals than for the LP animals for each of these important constituents, and the significance of these differences is beyond question.

TABLE 15.—COMPOSITION OF GAINS.

Low Protein							High Protein						
Rat No.	Water per cent	Protein per cent	Ash per cent	Ether Ext. per cent	Calc. Fat per cent	Energy calories per gm.	Rat No.	Water per cent	Protein per cent	Ash per cent	Ether Ext. per cent	Calc. Fat per cent	Energy calories per gm.
Group 1													
9460	65.3	16.0	3.1	16.5	14.8	2.34	9458	71.6	19.0	3.5	5.8	4.9	1.61
9572	58.6	16.6	3.8	21.0	19.5	2.86	9573	70.3	18.9	4.2	7.1	5.8	1.65
9847	58.7	17.2	3.5	20.7	18.4	2.75	9848	68.2	21.0	4.8	6.7	5.9	1.75
9858	63.0	20.3	4.2	12.2	12.0	2.28	9857	66.6	22.6	4.2	6.3	5.6	1.87
Average	61.84	17.57	3.63	17.18	15.75	2.52		69.06	20.56	4.18	6.38	5.50	1.73
Group 2													
338	51.5	14.6	3.1	30.6	29.4	3.63	333	64.8	19.7	3.5	12.4	11.4	2.19
360	56.5	16.2	3.8	23.7	23.1	3.17	354	68.2	22.6	4.0	5.2	4.9	1.79
4	56.9	14.2	3.3	26.0	25.2	3.15	3	71.0	20.0	3.8	5.4	4.6	1.58
563	58.4	18.0	4.1	21.7	18.6	2.79	568	67.7	20.6	3.4	9.5	7.6	1.93
567	58.5	19.0	4.2	21.1	17.8	2.77	569	68.1	20.7	3.6	8.9	7.3	1.87
Average	55.87	16.08	3.62	25.21	23.65	3.17		67.74	20.80	3.68	8.36	7.29	1.89
Group 3													
9461	66.5	16.3	3.5	14.0	12.8	2.20	9466	73.2	18.5	3.9	3.8	4.1	1.45
9574	64.7	16.5	4.3	15.5	14.0	2.31	9570	71.2	17.6	4.5	7.0	6.4	1.63
9845	60.6	19.3	3.8	17.7	14.6	2.56	9846	69.9	22.3	4.0	4.3	3.5	1.63
9853	62.8	19.4	4.3	12.8	12.9	2.29	9854	67.5	23.3	4.6	4.2	4.2	1.75
Average	63.57	18.04	3.91	14.90	13.49	2.34		70.49	20.66	4.20	4.57	4.31	1.60
Group 4													
332	54.4	14.8	3.2	28.1	26.5	3.32	334	65.2	20.0	3.4	11.7	10.9	2.20
357	58.1	16.6	4.1	20.5	20.8	2.96	359	68.4	23.2	3.8	5.7	4.9	1.80
2	57.6	15.8	4.1	23.0	21.9	3.01	1	68.8	19.5	3.5	8.6	7.9	1.86
565	60.0	16.2	3.8	22.6	19.2	2.74	564	66.5	21.0	3.8	10.3	7.9	2.00
Average	57.19	15.80	3.77	23.85	22.53	3.04		67.11	20.94	3.61	9.11	8.04	1.98
Grand Average	59.54	16.87	3.73	20.38	18.95	2.775		68.52	20.74	3.90	7.21	6.37	1.808

The empty weight of the animals at the beginning of the trial was assumed to be the same fraction of the whole weight as was found to be the case with the animals slaughtered initially. The dry matter of the experimental animals was determined likewise. This, multiplied by the composition of the dry matter of the representative initial bodies gives the amount of matter and energy present in the experimental animals initially. The percentage composition of the gains is given in Table 15. The column headed "Calculated Fat" was derived by the use of the carbon and nitrogen gains as follows (Armsby, 1928, p. 206):

Nitrogen x 6.25=protein
Protein x 0.525=carbon in protein
Total carbon—carbon in protein=carbon in fat
Carbon in fat x 1.307=fat.

The results of this calculation agree fairly well with, and are thought to be more accurate than, the ether extract figures. The question which to accept as the most reliable is not of major importance here. It was observed that the ether extract usually contained some ash (1 to 4 per cent), that the calculated energy agrees better with the bomb determinations when the calculated fat values were used, and that the sums of all nutrients (which should approach 100 per cent) were less variable when the calculated fat values were used in place of the ether extract values. This last observation is shown in Table 16.

TABLE 16.—THE AVERAGE SUM OF THE CONSTITUENTS OF THE GAIN.

	Number of animals	Mean per cent		Probable error	Widest variation from 100 per cent
Sum with Ether Extract	34	100.532	±	0.098	2.8
Sum with calculated fat	34	99.318	±	0.056	2.2

TABLE 17.—THE RATIO OF WATER TO PROTEIN IN RAT CARCASSES.

Author	Date	Age of Rats, days	Water/Protein
Inaba	1911	mature	3.6
Hatai	1917	32	4.46
Hatai	1917	294	2.95
Chanutin	1930	mostly mature	2.89
Mitchell et al.	1926a	30-40	4.3
Mitchell et al.	1926a	mature	3.366
Bierring et al.	1932	mature	3.1
Light et al.	1934	mature	3.25-3.95
Horst et al.	1934	mature	3.4

Another criterion of validity is the ratio of water to protein in the carcass or in its gain. Moulton (1923), and Armsby and Moulton (1925) have shown that this ratio is fairly constant after chemical maturity of the cells has been reached, which occurs early in the life of an animal. Various works were cited to show this ratio to be between

3 and 4 to 1, the average for cattle being about 3.25 :1, for swine 3.1 :1, and for sheep 3.5 :1. Inaba* (1911) reported the analysis of the mature rat as 74.6 per cent moisture and 3.3 per cent nitrogen, or 3.6 :1 :: water : protein, using the value 6.25 x N for protein. Several reports on the composition of rat carcasses give this ratio or sufficient data for it to be calculated. These have been grouped in Table 17.

The ratio of water to protein in the gains of the animals in the experiments now reported has been calculated. The average for the LP animals is 3.55 and for the HP animals 3.36 grams of water for every gram of protein gained.

It was mentioned previously that Swift et al. (1934) reported that the addition of cystine to a cystine-deficient ration permitted rats to retain a larger percentage of the energy consumed. The above calculations have been made with their data assuming that the gains contained 3.7 per cent of ash. On this basis it is found that, on the average, the gains are composed of 52.4 per cent water and 21.5 per cent protein, or a ratio of 2.44 :1. This figure is the lowest found in, or derived from, any data in the literature.

Table 15 shows that in every comparison between animals of a pair, one on the low and the other on the adequate protein diet, the HP animals gained a larger percentage of water, protein, and ash, while the LP animals gained a larger percentage of carbon, fat, and energy. The differences are quite distinct for each of the 17 comparisons. The composition of the gains of animals pair-fed under such conditions has been definitely modified by the nature of the diet, as is apparent from the appearance of the carcass samples. The initial carcasses usually required no ether extraction to render them workable for grinding and sieving. The final HP carcasses usually required one such extraction. Quite surprisingly, the LP carcasses were unworkable without two or three ether extractions, and when the ether extracts were combined with the powdered samples, the contrast in appearance between the carcasses of a pair was always striking.

(b) **Quantities of Materials Gained.**—In this study the question of primary importance is whether or not the changes in body composition are sufficient in themselves to account for the differences in growth rate observed. The average gains of each group and of all groups combined are given in Table 18. It is seen that the data of the earlier trials (Groups 1 and 3) agree well with the more satisfactory trials (Groups 2 and 4) and the data of the first 3 groups are in accord with the more complete studies (Group 4). The gross energy consumed is included in this table in order that the extent of equalization of

*Inaba, R., 1911, Arch. Physiol., p. 1.

TABLE 18.—ENERGY CONSUMED AND CHARACTER OF GAINS.

Diet	No. of Animals	Gross Energy Consumed	Gains in						
			Total weight grams	Water grams	Protein grams	Fat grams	Ash grams	Gross Energy calories	Metabolizable Energy calories
Group 1									
LP	4	606.0	30.5	18.8	5.4	4.8	1.1	76.7	70.0
HP	4	628.9	45.4	31.3	9.3	2.5	1.9	78.5	66.9
Group 2									
LP	5	378.2	25.7	14.4	4.1	6.1	0.9	81.5	76.4
HP	5	409.6	44.8	30.4	9.3	3.3	1.7	84.6	73.0
Group 3									
LP	4	607.4	27.6	17.5	5.0	3.7	1.1	64.6	58.4
HP	4	629.0	41.4	29.2	8.6	1.8	1.7	66.3	55.7
Group 4									
LP	4	398.5	26.9	15.4	4.3	6.1	1.0	81.9	76.6
HP	4	425.4	43.6	29.3	9.1	3.5	1.6	86.3	75.0
All Animals									
LP	17	490.5	27.6	16.4	4.7	5.2	1.0	76.5	70.7
HP	17	516.5	43.9	30.1	9.1	2.8	1.7	79.3	68.0

Difference and Significance of Energy Stored

	Gross	Metabolizable
Mean difference	2.81±1.75	2.74±1.75
Significance	5.14 : 1	4.98 : 1

energy intake may be noted in viewing the results in terms of stored energy. It must be kept in mind, however, that the original plan was to equalize the metabolizable rather than the gross energy intake.

Even though the HP animals gained 1.5 times as much weight and twice as much water and protein as the LP animals, the former gained only half as much fat, so the gains in total energy were practically the same. The table shows that the HP animals stored the larger amount of gross energy, while the LP animals stored the larger amount of metabolizable energy, but statistical analysis shows that neither difference is significant.

The data on gains by each individual are given in the appendix. Examination shows that there is no exception to the trend shown in the group averages. In the case of each pair the HP animal gained more water, protein, and ash than its LP pair-mate, while the latter outgained the former in fat with equal regularity.

The validity of the data on carcass gains of nutrients and energy may be examined by calculating energy storage by various methods and checking the results with the direct slaughter and combustion methods of determining energy gained. The following methods were employed, with the results shown in Table 19.

A. Direct bomb determinations of initial and final carcasses, the method used for the results given in Table 18, column "Gross Energy."

B. The storage of nutrients as calculated from the protein and carbon gained, multiplied by the appropriate factors.

C. The storage of nutrients as calculated from the protein and ether extract gained, multiplied by the appropriate factors.

D. The storage of energy calculated from the balance of energy on the living animal.

E. The storage of nutrients calculated from the balance of nitrogen and carbon on the living animal, multiplied by the nutrient heat factors.

The accuracy of methods D and E depends upon the accuracy of the balances on the living animal. When the work is exceedingly accurate methods D and E give results that agree with the method A closely, but very small losses of energy (method D), or carbon (method E) in the expired air, or even in the urine give quite erroneous results in energy or in carbon stored. This is due to the fact that only a small portion of the energy or carbon of the food is stored as body gain. This is illustrated in the excessively high results by the method of difference (D) for the Group 3 animals as shown in Table 19. The much more satisfactory balances of the Group 4 animals are again reflected in these data, showing that the total energy storage can be accurately estimated on the living animal, either from the balance of energy or from the balance of carbon and nitrogen. The average results for Group 4 animals by any of the 5 methods of calculation compare very favorably with each other, the average extremes being 5.08 and 5.75 calories for the LP and the HP animals, respectively, which is a maximal variation of 7 per cent of the average energy stored, or 1.35 per cent of the energy consumed.

Each method used for calculation of the energy stored shows (Table 19) that the HP animals stored a little more gross energy than the LP animals. The average differences for any group and by any particular method are small, and the method giving the largest differences is method A, which is probably the most accurate. Since none of the methods gave greater differences the conclusion must be drawn that growing albino rats pair-fed different levels of dietary protein do not store significantly different amounts of energy in their bodies.

In the study of this problem by Forbes and coworkers (1935) the opposite conclusion is reached, i. e., an inadequate level of protein in the diet of rats results in a decreased utilization of the energy of that diet for body gain. The reasons for this discrepancy are not entirely evident, though some possibilities might be considered. It has been stressed that an important point in experiments designed to examine utilization is to secure gains in live weight, and differences in gains, as economically as possible. The rapid growth of early age is very helpful, and the stage of advanced slower growth should not be included, so that the energy quota going into heat production may be reduced to a minimum. Especially is this so when the respiratory exchange is not observed. In addition, if there were a systematic error in food consumption it would be cumulative and a greater difference in energy

TABLE 19—CARCASS GAINS OF ENERGY, BY FIVE METHODS OF CALCULATION.

Rat No	Low Protein					Rat No.	High Protein				
	Method A calories	Method B calories	Method C calories	Method D calories	Method E calories		Method A calories	Method B calories	Method C calories	Method D calories	Method E calories
Group 1											
9460	86.07	85.41	91.30	------	------	9458	84.92	82.03	86.21	------	------
9572	55.00	53.66	56.31	- - -	------	9573	44.70	44.10	47.33	------	------
9847	90.61	89.69	96.81	------	------	9848	86.49	86.40	90.29	------	------
9858	75.17	75.75	76.42	------	------	9857	97.87	95.08	98.97	------	------
Average	76.71	76.13	80.26	------	------		78.50	76.90	80.70	------	------
Group 2											
338	119.77	119.78	123.39	------	------	333	119.07	120.28	125.31	------	------
360	101.68	99.98	101.78	------	------	354	95.79	93.88	95.59	------	------
4	78.70	80.03	81.84	------	------	3	60.93	60.61	63.65	------	------
563	53.15	53.18	58.88	------	------	568	75.27	73.80	81.02	------	------
567	54.30	54.21	60.39	------	------	569	72.10	72.14	78.03	------	------
Average	81.52	81.44	85.26	------	------		84.63	84.14	88.72	------	------
Group 3											
9461	72.19	70.51	74.22	94.72	------	9466	73.25	73.02	71.88	107.32	------
9574	35.62	35.00	37.19	41.16	------	9570	41.08	40.72	42.33	60.39	------
9845	79.62	77.08	86.30	112.50	------	9846	76.04	75.02	78.63	100.50	------
9853	70.85	72.20	71.82	103.48	------	9854	75.00	73.99	73.99	103.92	------
Average	64.57	63.70	67.38	87.96	------		66.34	65.69	66.71	93.03	------
Group 4											
332	112.57	113.92	119.15	120.76	117.58	334	118.72	117.46	121.54	123.69	124.59
357	86.94	86.01	85.06	91.09	88.41	359	84.41	83.61	87.12	92.89	87.23
2	70.82	70.28	72.75	77.16	77.12	1	72.04	72.15	74.52	77.21	75.09
565	57.11	57.37	64.11	58.77	56.22	564	70.21	68.36	76.15	68.54	66.33
Average	81.86	81.90	85.27	86.94	84.83		86.35	85.40	89.83	90.58	88.31
Grand Average	76.48	76.12	79.88	------	------		79.29	78.39	81.92	------	------

storage would be indicated by a long experiment than by a short one. In these respects the present work and that of Forbes and coworkers are quite different, as may be seen from Table 20.

TABLE 20.—A COMPARISON OF EXPERIMENTS AS TO ECONOMY OF GAINS.

	Forbes et al.		Present Work	
	1st Expt.	2nd Expt.	Average of all animals	Average of Series 5-6-7-8
Length of feeding period, days	70	70	27	17.2
Energy intake, calories:				
High protein	2164	2076	517	417
Low protein	2164	2076	491	387
Body gains, grams:				
High protein	119	100	44	45.5
Low protein	79	66	28	27
Difference in gain, grams	40	34	16	18.5
Calories fed per animal to produce 1 gram difference in gain	541	611	315	217
Energy stored, as fraction of food energy, per cent:				
High protein	13.9	12.9	15.4	20.5
Low protein	10.4	10.9	15.6	21.2

The data of Series 5, which supplied the most satisfactory balance trials and growth rates, are given in Table 21.

TABLE 21.—RESUME OF BALANCES AND FOOD ENERGY STORED BY ANIMALS OF SERIES 5.

Rat No.	Diet	Food N accounted for per cent	Food C accounted for per cent	Food energy accounted for per cent	Energy stored, as fraction of food energy per cent
338	LP	100.25	-----	-----	25.39
333	HP	99.44	-----	-----	25.65
332	LP	100.12	99.33	98.36	23.86
334	HP	99.46	98.76	99.01	23.58

From the above data, in which almost one-fourth of the gross energy intake was stored as body gain, and in which the balances are highly satisfactory, only one conclusion is permissible, i. e., the food utilization is not lowered by lowering the protein level to the point of definite inadequacy. The less complete data, with slower rates of food storage in other experiments, showed the same results.

Much smaller differences in the percentage of fat in the animals on the various levels of protein were noted by Forbes and collaborators than were found in our studies. In the former case the animals were carried past the point of most rapid growth rate. The high protein diets were becoming unnecessarily high as the experiment advanced, and at later stages the animals would be using more protein for fat storage and for heat production and less for protein storage than they would at an earlier age. The difference in adequacy of the diets di-

TABLE 22.—DISTRIBUTION OF ENERGY, IN CALORIES, FOR ANIMALS ON WHICH THE BALANCE OF ENERGY WAS DETERMINED.

Animal No.	Gross Energy Intake	Heat Liberated	Excreta		Gains in		Total	Unaccounted for
			Feces	Urine	Protein	Fat		
			Group 3—Low Protein					
9461	720.76	586.01	42.15		30.61	39.90	72.19	20.41
9574	337.83	276.33	20.43		14.48	20.52	35.62	5.45
9845	641.76	494.45	51.58		34.14	42.94	79.62	16.11
9853	729.30	580.71	58.94		34.20	38.00	70.85	18.80
Average	607.41	484.38	43.28		28.36	35.34	64.57	15.19
			Group 3—High Protein					
9466	746.50	596.09	43.17		53.35	19.67	73.25	13.91
9570	348.96	267.39	22.07		25.42	15.30	41.08	18.42
9846	666.08	517.96	53.61		59.34	15.68	76.04	18.47
9854	754.47	599.61	63.95		56.89	17.10	75.00	15.91
Average	629.00	495.26	45.70		48.75	16.94	66.34	21.70
			Group 4—Low Protein					
332	471.74	322.05	20.42	8.51	28.61	85.31	112.57	8.19
357	471.74	350.11	22.56	7.98	27.87	58.14	86.94	4.15
2	325.18	225.10	16.32	6.60	21.26	49.02	70.82	6.34
565	325.18	243.25	17.65	5.51	19.27	38.10	57.11	1.66
Average	398.46	285.13	19.24	7.15	24.25	57.64	81.86	5.09
			Group 4—High Protein					
334	503.57	343.00	21.70	15.18	61.50	55.96	118.72	4.97
359	503.57	373.12	22.23	15.33	61.85	21.76	84.41	8.48
1	347.12	245.12	14.42	10.37	42.98	29.17	72.04	6.12
564	347.12	249.91	17.57	11.10	41.95	26.41	70.21	1.67*
Average	425.35	302.79	18.98	13.00	52.07	33.33	86.35	4.48

*Excess.

minishes as the growth rate diminishes, and the early differences in body composition tend to be obscured as the weight increases and as the rate of growth decreases.

Utilization of Energy, A Summary of the Data

The entire data of the several energy fractions (food, excreta, heat, body gain) may now be collected in one table to review the relative distribution of energy in growing animals as affected by the level of protein in the diet.

Table 22 shows the distribution of energy for those animals on which the total heat production was determined. Table 23 shows the same relations for all animals, the heat production being assumed to be

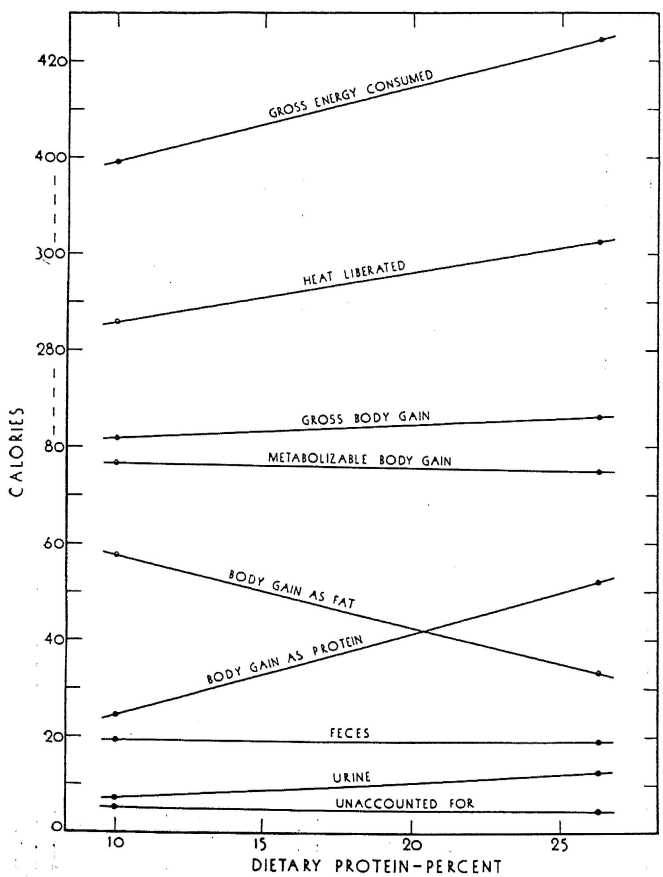

Fig. 5.—Utilization of Energy—Series 5.

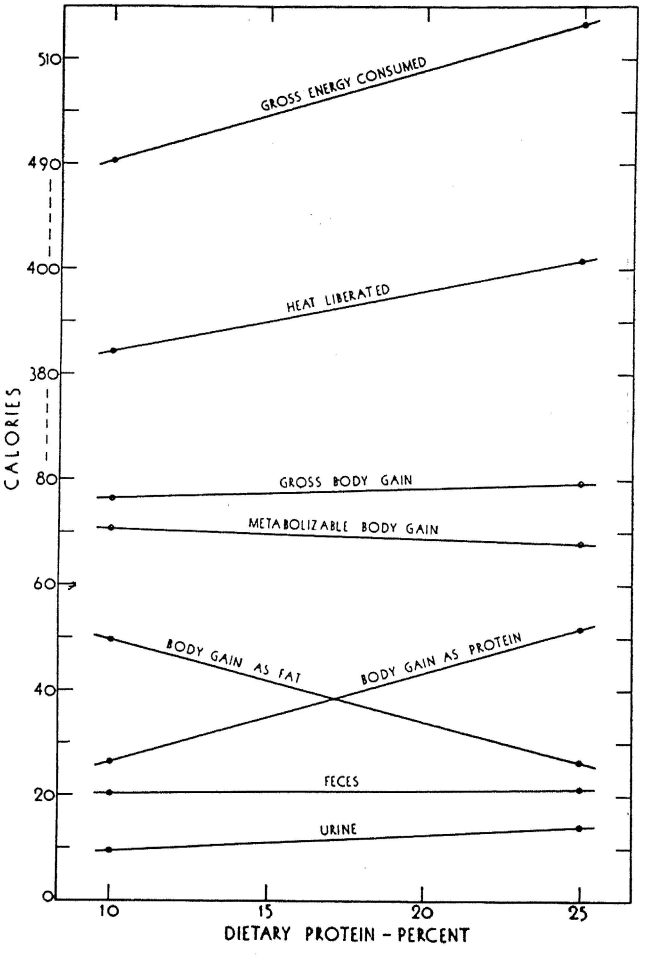

Fig. 6.—Utilization of Energy—All Animals.

the energy unaccounted for in the excreta and body gains. For the sake of comparison with the data of Forbes et al. (1935) the average data in Table 22 for Series 5, and those in Table 23 for all animals are represented in chart form in Figures 5 and 6, in the same manner as used by Forbes and associates. The position of each curve is determined by only two points, its purpose being a conventional comparison between the two levels of protein used, rather than to express an exactly linear relationship between the level of protein in the diet and the various energy fractions.

Figures 5 and 6 show that the HP animals received distinctly more gross energy in the food than did the LP animals, but that the storage

TABLE 23.—DISTRIBUTION OF ENERGY, IN CALORIES, WITH HEAT PRODUCTION BY DIFFERENCE.

Low Protein							High Protein						
Rat No.	Gross Energy Intake	Heat Liberated	Excreta	Gains in			Rat No.	Gross Energy Intake	Heat Liberated	Excreta	Gains in		
				Protein	Fat	Total					Protein	Fat	Total
Group 1													
9460	720.03	593.99	39.97	33.63	51.78	86.07	9458	746.43	618.41	43.10	57.23	24.80	84.92
9572	337.69	262.37	20.32	18.13	35.53	55.00	9573	348.73	282.84	21.19	29.18	14.92	44.70
9847	637.04	511.76	34.65	32.21	57.48	90.61	9848	666.08	531.97	47.62	58.94	27.46	86.49
9858	729.30	609.02	45.11	38.13	37.62	75.17	9857	754.47	605.66	50.94	67.43	27.65	97.87
Average	606.02	494.29	35.02	30.53	45.60	76.71		628.93	509.72	40.71	53.20	23.71	78.50
Group 2													
338	471.74	322.66	29.31	27.53	92.25	119.77	333	503.57	347.62	36.88	61.28	59.00	119.07
360	471.74	341.90	28.16	29.58	70.40	101.68	354	503.57	370.11	37.67	69.08	24.80	95.79
4	325.18	226.83	19.65	20.18	59.85	78.70	3	347.12	259.58	26.61	43.89	16.72	60.93
563	309.47	233.53	22.79	19.55	33.63	53.15	568	346.97	247.03	24.67	45.77	28.03	75.27
567	313.00	237.43	21.27	21.15	33.06	54.30	569	346.92	247.77	27.05	45.54	26.60	72.10
Average	378.23	272.47	24.24	23.60	57.84	81.52		409.63	294.42	30.58	53.11	31.03	84.63
Group 3													
9461	720.76	608.54	40.03	30.61	39.90	72.19	9466	746.50	630.16	43.09	53.35	19.67	73.25
9574	337.83	281.87	20.34	14.48	20.52	35.62	9570	348.96	286.70	21.18	25.42	15.30	41.08
9845	641.76	527.33	34.81	34.14	42.94	79.62	9846	666.08	542.42	47.62	59.34	15.68	76.04
9853	729.30	612.34	45.11	34.20	38.00	70.85	9854	754.47	628.53	50.94	56.89	17.10	75.00
Average	607.41	507.77	35.07	28.36	35.34	64.57		629.00	521.95	40.71	48.75	16.94	66.34
Group 4													
332	471.74	330.24	28.93	28.61	85.31	112.57	334	503.57	347.97	36.88	61.50	55.96	118.72
357	471.74	354.26	30.54	27.87	58.14	86.94	359	503.57	381.60	37.56	61.85	21.76	84.41
2	325.18	231.44	22.92	21.26	49.02	70.82	1	347.12	250.29	24.79	42.98	29.17	72.04
565	325.18	244.91	23.16	19.27	38.10	57.11	564	347.12	248.24	28.67	41.95	26.41	70.21
Average	398.46	290.21	26.39	24.25	57.64	81.86		425.35	307.03	31.98	52.07	33.33	86.35
Grand Av.	490.51	384.20	29.83	26.50	49.62	76.48		516.54	401.58	35.67	51.86	26.53	76.29

of energy, either gross or metabolizable, for body gain was quite similar. It must be concluded, therefore, that a further restriction of the food consumed by the HP animals so that the gross or metabolizable energy intake is exactly equalized for all animals, could not result in a greater storage of energy by the HP than by the LP animals.

Tables 22 and 23 summarize the results of the various tables presented earlier. Table 22 shows that a careful analysis of the income, outgo, and storage of energy of animals on different levels of protein reveals no more complete utilization of energy by growing rats on an adequate than on an inadequate level. If there is an advantage in energy utilization it lies with the LP animals, since they received significantly less energy, had a significantly lower total heat production, and stored slightly more metabolizable energy than their pair-mates on the HP diets. That the animals on the higher protein diets are at a disadvantage in the utilization of energy under the experimental conditions imposed is not surprising in view of the fact that the maintenance requirement of these animals very probably is higher than that of the LP animals. The HP animals are larger, and, weight for weight, their tissues contain a higher proportion of active protoplasm (protein) than do the tissues of the LP animals.

The data for all animals (Table 23) are in agreement with those of Table 22. The conclusions are the same, then, whether the less complete data are included or not.

DISCUSSION

In the studies described it was seen that the gains of the animals on the adequate level of protein are of higher protein content, with its accompanying water, than is the case for the animals on the inadequate level, while the latter animals became definitely over-fat. The over-fat condition was the direct result of the consumption of the low protein diet, while the fat-poor condition of the HP animals was the result of quantitative underfeeding. The ration was satisfactory for growth of bone and muscle but was allowed in insufficient quantity to satisfy the normal needs of the animal for energy.

This effect of dietary protein on the composition of body gains, under the conditions imposed, is compatible with present physiological knowledge. The low protein ration can not be consumed in sufficient quantity for the most rapid protein retention, and the high calorie to protein ratio is the factor which limits the amount of food consumed. The animals are calorie sick. The low protein diet, and the animals on that diet, are calorie rich and protein poor; the high protein diet, and the animals on that diet, are protein rich and calorie poor.

In 1908 Waters reported on extensive studies of the effects of retarded growth by quantitative underfeeding of cattle. A later report on these studies was made by Moulton, Trowbridge, and Haigh (1922). It was shown that growth of muscle and bone occurred on weight maintenance allowances of a balanced ration. This was brought about through depletion of the fat stores. Actually then the animals were becoming thinner and losing energy while maintaining weight and increasing to some extent in bone and muscle. In other animals which were allowed to grow ½ lb. daily, the percentage of fat in the carcasses decreased, thus decreasing the per-pound energy value of the bodies as the slow growth proceeded. It follows that growth in dimensions and in weight may take place when a young animal with an initial reserve of fat is allowed only enough of a balanced ration to remain in energy equilibrium. This explains the initial increase in weight that Jackson (1929) observed when his experimental animals received a tryptophane supplement; the controls were unable to make any gain in weight although they consumed the same quantities of the basal diet. When the fat stores are depleted, however, the daily ration must be oxidized for the energy requirement of maintenance, leaving nothing for further growth.

Since the HP animals could store little fat although their LP pair-mates were constantly becoming fatter, it is easily seen why differences in growth rate occur on identical intake and energy storage. It is also seen why the growth curves did not spread when the consumption of food dropped to low levels. At these times it was observed that the HP animals maintained their weights no better than did the LP animals, and sometimes lost weight more rapidly. When body stores were called upon for energy the HP animals were at a distinct disadvantage.

Lee and Schaffer (1934) described the same type of body changes as are under discussion. In their experiments a balanced ration was fed to both animals of each pair, but one of each pair was injected with anterior pituitary growth substance. This substance stimulates protein storage (Schaffer and Lee, 1935), and to answer this stimulus the surplus fat was used to meet energy requirements while the protein constituents of the diet were used more economically for tissue building. As a result these animals grew to considerably heavier weights than did their uninjected pair-mates, but their bodies were of lower energy value, both in per unit gain and in total gain. This striking difference in body composition does not occur with *ad libitum* feeding in otherwise similar experiments (Bierring and Nielson, 1932).

The case of the LP animals is just the opposite of energy starvation. Almost one-half of the dry matter gained by the LP animals was fat, while only one-fifth of the dry matter gained by the HP animals

was fat. The contrast in average percentages of dry matter, protein, and fat in the gains of the 17 LP and the 17 HP animals is sufficiently striking to deserve special emphasis. These data are repeated in Table 24.

TABLE 24.—THE AVERAGE COMPOSITION OF THE GAINS, ALL ANIMALS.

Diet	Dry matter per cent	Protein per cent	Fat per cent	Energy calories per gram
LP	40.5	16.9	19.0	2.775
HP	31.5	20.7	6.4	1.808

The deficiency in the diet of the LP animals is protein, and an animal on such a diet consumes all the calories its physiological limitations will permit, as Jackson (1929) postulated. The composition of the animal changes materially, and when appetite fails the evidence is strong that the limit of fat storage is being approached, and from that time on food consumption and energy storage on that diet will be restricted and controlled by the amount of protein which can be retained, and by the amount of energy liberated as heat. Under these conditions there can be no appreciable acceleration of the rate of growth until the percentage of protein in the diet has been sufficiently increased to permit a further degree of protein storage. Under ordinary conditions, the ratio of protein stored to fat stored decreases with increasing age, and, as shown in current feeding standards, the nutritive ratio of the diet can be decreased accordingly. This occurs, of course, only when the dietary protein has been adequate, and the composition of the gain has been normal. It is now clear why the normal course does not follow when the initial dietary protein has been inadequate. Osborne and Mendel (1916), in a study of protein minima for growth, made the following observation:

> "We have frequently noted that when the protein concentration of the food becomes very low, the animals do not eat satisfactorily, and frequently fail to respond to moderate increments of protein intake which ought promptly to induce renewed growth."

The reason for this is seen in the data just presented, showing a changed composition of animals on low protein diets. Immature animals kept at weight maintenance by *ad libitum* consumption of a diet too low in protein, must be in a positive energy balance, storing fat and losing protein and water. After a time such animals become sufficiently fat so that they restrict their intakes to the energy requirements. At this point a small increase in protein, still insufficient for normal growth unless a disproportionate amount of fat is deposited, will not stimulate

appetite sufficiently to definitely renew growth. The increase in protein must be sufficient to permit storage of protein and fat at such a rate that the carcass does not increase materially in fatness.

If the protein adequacy of the diet is not sufficiently restricted to prevent growth to maturity, it is reasonable to suppose that the mature composition will not be abnormal, for a mature animal, in comparison with a growing animal, is normally quite fat. As the animal grows older its demand for protein normally diminishes, and a protein content too low for maximum growth in the most rapidly growing period becomes adequate for the slower growth of advancing age. The pair-fed animal with an adequate protein intake stores less protein as maturity approaches, and converts a larger proportion of its limited excess energy into fat. One would therefore expect to find that as the growth period is prolonged the differences in composition of the carcasses become less pronounced.

It is possible that other dietary deficiencies, studied by the paired-feeding method, could also cause gains of unequal energy value and thus explain the growth differences sometimes noted. It may even be unnecessary to make the assumption of an increased specific dynamic action of unbalanced nutrients aggregating in the body (Mitchell, 1934), or of decreased food utilization due to increased activity (Forbes et al., 1935) or of any specific physiological process. One may speculate as to the factors other than protein that may give a similar result, that is, produce gains of unlike energy value. Presumably any factor which has a direct effect on protein storage would give this result, as does the growth-promoting hormone of the anterior pituitary. It might be expected that a deficiency of water or of certain minerals might limit protein storage directly, since these materials are necessary components of muscle and bone. If the constituent under question also restricts fat storage directly, or is needed in the daily metabolism of the food consumed, the effect may be solely to restrict appetite. The work of Jackson and Smith (1931) upon the effects of a deficiency of water under paired feeding conditions indicates that the animals on a restricted water intake gained less than their pair-mates because their tissues were more dehydrated, and therefore were of higher energy value. A deficiency of sodium chloride limits growth and nitrogen storage, and also lowers utilization of energy, according to Mitchell and Carman (1926b) but the authors did not examine this latter point. Earlier work by Kennard, Holder, and White (1922) upon this same problem indicates rather strongly that energy utilization is not lowered in this case, but that body composition changes occur which account for the difference in live weight gains, in the same manner as just described in the case of protein deficiency. In the following table some of their average figures are reproduced. The last

column has been calculated from their data, on the basis of 9.5 and 5.7 calories per gram for fat protein, respectively. Table 25 shows that

TABLE 25.—GAINS OF MATTER AND ENERGY DURING A 14-DAY FEEDING TRIAL WITH CHICKS.

Ration	Gain (chilled wt.) grams	Gain (edible) grams	Composition of the edible gain			
			grams Fat	Protein grams	Water grams	Energy calories
Basal	197	111	74	10	28	760
Basal + NaCl	265	170	56	26	87	680

the larger gain in weight was made by the animals receiving the added sodium chloride. Most of this gain was of edible portion. The gains of the animals that received no mineral supplement were mostly fat, and the edible gain contained more energy than that of the mineral supplemented group. It is improbable that the composition of the non-edible portion would have changed the results.

Phosphorus deficiency is said to restrict growth directly as well as though decreasing the appetite (Eckles, Gullickson, and Palmer, 1932). Some data were presented by Riddle, Hughes, and Fitch (1934) to show that heat production is increased in such cases. The evidence is not conclusive, however, because food refusals prevented strict adherence to paired feeding methods. In addition, body composition was not examined.

Smith and collaborators (1934) reported data indicating that food utilization is markedly reduced on low-ash diets. Several possibilities were investigated with negative results, so the authors concluded that the loss of energy was chiefly through the feces, due to the severe diarrhea suffiered by animals on the low salt regime (Swanson and Smith, 1934).

Two different factors which affect protein storage directly, the level of protein in the diet, and the amount of anterior pituitary growth hormone supplied, have been shown to lead to differences in gains under paired feeding conditions explained entirely by differences in body composition, and leading to no difference in total energy storage. It is conceivable that the other factors mentioned above, i. e., sodium chloride, phosphorus, water, and possibly others, may likewise affect protein storage directly and bring about results similar to those described in this report. Further study is necessary to decide these points. However, there is no indication that any vitamin plays such a role. So far as we know storage of muscle tissue does not necessarily require a definite quantitative storage of any vitamin. A vitamin inadequacy hampers certain functions, and in almost every case restricts appetite sharply.

The animal on a diet low in vitamin B does not increase its consumption above its energy needs in order to consume more of the vitamin, and therefore it does not fatten on such a diet. Findlay (1928) has shown that a deficiency of vitamin B results in a carcass showing all the evidences of energy starvation. For this reason, and because of the many failures to obtain differences in growth in vitamin studies when the paired feeding method has been used, one may conclude for the present that a deficiency of vitamin B restricts the consumption of energy but does not reduce its utilization.

There is no doubt that the paired feeding method may be employed usefully in a study of the biological value of proteins, or amino acid deficiencies, and of amino acid requirements. However, even in these cases the most valid criterion of adequacy is growth or nitrogen storage, and not the utilization of energy.

It is our view, however, that additional studies are necessary before the universal applicability, or necessity, of the paired feeding method may be taken for granted. In studies of vitamin B, for example, this method assumes that the animal which receives the less adequate supply will retain a smaller proportion of the energy it consumes, and thus a larger proportion is used for some other purpose. In the first place it has not yet been sufficiently well established that there is such a difference. The probability that it does exist is still further reduced by the fact that the gains in weight are practically identical. However, there still remains one point which should receive some attention. If differences in the utilization of energy are reported, the magnitude of these differences should be carefully scrutinized. If one animal stores 117 calories and another stores 98, the difference appears striking, but if this difference is only 1.1 per cent of the total energy consumed it gives a very different impression. If the animal on the more adequate diet stores even one per cent more of the total energy consumed than does the other the fact would be significant, if it indicates some fundamental physiological adaptation. Any significance is doubtful, however, unless the distribution of energy is accounted for in the most rigorous manner, without leaving a single loophole for an alternative explanation. The energy balance of each animal should be complete to the last detail.

Furthermore the ad libitum method has advantages of its own. It makes it possible to observe the effect of an incomplete diet on food consumption. This effect can be accurately measured, and there is no *a priori* reason for supposing that it is inferior in physiological significance to an effect on physical activity, or on specific dynamic action. A somewhat similar view has been expressed by Morrison (1933). Brody (1935) recently reviewed the literature on the technique to be used in nutrition studies and came to essentially the same conclusion.

BIBLIOGRAPHY

Armsby, H. P., 1921. *Cooperative Experiments upon the Protein Requirements for the Growth of Cattle.* Bull. Nat. Res. Council, No. 12, *2*, 219-288.

Armsby, H. P., 1928. *The Nutrition of Farm Animals.* The MacMillan Co., New York.

Armsby, H. P., and Moulton, C. R., 1925. *The Animal as a Converter of Matter and Energy.* A. C. S. Monograph, The Chemical Catalog Co., New York.

Bierring, E., and Nielsen, E., 1932. *Composition of Tissues of Albino Rats Treated with Alkaline Pituitary Extracts.* Biochem. J., *26*, 1015-21.

Braman, W. W., Black, Alex, Kahlenberg, O. J., Voris, LeRoy, Swift, R. W., and Forbes, E. B. 1935. *The Utilization of Energy-Producing Nutriment and Protein in White and Yellow Corn and in Diets Deficient in Vitamins A. D. and G.* J. Agr. Res., *50*, 1-37.

Brody, Samuel, 1935. *The Annual Review of Biochemistry,* Nutrition section. Stanford University Press, Stanford University, California.

Chanutin, Alfred, 1930. *Studies on the Creatine and Nitrogen Content of the Whole Rat after the Feeding of a Variety of Diets and after Nephrectomy.* J. Biol. Chem., *89*, 765-74.

Cowgill, G. R., 1921. *A Contribution to the Study of the Relation between Vitamin-B and the Nutrition of the Dog.* Am. J. Physiol., *57*, 420-36.

Cowgill, G. R., 1934. *The Vitamin B Requirement of Man.* Yale University Press, New Haven, Conn.

Daniels, Farrington, 1916. *An Adiabatic Calorimeter.* J. Amer. Chem Soc., 38, 1473-80.

Drury, A. N., Harris, L. J., and Maudsley, Cecil, 1930. *Vitamin B Deficiencf in the Rat. Bradycardia as a Distinctive Feature.* Biochem. J., *24*, 1632-49.

Dunlop, George, 1935. *The Effect of the Growth-Promoting Appetite-Stimulating or "Physin" Factor on the Live Weight Increase of Swine.* J. Agr. Sci., *25*, 445.

Eckles, C. H., Gullickson, T. W., and Palmer, L. S., 1932. *Phosphorus Deficiency in the Rations of Cattle.* Minn. Agr. Exp. Sta. Tech. Bull. 91.

Findlay, G. M., 1928. *Pellagra-Like Lesions Associated with Deficiency of Vitamin B_2 in the Rat.* J. Patho. and Bact., *31*, 353-64.

Forbes, E. B., Swift, R. W., Black, Alex, and Kahlenberg, O. J., 1935. *The Utilization of Energy-Producing Nutriment and Protein as Affected by Individual Nutrient Deficiencies. III. The Effects of the Plane of Protein Intake.* J. Nutr., *10*, 461-479.

Graham, Claire E., and Griffith, Wendell H., 1933a. *Studies on Growth. I. Growth Factors in Liver.* J Nutr., *6*, 179-94.

Graham, Claire E. and Griffith, Wendell H., 1933b. *Studies on Growth. II. The Effect of Vitamins B and G on the Consumption and Utilization of Food.* J. Nutr., *6*, 195-204.

Gulick, A., 1922. *The Influence of a Beri-Beri Diet upon the Metabolic Rate of the White Rat.* Am. J. Physiol., *59*, 483-4.

Gulick, A., 1924. *The Basal Metabolism of White Rats in Relation to the Intake of Vitamin B.* Am. J. Physiol., *68*, 131-2.

Haag, J. R., 1931. *The Physiological Effect of Rations Restricted Solely to the Alfalfa Plant. II. Cystine as a Limiting Factor in the Nutritive Value of Alfalfa Proteins.* J. Nutr., *4*, 363-70.

Haldane, J. S., 1892. *A New Form of Apparatus for Measuring the Respiratory Exchange in Animals.* J. Physiol., *13*, 419-30.

Hatai, S., 1917. *Changes in the Composition of the Entire Body of the Albino Rat During the Life Span.* Am. J. Anat., *21*, 23-38.

Hoet, J., 1923. *Etude de l'Alimentation Artificielle chez le Pigeon et de la Deficience en Vitamins.* Biochem. J., *17*, 220-9.

Hogan, A. G., and Pilcher, R. W., 1933. *Effects of Variations in the Amounts of Vitamin B and Protein in the Ration.* Mo. Agr. Exp. Sta. Res. Bull. 195.

Horst, Kathryn, Mendel, L. B., and Benedict, F. G., 1934. *The Influence of Previous Exercise upon the Metabolism, the Rectal Temperature, and the Body Composition of the Rat.* J. Nutr., *7*, 251-75.

Jackson, C. M., and Smith, V. D. E., 1931. *The Effects of Deficient Water Intake on the Growth of the Rat.* Am. J. Physiol., *97*, 146-53.

Jackson, R. W., 1929. *Indole Derivatives in Connection with a Diet Deficient in Tryotophane. II.* J. Biol. Chem., *84*, 1-21.

Jackson, R. W., and Block, R. J., 1932. *The Metabolism of Cystine and Methionine. The Availability of Methionine in Supplementing a Diet Deficient in Cystine.* J. Biol. Chem., *98*, 465-77.

Johnson, D. W., and Palmer, L. S., 1934. *The Appetite-Stimulating and Growth-Promoting Property of Liver.* J. Nutr., *8*, 285.

Kennard, D. C., Holder, R. C., and White, P. S., 1922. *Poultry Fleshing Investigations. The Utilization of Soy Bean and Corn Proteins as Affected by Suitable Mineral Supplements.* Am. J. Physiol., *59*, 298.

Kon, S. K., 1929. *On the Carbon : Nitrogen (C/N) Ratio in the Urine of Rats Deprived of One or Both Factors of the Vitamin B Complex.* J. Nutr., *1*, 467-73.

Kon, S. K., and Drummond, J. C., 1927. *The Physiological Role of Vitamin B. Part III. Study of Vitamin B Deficiency in Pigeons.* Biochem. J., *21*, 632-52.

Kriss, Max, Forbes, E. B., and Miller, R. C., 1934. *The Specific Dynamic Effects of Protein, Fat, and Carbohydrate as Determined with the Albino Rat at Different Planes of Nutrition.* J. Nutr., *8*, 509-34.

Kriss, Max, and Miller, R. C., 1934. *The Derivation of Factors for Computing the Gaseous Exchange and the Heat Production in the Metabolism of Casein by the Albino Rat.* J. Nutr., *8*, 669-74.

Lee, Milton O., and Schaffer, Norwood K., 1934. *Anterior Pituitary Growth Hormone and the Composition of Growth.* J. Nutr., *7*, 337-63.

Light, Amos E., Smith, P. K., Smith, A. H., and Anderson, W. E., 1934. *Inorganic Salts in Nutrition. XI. Changes in Composition of the Whole Animal Induced by a Diet Poor in Salts.* J. Biol. Chem., *107*, 689-95.

Love, H. H., 1924. *A Modification of Student's Table for Use in Interpreting Experimental Results.* J. Am. Soc. Agronomy, *16*, 68-73.

Lusk, Graham, 1928. *The Elements of the Science of Nutrition.* 4th Ed. W. B. Saunders Co., Philadelphia.

McClure, F. J., Voris, LeRoy, and Forbes, E. B., 1934. *The Utilization of Energy Producing Nutriment and Protein as Affected by Individual Nutrient Deficiencies. II. The Effects of Vitamin B Deficiency.* J. Nutr., *8*, 295-308.

Mendel, L. B., 1923. *Nutrition: The Chemistry of Life.* Yale University Press, New Haven, Conn.

Mitchell, H. H., 1924. *The Biological Value of Proteins at Different Levels of Intake.* J. Biol. Chem., *58*, 905-922.

Mitchell, H. H., 1927. *Does the Amount of Food Consumed Influence the Growth of an Animal.* Science, *66*, 596-600.

Mitchell, H. H., 1930-31. Forty-Fourth Ann. Rept. Ill. Agr. Exp. Sta., 118-9.

Mitchell, H. H., 1933. *An Application of the Paired Feeding Method to the Quantitative Estimation of the Relative Vitamin B Content of Foods and Artificial Concentrates.* Am. J. Physiol., *104*, 594-607.

Mitchell, H. H., 1934. *Balanced Diets, Net Energy Values and Specific Dynamic Effects.* Science, *80*, 558-60.

Mitchell, H. H., and Beadles, J. R., 1930. *The Paired Feeding Method in Nutrition Experiments and Its Application to the Problem of Cystine Deficiencies in Food Proteins.* J. Nutr., *2*, 225-43.

Mitchell, H. H., and Carman, G. G., 1926a. *The Composition of the Gains in Weight and the Utilization of Food Energy in Growing Rats.* Am. J. Physiol., *76*, 398-410.

Mitchell, H. H., and Carman, G. G., 1926b. *Does the Addition of Sodium Chloride Increase the Value of a Corn Ration for Growing Animals.* J. Biol. Chem., *68*, 165-81.

Mitchell, H. H., and Kick, C. H., 1927. *The Supplementary Relation Between the Proteins of Corn and Tankage.* J. Agr. Res., *35*, 857-64.

Mitchell, H. H., and Smuts, D. B., 1932. *The Amino Acid Deficiencies of Beef, Wheat, Corn, Oats, and Soybeans for Growth in the White Rat.* J. Biol. Chem., *95*, 263-81.

Morrison, F. B., 1933. *Getting More Value From Feeding Experiments.* Proc. Am. Soc. Animal Prod., Ann. Meeting, pp. 27-34.

Moulton, C. R., 1923. *Age and Chemical Development in Mammals.* J. Biol. Chem., *57*, 79-97.

Moulton, C. R., Trowbridge, P. F., and Haigh, L. D., 1922. *Studies in Animal Nutrition. III. Changes in Chemical Composition on Different Planes of Nutrition.* Mo. Agr. Exp. Sta. Res. Bull. 55.

Official and Tentative "Methods of the Association of Official Agricultural Chemists," 3rd Ed., 1930. Washington, D. C.

Osborne, T. B. and Mendel, L. B., 1915a. *The Comparative Nutritive Value of Certain Proteins in Growth, and the Problem of the Protein Minimum.* J. Biol. Chem., *20*, 351-78.

Osborne, T. B. and Mendel, L. B., 1915b. *Protein Minima for Maintenance.* J. Biol. Chem., *22*, 241-58.

Osborne, T. B. and Mendel, L. B., 1916. *A Quantitative Comparison of Casein, Lactalbumin, and Edestin for Growth or Maintenance.* J. Biol. Chem., *26*, 1-23.

Osborne, T. B. and Mendel, L. B., 1918. *Nutritive Factors in Plant Tissues. I. The Protein Factor in the Seeds of Cereals.* J. Biol. Chem., *34*, 521-35.

Osborne, T. B., and Mendel, L. B., 1919. *The Nutritive Value of the Wheat Kernel and Its Milling Products.* J. Biol. Chem., *37*, 557-601.

Palmer, L. S., and Kennedy, Cornelia, 1930. *The Fundamental Food Requirements for the Growth of the Rat. VI. The Influence of the Food Consumption and the Efficiency Quotient of the Animal.* J. Biol. Chem., Proceedings, *87*, xlv.

Palmer, L. S., and Kennedy, Cornelia, 1931. *The Fundamental Food Requirements for the Growth of the Rat. VI. The Influence of the Food Consumption and the Efficiency Quotient of the Animal.* J. Biol. Chem., *90*, 545-64.

Pilcher, Robert Warren, 1930. *Quantitative Studies on the Relation between Vitamin B and the Protein Content of the Ration.* Ph.D. Thesis, University of Missouri.

Record, P. R., Bethke, R. M., and Wilder, O. H. M., 1934. *Effect of Method of Manufacture on the Nutritive Value of Fishmeals as Determined by Growth Studies with Chicks.* J. Agr. Res., *49*, 715-22.

Riddell, W. H., Hughes, J. S., and Fitch, J. B., 1934. *The Relation of Phosphorus Deficiency to the Utilization of Feed in Dairy Cattle.* Kansas Agr. Exp. Sta. Tech. Bull. 36.

Rose, W. B., Stucky, C. J., and Mendel, L. B., 1929-30. *Physiology of Vitamins. IX. Hemoglobin, Sugar, and Chloride Changes in the Blood of Vitamin B-Deficient Rats.* Am. J. Physiol., *91*, 520-30.

Rubner, Max, 1894. *Die Quelle der thierischen Warme.* Zeitschr. f. Biol., *30*, 73-142.

Schaffer, Norwood K., and Lee, Milton, 1935. *The Effect of the Anterior Pituitary Growth Hormone on Protein Metabolism.* J. Biol. Chem., *108*, 355-71.

Seegars, W. H., and Smith, H. G., 1932. *Influence of Raw and Whole Dried Liver of Food Consumption and Utilization.* Proc. Soc. Exp. Biol. and Med., *30*, 365.

Sherman, H. C., 1932. *Chemistry of Food and Nutrition.* 4th Ed. The MacMillan Co., New York.

Smith, A. H., and Smith, P. K., 1934. *Inorganic Salts in Nutrition. X. Electrolyte Balance in the Serum of Rats Receiving a Diet Deficient in Inorganic Constituents.* J. Biol. Chem., *107*, 681-88.

"Student," 1908. *The Probable Error of a Mean.* Biometrika, *6*, 1-25.

Sure, Barnett, 1928. *A Detailed Study of the Role of Vitamin B in Anorexia in the Albino Rat.* J. Nutr., *1*, 49-56.

Sure, Barnett, 1932. *Avitaminosis. XI. The Specific Effect of Vitamin B on Growth as Evidenced by the Use of Vitamin B Concentrates.* J. Biol. Chem., *97*, 133-9.

Sure, Barnett, Kik, M. C., Walker, D. J., and Smith, M. E., 1933. *Dietary Requirements for Fertility and Lactation. IV. The Specific Effect of Vitamin B on Lactation and Growth.* Ark. Agr. Exp. Sta. Bull. 284.

Swanson, Pearl P., and Smith, A. H., 1934. *Inorganic Salts in Nutrition. IX. Correlation between Suppressed Growth and the Development of Polycythemia Induced by Feeding a Ration Poor in Salts.* J. Nutr., *8*, 659-67.

Swift, R. W., Kahlenberg, B. J., Voris, LeRoy, and Forbes, E. B., 1934. *The Utilization of Energy-Producing Nutriment and Protein as Affected by Individual Nutrient Deficiencies. I. The Effects of Cystine Deficiency.* J. Nutr., *8*, 197-219.

Waters, H. J., 1908. *The Capacity of Animals to Grow Under Adverse Conditions.* Proc. 29th Ann. Meeting of Society for Promotion of Agr. Science, p. 71-96.

Weichselbaum, T. E., Weichselbaum, M. B., and Steward, C. P., 1932. *Feeding Experiments with Methionine.* Nature, *129*, 795.

White, J. W., and Holben, F. J., 1925. *Perfection of Chromic Acid Method for Determining Organic Carbon.* J. Ind. Eng. Chem., *17*, 83-5.

White, J. W., and Holben, F. J., 1934. *Supplementary Notes on the Perfected Chromic Acid Method for Determining Organic Carbon.* J. Asso. Official Agr. Chemists, *17*, 334-6.

TABLE 26.—INITIAL CONTROL ANIMALS, WEIGHT AND COMPOSITION OF BODY.

Animal No.	Before Slaughter		Whole Wet Weight grams	Empty Weight grams	Dry Weight grams	Composition of Dry Carcas					
	Time on Diet days	Gain in Weight grams				Carbon	Ash	Water	N	Ether Ext.	Energy calories per gm.
						per cent					
Low Protein for Series 1, 2, 3, 4 (Groups 1 and 3)											
9465	3	−0.4	32.65	------	8.69	48.3	13.37	1.22	10.08	19.69	5.378
9571	7	2.5	30.50	------	8.26	47.3	17.56	1.33	9.97	18.35	5.101
Total	10	2.1	63.15	------	16.95	95.6	30.93	2.55	20.05	38.04	10.479
Average	5	1.05	31.58	------	8.48	47.8	15.46	1.27	10.02	19.02	5.240
High Protein for Series 1, 2, 3, 4 (Groups 1 and 3)											
9464	3	2.5	29.46	------	7.67	46.2	15.29	0.58	10.92	12.49	5.070
9569	7	6.0	29.59	------	7.84	47.3	15.14	1.20	10.66	16.42	5.320
Total	10	8.5	59.05	------	15.51	93.5	30.43	1.78	21.58	28.91	10.390
Average	5	4.25	29.53	------	7.76	46.8	15.21	0.89	10.79	14.45	5.105
Low Protein for Series 5, 6, 7, 8 (Groups 2 and -)											
421	3	4.3	32.28	30.96	9.06	50.8	11.66	0.34	9.347	23.92	5.899
420	3	4.9	33.39	31.27	9.26	51.1	12.17	0.52	9.156	24.70	5.854
419	3	4.3	33.28	31.88	9.49	50.7	12.68	0.25	9.157	25.16	5.868
469	3	3.9	34.38	32.82	9.39	50.6	12.01	0.06	9.747	21.05	5.751
Total	12	17.4	133.33	126.93	37.20	203.2	48.52	1.17	37.407	94.83	23.372
Average	3	4.35	33.33	31.73	9.30	50.8	12.13	0.29	9.352	23.71	5.843
High Protein for Series 5, 6, 7, 8 (Groups 2 and 4)											
424	3	7.8	34.81	33.57	9.23	50.4	11.31	0.42	10.213	19.92	5.719
425	3	8.2	32.16	30.71	8.45	50.2	12.24	0.65	9.926	20.10	5.709
335	3	5.0	33.52	32.20	8.93	49.5	12.59	0.52	9.931	19.84	5.615
468	3	5.4	35.37	33.33	9.77	49.6	11.90	0.23	10.270	18.13	5.666
Total	12	26.4	135.86	129.81	36.38	199.7	48.04	1.82	40.340	77.99	22.709
Average	3	6.60	33.96	32.45	9.09	49.9	12.01	0.46	10.085	19.50	5.677

TABLE 27.—COMPOSITION OF CARCASSES OF EXPERIMENTAL RATS, DRY BASIS.

Animal No.	Nitrogen per cent	Protein per cent	Ether Extract per cent	Ash per cent	Water per cent	Energy cals. per gram	Total per cent	Carbon		Calc. Fat per cent	Total per cent		Calculated Energy, cals.		
								in protein per cent	in fat per cent		using Et. Ex.	using Calc.Fat	of Protein	of Calc. Fat	Total
								Low Protein							
9461	8.77	54.81	32.04	12.63	0.42	6.001	51.80	28.72	23.08	30.17	99.90	98.03	3.124	2.866	5.990
9460	8.42	52.63	36.31	11.57	0.49	6.151	53.30	27.58	25.72	33.62	101.00	98.31	3.000	3.194	6.194
9574	9.03	56.44	28.52	14.16	0.78	5.734	50.40	29.57	20.83	27.22	99.90	98.60	3.217	2.586	5.803
9572	8.26	51.63	33.94	12.39	1.17	6.006	51.90	27.05	24.85	32.48	99.13	97.67	2.943	3.086	6.029
9845	8.70	54.38	34.75	11.92	0.47	6.018	51.60	28.50	23.10	30.19	101.52	96.96	3.100	2.868	5.968
9847	7.84	49.00	39.25	10.97	0.38	6.177	53.10	25.68	27.42	35.84	99.60	96.19	2.793	3.405	6.198
9853	9.02	56.38	28.20	13.07	0.50	5.787	51.40	29.54	21.86	28.57	98.15	98.52	3.214	2.714	5.928
9858	9.27	57.94	27.25	12.97	0.58	5.780	51.20	30.36	20.84	27.24	98.74	98.73	3.303	2.588	5.891
332	6.77	42.31	46.83	8.99	0.46	6.701	57.13	22.17	34.96	45.69	98.59	97.45	2.412	4.341	6.753
338	6.45	40.31	47.86	8.46	1.09	6.801	57.40	21.12	36.28	47.42	97.72	97.28	2.298	4.505	6.803
357	7.68	48.00	37.58	10.83	0.40	6.504	55.11	25.15	29.96	39.16	96.81	98.39	2.736	3.720	6.456
360	7.33	45.81	41.84	10.06	0.36	6.684	56.20	24.00	32.20	42.09	98.07	98.32	2.611	3.999	6.610
2	7.496	46.85	39.86	10.71	0.71	6.476	54.95	24.55	30.40	39.73	98.13	98.00	2.670	3.774	6.444
4	7.014	43.84	43.66	9.58	0.96	6.599	56.50	22.97	33.53	43.82	98.04	98.20	2.499	4.163	6.662
565	8.004	50.03	39.24	10.94	0.06	6.322	54.25	26.22	28.03	36.64	100.27	97.67	2.852	3.481	6.333
563	8.227	51.42	36.89	11.07	0.18	6.241	53.61	26.94	26.67	34.86	99.56	97.53	2.931	3.312	6.243
567	8.41	52.56	36.21	11.23	0.18	6.231	53.53	27.54	25.99	33.97	100.68	97.94	2.996	3.227	6.223
Total	136.691	854.34	630.23	191.55	9.19	106.213	913.38	447.66	465.72	608.71	1685.31	1663.79	48.699	57.829	106.528
Average	8.041	50.26	37.07	11.27	0.54	6.248	53.73	26.33	27.40	31.81	99.14	97.87	2.865	3.402	6.266
								High Protein							
9466	10.90	68.13	14.34	14.83	0.67	5.299	47.20	35.70	11.50	15.03	97.97	98.66	3.883	1.428	5.311
9458	10.72	67.00	18.25	13.42	0.56	5.491	47.80	35.11	12.69	16.59	99.23	97.57	3.819	1.576	5.395
9570	10.29	64.31	19.18	15.39	0.89	5.389	47.70	33.70	14.00	18.30	99.77	98.89	3.666	1.739	5.405
9573	10.43	65.19	18.74	14.65	1.20	5.330	47.20	34.16	13.04	17.04	99.78	98.08	3.716	1.619	5.335
9846	11.41	71.31	14.42	13.92	0.70	5.309	47.20	37.37	9.83	12.85	100.35	98.78	4.065	1.221	5.286
9848	10.61	66.31	18.77	15.14	0.58	5.394	47.90	34.75	13.15	17.19	100.80	99.22	3.780	1.633	5.413
9854	11.18	69.88	13.48	14.46	0.60	5.300	47.10	36.62	10.48	13.70	98.42	98.64	3.983	1.302	5.285
9857	10.78	67.38	17.48	13.33	0.53	5.452	47.60	35.31	12.29	16.06	98.72	97.30	3.841	1.526	5.367
334	9.46	59.13	28.74	10.54	0.52	6.080	52.50	30.98	21.52	28.13	98.93	98.32	3.370	2.672	6.042
333	9.29	58.06	30.01	10.58	0.52	6.015	52.57	30.42	22.15	28.95	99.17	98.11	3.309	2.750	6.059
359	11.00	68.75	18.48	11.89	0.80	5.657	49.79	36.03	13.76	17.98	99.92	99.42	3.919	1.708	5.627
354	10.89	68.06	17.47	12.30	0.37	5.631	49.26	35.66	13.60	17.78	98.20	98.51	3.879	1.689	5.568
1	10.026	62.66	24.15	11.57	0.34	5.843	51.20	32.83	18.37	24.01	98.72	98.58	3.572	2.281	5.853
3	10.618	66.36	19.01	12.69	0.22	5.554	48.96	34.77	14.19	18.55	98.28	97.82	3.783	1.762	5.545
564	10.037	62.73	25.63	11.56	0.26	5.836	50.42	32.87	17.55	22.94	100.18	97.49	3.576	2.179	5.755
568	10.124	63.28	25.14	11.09	0.42	5.834	50.60	33.16	17.44	22.79	99.93	97.58	3.607	2.165	5.772
569	10.235	63.97	24.22	11.59	0.39	5.766	50.65	33.52	17.13	22.39	100.17	98.34	3.646	2.127	5.773
Total	178.000	1112.51	347.51	218.95	9.57	95.180	835.65	582.96	252.69	330.28	1688.54	1671.31	63.414	31.377	94.791
Average	10.471	65.44	20.44	12.88	0.56	5.599	49.16	34.29	14.86	19.43	99.33	98.31	3.730	1.846	5.576

TABLE 28.—INITIAL WEIGHTS AND GAINS.

Series	Duration of trial days	LP			HP			
		Animal No.	Initial weight, grams	Gain, grams	Animal No.	Initial weight, grams	Gain, grams	Difference in gain, grams
1D*	40	9460	30.635	38.643	9458	30.815	55.725	17.082
1H*	40	9461	30.870	34.736	9466	32.140	54.695	19.959
2D	24	9572	32.855	20.633	9573	33.730	28.625	7.992
2H	24	9574	32.800	16.934	9570	30.730	27.662	10.728
3D	40	9847	27.210	35.159	9848	30.775	53.091	17.932
3H	40	9845	29.389	32.909	9846	31.007	49.295	16.386
4D	48	9858	31.380	35.075	9857	31.580	54.742	19.667
4H	48	9853	29.016	33.156	9854	31.565	45.653	12.497
Average	38	----	30.519	30.906		31.543	46.186	15.280
5D	20	338	34.130	33.592	333	33.740	55.921	22.329
5H	20	332	34.700	35.449	334	35.930	55.498	20.049
6D	20	360	34.400	34.149	354	35.957	54.798	20.649
6H	20	357	35.575	30.179	359	36.793	47.932	17.753
7D	15	4	30.772	25.103	3	32.443	39.524	14.421
7H	15	2	30.917	23.921	1	32.932	39.202	15.281
8D	15	563	32.813	19.849	568	35.597	39.770	19.921
8D	15	567	33.839	21.083	569	33.968	39.613	18.530
8H	15	565	33.405	21.380	564	35.169	36.566.	15.186
Average	17.2		33.395	27.190		34.725	45.425	18.235
Grand Average	27		32.042	28.938		33.228	45.783	16.845

*D = digestion cage. H = Haldane chamber.

TABLE 29.—BALANCES OF NITROGEN, CARBON, AND ENERGY. A—LOW PROTEIN.

Animal No.	Nitrogen				Carbon				Energy			
	Consumed grams	Recovered grams	Difference		Consumed grams	Recovered grams	Difference		Consumed cals.	Recovered cals.	Difference	
			grams	per cent of intake			grams	per cent of intake			cals.	per cent of intake
Group 1												
9460	2.438	2.271	−.167	6.85	-----	-----	----	----	720.03	------	-----	----
9572	1.221	1.238	.017	1.39	-----	-----	----	----	337.69	------	-----	----
9847	2.216	2.089	−.127	5.73	-----	-----	----	----	637.04	------	-----	----
9858	2.626	2.721	.095	3.62	-----	-----	----	----	729.30	------	-----	----
Average	2.125	2.080	−.045	2.14	-----	-----	----	----	606.02	------	-----	----
Group 2												
338	1.623	1.627	.004	0.25	-----	-----	----	----	471.74	------	-----	----
360	1.623	1.622	−.001	0.06	-----	-----	----	----	471.74	------	-----	----
4	1.119	1.092	−.027	2.41	-----	-----	----	----	325.18	------	-----	----
563	1.065	1.097	.032	3.00	-----	-----	----	----	309.47	------	-----	----
567	1.077	1.146	.069	6.41	-----	-----	----	----	313.00	------	-----	----
Average	1.301	1.317	.015	1.18	-----	-----	----	----	378.23	------	-----	----
Group 3												
9461	2.429	1.950	−.479	19.72	69.22	67.08	−2.14	3.09	720.76	700.35	−20.41	2.83
9574	1.221	0.945	−.276	22.60	32.26	31.31	−0.95	2.94	337.83	332.38	− 5.45	1.61
9845	2.233	2.024	−.209	9.36	61.45	59.88	−1.57	2.55	641.76	625.65	−16.11	2.51
9853	2.626	2.489	−.137	5.22	69.38	68.10	−1.28	1.88	729.30	710.50	−18.80	2.58
Average	2.127	1.852	−.275	12.94	58.08	56.59	−1.49	2.56	607.41	592.22	−15.19	2.50
Group 4												
332	1.623	1.625	.002	0.12	45.00	44.70	−0.30	0.67	471.74	463.55	−8.19	1.74
357	1.623	1.612	−.011	0.68	45.00	44.80	−0.20	0.44	471.74	467.59	−4.15	0.88
2	1.119	1.230	.111	9.92	31.02	30.51	−0.51	1.64	325.18	318.84	−6.34	1.95
565	1.119	1.128	.009	0.80	31.02	31.12	0.10	0.32	325.18	323.52	−1.66	0.51
Average	1.371	1.399	.028	2.02	38.01	37.78	−0.23	0.60	398.46	393.38	−5.09	1.28

TABLE 29.—BALANCES OF NITROGEN, CARBON, AND ENERGY (Cont.) B—HIGH PROTEIN.

Animal No.	Nitrogen				Carbon				Energy			
	Consumed grams	Recovered grams	Difference		Consumed grams	Recovered grams	Difference		Consumed cals.	Recovered cals.	Difference	
			grams	per cent of intake			grams	per cent of intake			cals.	per cent of intake
Group 1												
9458	5.096	4.742	−0.354	6.95	-----	-----	----	----	746.43	------	-----	----
9573	2.378	2.261	−0.117	4.92	-----	-----	----	----	348.73	------	-----	----
9848	4.586	4.464	−0.122	2.66	-----	-----	----	----	666.08	------	-----	----
9857	5.147	5.238	0.091	1.77	-----	-----	----	----	754.47	------	-----	----
Average	4.302	4.176	−0.126	2.92	-----	-----	----	----	628.93	------	-----	----
Group 2												
333	4.259	4.235	−0.024	0.56	-----	-----	----	----	503.57	------	-----	----
354	4.259	4.222	−0.037	0.87	-----	-----	----	----	503.57	------	-----	----
3	2.936	2.953	0.017	0.58	-----	-----	----	----	347.12	------	-----	----
568	2.935	2.949	0.014	0.48	-----	-----	----	----	346.97	------	-----	----
569	2.934	3.014	0.080	2.73	-----	-----	----	----	346.92	------	-----	----
Average	3.465	3.475	0.010	0.29	-----	-----	----	----	409.63	------	-----	----
Group 3												
9466	5.096	3.084	−2.012	39.48	70.91	67.56	−3.35	4.72	746.50	712.51	−33.99	4.55
9570	2.379	1.464	−0.915	38.46	33.10	30.99	−2.11	6.37	348.96	330.54	−18.42	5.28
9846	4.586	3.747	−0.839	18.29	63.31	61.56	−1.75	2.76	666.08	647.61	−18.47	2.77
9854	5.147	4.288	−0.859	16.69	71.45	70.12	−1.33	1.86	754.47	738.56	−15.91	2.15
Average	4.302	3.146	−1.156	26.88	59.69	57.56	−2.13	3.58	629.00	607.31	−21.70	3.45
Group 4												
334	4.259	4.236	−0.023	0.54	46.94	46.36	−0.58	1.24	503.57	498.60	−4.97	0.99
359	4.259	4.272	0.013	0.31	46.94	46.66	−0.28	0.60	503.57	495.09	−8.48	1.68
1	2.936	2.765	−0.171	5.82	32.35	32.11	−0.24	0.74	347.12	341.00	−6.12	1.76
564	2.936	2.990	0.054	1.84	32.35	32.54	0.19	0.59	347.12	348.79	1.67	0.48
Average	3.598	3.566	−0.032	0.88	39.65	39.42	−0.23	0.57	425.35	420.87	−4.48	1.05

TABLE 30.—DIGESTIBILITY OF DRY MATTER, NUTRIENTS, AND ENERGY.

Low Protein						High Protein					
Animal No.	Dry Matter per cent	Protein per cent	N. F. E. per cent	Ether Ext. per cent	Energy per cent	Animal No.	Dry Matter per cent	Protein per cent	N. F. E. per cent	Ether Ext. per cent	Energy per cent
Group 1											
9460	95.65	88.36	98.49	97.59	96.39	9458	95.89	92.83	98.68	98.48	96.82
9572	95.09	88.19	98.45	97.85	96.31	9573	94.93	92.55	98.16	98.36	96.46
9847	93.10	86.99	98.10	99.25	96.45	9848	92.33	90.98	96.99	99.26	95.36
9858	92.81	85.07	98.01	98.66	96.13	9857	92.62	90.44	97.56	98.99	95.90
Average	94.03	86.96	98.24	98.38	96.32		93.82	91.58	97.83	98.83	96.10
Groups 2 and 4											
338	92.47	87.23	97.61	97.69	95.63	333	92.65	92.32	97.86	97.32	95.71
332	92.40	88.30	97.78	96.96	95.67	334	92.29	92.14	97.69	97.78	95.69
360	92.50	86.06	97.55	98.75	95.64	354	92.38	92.69	96.98	98.23	95.25
357	92.14	85.48	97.37	97.75	95.22	359	92.18	92.03	97.32	98.63	95.59
4	92.24	86.70	97.36	98.75	95.51	3	92.31	92.02	96.98	98.58	95.32
2	92.10	87.13	96.65	98.85	94.98	1	92.37	92.88	97.17	98.48	95.85
563	92.02	86.18	96.87	98.49	94.38	568	92.45	92.34	97.45	98.86	95.79
567	92.11	86.64	97.14	98.71	94.96	569	92.22	92.44	96.77	98.58	95.25
565	91.70	84.16	96.99	98.47	94.57	564	92.07	92.39	96.67	98.39	94.94
Average	92.22	86.49	97.31	98.19	95.24		92.33	92.35	97.25	98.26	95.50
Grand Avg.	92.97	86.69	97.71	98.26	95.68		92.94	92.08	97.51	98.48	95.74

TABLE 31.—THE ABSORPTION OF ENERGY.

Low Protein			High Protein		
	Energy Absorbed*			Energy Absorbed*	
Animal No.	Direct. calories	Calculated, calories	Animal No.	Direct, calories	Calculated, calories
Group 1—					
9460	694.07	694.88	9458	722.68	724.74
9572	325.24	325.16	9573	336.37	336.68
9847	614.40	617.04	9848	635.17	639.28
9858	701.08	698.59	9857	723.44	721.61
Average	583.70	583.92		604.42	605.58
Groups 2 and 4					
338	451.14	448.73	333	481.97	478.60
332	451.32	448.78	334	481.87	478.66
360	451.17	449.41	354	479.66	478.79
357	449.18	447.18	359	481.34	479.01
4	310.59	309.71	3	330.88	329.63
2	308.86	308.68	1	332.70	330.70
563	292.07	293.43	568	332.35	330.76
567	297.22	297.68	569	330.44	329.60
565	307.53	307.74	564	329.55	329.42
Average	368.79	367.93		397.86	396.13
Grand Average	434.91	434.39		461.42	460.58

*"Direct" refers to energy determination of feed and feces by the bomb calorimeter; "calculated" refers to the method of determining the nutrierts absorbed and multiplying by appropriate energy factors.

TABLE 32.—THE URINARY EXCRETION OF NITROGEN AND ENERGY.

Animal No.	Low Protein		Animal No.	High Protein	
	Urinary Nitrogen grams	Urinary Energy calories		Urinary Nitrogen grams	Urinary Energy calories
Group 1					
9460	1.038	14.01	9458	2.764	19.35
9572	0.583	7.87	9573	1.261	8.83
9847	0.891	12.03	9848	2.387	16.71
9858	1.251	16.89	9857	2.844	19.91
Average	0.941	12.70		2.314	16.20
Group 2					
338	0.645	8.71	333	2.183	15.28
360	0.562	7.59	354	1.966	13.76
4	0.375	5.06	3	1.482	10.37
563	0.399	5.39	568	1.436	10.05
567	0.407	5.49	569	1.510	10.57
Average	0.478	6.45		1.715	12.01
Group 3*					
9461	1.038	14.01	9466	2.764	19.35
9574	0.583	7.87	9570	1.261	8.83
9845	0.891	12.03	9846	2.387	16.71
9853	1.251	16.89	9854	2.844	19.91
Average	0.941	12.70		2.314	16.20
Group 4					
332	0.630	8.51	334	2.169	15.18
357	0.591	7.98	359	2.190	15.33
2	0.489	6.60	1	1.482	10.37
565	0.408	5.51	564	1.586	11.10
Average	0.530	7.15		1.857	13.00
Grand Average	0.708	9.56		2.030	14.21

*Urinary Nitrogen of Pair-mates on Digestion Trial.

TABLE 33.—HEAT PRODUCTION AS DETERMINED FROM PROTEIN METABOLISM AND RESPIRATORY EXCHANGE.

Animal No.	Urinary N gms.	Protein Oxidized gms.	O_2 Consumed gms.	CO_2 Liberated gms.	Due to protein oxidation		Non-protein O_2 gms.	Non-protein CO_2 gms.	Non-protein R. Q.	Heat Liberated from		
					O_2 gms.	CO_2 gms.				Protein cals.	Non-protein cals.	Total cals.
Group 3 LP												
9461	1.038	6.62	171.11	208.61	9.62	10.68	161.49	197.93	.891	30.32	555.69	586.01
9574	0.583	3.72	81.30	96.25	5.41	6.00	75.89	90.25	.865	17.04	259.29	276.33
9845	0.891	5.68	144.13	176.92	8.25	9.17	135.88	167.75	.897	26.01	468.44	494.45
9853	1.251	7.98	169.70	206.53	11.59	12.88	158.11	193.65	.890	36.55	544.16	580.71
Total	3.763	24.00	566.24	688.31	34.87	38.73	531.37	649.58	.889	109.92	1827.58	1937.50
Average	0.941	6.00	141.56	172.08	8.72	9.68	132.84	162.40	.889	27.48	456.90	484.38
Group 4 LP												
332	0.630	3.98	92.89	119.43	5.61	6.23	87.28	113.20	.943	17.95	304.10	322.05
357	0.591	3.74	101.60	127.05	5.27	5.85	96.33	121.20	.915	16.87	333.24	350.11
2	0.489	3.09	65.12	82.74	4.36	4.84	60.76	77.90	.932	13.94	211.16	225.10
565	0.408	2.58	70.70	87.78	3.64	4.04	67.06	83.74	.908	11.64	231.61	243.25
Total	2.118	13.39	330.31	417.00	18.88	20.96	311.43	396.04	.925	60.40	1080.11	1140.51
Average	0.530	3.35	82.58	104.25	4.72	5.24	77.86	99.01	.925	15.10	270.03	285.13
Group 3 HP												
9466	2.764	17.63	175.98	209.43	25.62	28.45	150.36	180.98	.875	80.75	515.34	596.09
9570	1.261	8.05	79.33	92.58	11.70	12.99	67.63	79.59	.856	36.87	230.52	267.39
9846	2.387	15.23	152.73	182.68	22.13	24.58	130.60	158.10	.880	69.75	448.21	517.96
9854	2.844	18.14	177.19	210.17	26.36	29.28	150.83	180.89	.872	83.08	516.53	599.61
Total	9.256	59.05	585.23	694.86	85.81	95.30	499.42	599.56	.873	270.45	1710.60	1981.05
Average	2.314	14.76	146.31	173.72	21.45	23.83	124.86	149.89	.873	67.61	427.65	495.26
Group 4 HP												
334	2.169	13.79	101.33	121.52	19.82	22.01	81.51	99.51	.888	62.88	280.12	343.00
359	2.190	13.93	110.26	131.34	20.02	22.23	90.24	109.11	.879	63.52	309.60	373.12
1	1.346	8.56	72.38	86.29	12.30	13.66	60.08	72.63	.879	39.03	206.09	245.12
564	1.586	10.09	74.13	87.20	14.50	16.10	59.63	71.10	.867	46.01	203.90	249.91
Total	7.291	46.37	358.10	426.35	66.64	74.00	291.46	352.35	.879	211.44	999.71	1211.15
Average	1.823	11.59	89.53	106.59	16.66	18.50	72.87	88.09	.879	52.86	249.93	302.79

TABLE 34.—HEAT PRODUCTION AND STORAGE OF ENERGY AS CALCULATED FROM ABSORPTION AND BALANCE DATA, GROUP 4.

Animal No.	Absorption of				Urinary Nitrogen gms.	Protein Oxidized gms.	Carbon Expired gms.	Urinary Carbon gms.	Total C Outgo gms.	Carbon Stored gms.	Nitrogen Stored gms.	Protein Stored gms.
	Carbon gms.	Nitrogen gms.	Fat gms.	N. F. E. gms.								
Low Protein												
332	43.18	1.431	14.68	61.35	0.630	3.98	32.57	0.81	33.38	9.80	0.801	5.01
357	42.98	1.384	14.80	61.09	0.591	3.74	34.65	0.89	35.54	7.44	0.793	4.96
2	29.61	0.974	10.32	41.79	0.489	3.09	22.56	0.64	23.20	6.41	0.485	3.03
565	29.46	0.940	10.28	41.94	0.408	2.58	23.94	0.78	24.72	4.74	0.532	3.33
Total	145.23	4.729	50.08	206.17	2.118	13.39	113.72	3.12	116.84	28.39	2.611	16.33
Average	36.31	1.182	12.52	51.54	0.530	3.35	28.43	0.78	29.21	7.10	0.653	4.08
High Protein												
334	45.07	3.919	14.95	45.60	2.169	13.79	33.14	1.19	34.33	10.74	1.750	10.94
359	44.98	3.913	15.08	45.43	2.190	13.93	35.82	1.44	37.26	7.72	1.723	10.77
1	31.08	2.697	10.38	31.26	1.482	9.43	23.53	1.01	24.54	6.54	1.215	7.59
564	30.85	2.709	10.37	31.10	1.586	10.09	23.78	1.27	25.05	5.80	1.123	7.02
Total	151.98	13.238	50.78	153.39	7.427	47.24	116.27	4.91	121.18	30.80	5.811	36.32
Average	38.00	3.310	12.70	38.35	1.857	11.81	29.07	1.23	30.30	7.70	1.453	9.08

Animal No.	Carbon in Protein Stored gms.	Carbon in Fat Stored gms.	Fat Stored gms.	Fat Oxidized gms.	Heat from Oxidation of			Total Heat Production cals.	Energy of Fat Stored cals.	Energy of Protein Stored cals.	Total Energy Stored cals.
					Fat cals.	Protein cals.	Carbohyd'te cals.				
Low Protein											
332	2.63	7.17	9.37	5.31	50.45	17.95	256.75	325.15	89.02	28.56	117.58
357	2.60	4.84	6.33	8.47	80.47	16.87	255.66	353.00	60.14	28.27	88.41
2	1.59	4.82	6.30	4.02	38.19	13.94	174.89	227.02	59.85	17.27	77.12
565	1.74	3.00	3.92	6.36	60.42	11.64	175.52	247.58	37.24	18.98	56.22
Total	8.56	19.83	25.92	24.16	229.53	60.40	862.82	1152.75	246.25	93.08	339.33
Average	2.14	4.96	6.48	6.04	57.38	15.10	215.71	288.19	61.56	23.27	84.83
High Protein											
334	5.73	5.01	6.55	8.40	79.80	62.88	190.84	333.52	62.23	62.36	124.59
359	5.64	2.08	2.72	12.36	117.42	63.52	190.12	371.06	25.84	61.39	87.23
1	3.98	2.56	3.35	7.03	66.79	43.00	130.82	240.61	31.83	43.26	75.09
564	3.68	2.12	2.77	7.60	72.20	46.01	130.15	248.36	26.32	40.01	66.33
Total	19.03	11.77	15.39	35.39	336.21	215.41	641.93	1193.55	146.22	207.02	353.24
Average	4.76	2.94	3.85	8.85	84.05	53.85	160.48	298.39	36.56	51.76	88.31

TABLE 35.—HEAT PRODUCTION AS CALCULATED BY THE METHOD OF DIFFERENCE, IN CALORIES.

Animal No.	Energy			Heat Liberated	Animal No.	Energy			Heat Liberated
	Absorbed	of Urine	Stored			Absorbed	of Urine	Stored	
Group 1—LP					Group 1—HP				
9460	694.07	14.01	86.07	593.99	9458	722.68	19.35	84.92	618.41
9572	325.24	7.87	55.00	262.37	9573	336.37	8.83	44.70	282.84
9847	614.40	12.03	90.61	511.76	9848	635.17	16.71	86.49	531.97
9858	701.08	16.89	75.17	609.02	9857	723.44	19.91	97.87	605.66
Total	2334.79	50.80	306.85	1977.14		2417.66	64.80	313.98	2038.88
Average	583.70	12.70	76.71	494.29		604.42	16.20	78.50	509.72
Group 2—LP					Group 2—HP				
338	451.14	8.71	119.77	322.66	333	481.97	15.28	119.07	347.62
360	451.17	7.59	101.68	341.90	354	479.66	13.76	95.79	370.11
4	310.59	5.06	78.70	226.83	3	330.88	10.37	60.93	259.58
563	292.07	5.39	53.15	233.53	568	332.35	10.05	75.27	247.03
567	297.22	5.49	54.30	237.43	569	330.44	10.57	72.10	247.77
Total	1802.19	32.24	407.60	1362.35		1955.30	60.03	423.16	1472.11
Average	360.44	6.45	81.52	272.47		391.06	12.01	84.63	294.42
Group 3—LP					Group 3—HP				
9461	694.74	14.01	72.19	608.54	9466	722.76	19.35	73.25	630.16
9574	325.36	7.87	35.62	281.87	9570	336.61	8.83	41.08	286.70
9845	618.98	12.03	79.62	527.33	9846	635.17	16.71	76.04	542.42
9853	701.08	16.89	70.85	613.34	9854	723.44	19.91	75.00	628.53
Total	2340.16	50.80	258.28	2031.08		2417.98	64.80	265.37	2087.81
Average	585.04	12.70	64.57	507.77		604.50	16.20	66.34	521.95
Group 4—LP					Group 4—HP				
332	451.32	8.51	112.57	330.24	334	481.87	15.18	118.72	347.97
357	449.18	7.98	86.94	354.26	359	481.34	15.33	84.41	381.60
2	308.86	6.60	70.82	231.44	1	332.70	10.37	72.04	250.29
565	307.53	5.51	57.11	244.91	564	329.55	11.10	70.21	248.24
Total	1516.89	28.60	327.44	1160.85		1625.46	51.98	345.38	1228.10
Average	379.22	7.15	81.86	290.21		406.37	13.00	86.35	307.03
Grand Total	7994.03	162.44	1300.17	6531.42		8416.40	241.61	1347.89	6826.90
Grand Avg.	470.24	9.56	76.48	384.20		495.08	14.21	79.29	401.58

TABLE 36.—HEAT PRODUCTION AS CALCULATED FROM NUTRIENTS ABSORBED AND SLAUGHTER DATA, LP.

Animal No.	Absorbed, gms.			Nutrients Stored, Oxidized, gms.					Heat Liberated, Calories from			
	Carbohydrate	Fat	Nitrogen	Nitrogen Stored	Nitrogen Catabolized	Protein Oxidized	Fat Stored	Fat Oxidized	Protein	Fat	Carbohydrate	Total
Group 1—LP												
9460	99.96	20.62	2.149	0.944	1.205	7.69	5.45	15.17	35.22	144.12	418.33	597.67
9572	46.36	9.56	1.074	0.508	0.566	3.61	3.74	5.82	16.53	55.29	194.02	265.84
9847	87.89	18.63	1.922	0.904	1.018	6.49	6.05	12.58	29.72	119.51	367.82	517.05
9858	100.21	20.57	2.226	1.070	1.156	7.38	3.96	16.61	33.80	157.80	419.38	610.98
Total	334.42	69.38	7.371	3.426	3.945	25.17	19.20	50.18	115.27	476.72	1399.55	1991.54
Average	83.61	17.35	1.843	0.857	0.986	6.29	4.80	12.55	28.82	119.18	349.89	497.89
Group 2—LP												
338	61.24	14.79	1.414	0.773	0.641	4.05	9.71	5.08	18.27	48.26	256.29	322.82
360	61.20	14.95	1.394	0.831	0.563	3.56	7.41	7.54	16.06	71.63	256.12	343.81
4	42.10	10.31	0.969	0.567	0.402	2.54	6.30	4.01	11.46	38.10	176.19	225.75
563	39.87	9.78	0.916	0.549	0.367	2.32	3.54	6.24	10.46	59.28	166.86	236.60
567	40.43	9.92	0.932	0.594	0.338	2.14	3.48	6.44	9.65	61.18	169.20	240.03
Total	244.84	59.75	5.625	3.314	2.311	14.61	30.44	29.31	65.90	278.45	1024.66	1369.01
Average	48.97	11.95	1.125	0.663	0.462	2.92	6.09	5.86	13.18	55.69	204.93	273.80
Group 3—LP												
9461	100.14	20.63	2.141	0.859	1.282	8.18	4.20	16.43	37.46	156.09	419.09	612.64
9574	46.39	9.56	1.074	0.406	0.668	4.26	2.16	7.40	19.51	70.30	194.14	283.95
9845	88.51	18.79	1.937	0.958	0.979	6.25	4.52	14.27	28.63	135.57	370.41	534.61
9853	100.21	20.57	2.226	0.960	1.266	8.08	4.00	16.57	37.01	157.42	419.38	613.81
Total	335.25	69.55	7.378	3.183	4.195	26.77	14.88	54.67	122.61	519.38	1403.02	2045.01
Average	83.81	17.39	1.845	0.796	1.049	6.69	3.72	13.67	30.65	129.85	350.76	511.25
Group 4—LP												
332	61.35	14.68	1.431	0.803	0.628	3.97	8.98	5.70	17.90	54.15	256.75	328.80
357	61.09	14.80	1.384	0.782	0.602	3.80	6.12	8.68	17.14	82.46	255.66	355.26
2	41.79	10.32	0.974	0.596	0.378	2.39	5.16	5.16	10.78	49.02	174.89	234.69
565	41.94	10.28	0.940	0.541	0.399	2.52	4.01	6.27	11.37	59.57	175.52	246.46
Total	206.17	50.08	4.729	2.722	2.007	12.68	24.27	25.81	57.19	245.20	862.82	1165.21
Average	51.54	12.52	1.182	0.681	0.502	3.17	6.07	6.45	14.30	61.30	215.71	291.30
Grand Total	1120.68	248.76	25.103	12.645	12.458	79.23	88.79	159.97	360.97	1519.75	4690.05	6570.77
Grand Avg.	65.92	14.63	1.477	0.744	0.733	4.66	5.22	9.41	21.23	89.40	275.89	386.52

TABLE 36 (Continued)—HIGH PROTEIN.

Animal No.	Absorbed, gms.			Nutrients Stored, Oxidized, gms.					Heat Liberates, Calories from			
	Carbohydrate	Fat	Nitrogen	Nitrogen Stored	Nitrogen Catabolized	Protein Stored	Fat Stored	Fat Oxidized	Protein	Fat	Carbohydrate	Total
Group 1—HP												
9458	83.69	20.80	4.724	1.606	3.118	19.89	2.61	18.19	91.10	172.81	350.24	614.15
9573	38.97	9.61	2.197	0.819	1.378	8.79	1.57	8.04	40.26	76.38	163.09	279.73
9848	72.82	18.78	4.164	1.655	2.509	16.01	2.89	15.89	73.33	150.96	304.75	529.04
9857	83.88	20.67	4.645	1.892	2.753	17.56	2.91	17.76	80.42	168.72	351.04	600.18
Total	279.36	69.86	15.730	5.972	9.758	62.25	9.98	59.88	285.11	568.87	1169.12	2023.10
Average	69.84	17.47	3.933	1.493	2.440	15.56	2.50	14.97	71.28	142.22	292.28	505.78
Group 2—HP												
333	45.68	14.88	3.927	1.720	2.207	14.04	6.21	8.67	64.02	82.37	191.17	337.56
354	45.27	15.02	3.942	1.939	2.003	12.74	2.61	12.41	58.09	117.90	189.45	365.44
3	31.20	10.39	2.697	1.232	1.465	9.32	1.76	8.63	42.50	81.99	130.57	255.06
568	31.34	10.41	2.706	1.284	1.422	9.04	2.95	7.46	41.22	70.87	131.16	243.25
569	31.12	10.38	2.709	1.279	1.430	9.09	2.80	7.58	41.45	72.01	130.24	243.70
Total	184.61	61.08	15.981	7.454	8.527	54.23	16.33	44.75	247.28	425.14	772.59	1445.01
Average	36.92	12.22	3.196	1.491	1.705	10.85	3.27	8.95	49.46	85.03	154.52	289.00
Group 3—HP												
9466	83.70	20.80	4.724	1.498	3.226	20.58	2.07	18.73	94.26	177.94	350.28	622.48
9570	39.00	9.61	2.198	0.714	1.484	9.47	1.61	8.00	43.37	76.00	163.22	282.59
9846	72.82	18.78	4.164	1.665	2.499	15.94	1.65	17.13	73.01	162.74	304.75	540.50
9854	83.88	20.67	4.645	1.596	3.049	19.45	1.80	18.87	89.08	179.27	351.04	619.39
Total	279.40	69.86	15.731	5.473	10.258	65.44	7.13	62.73	299.72	595.95	1169.29	2064.96
Average	69.85	17.47	3.933	1.368	2.565	16.36	1.78	15.68	74.93	148.99	292.32	516.24
Group 4—HP												
334	45.60	14.95	3.919	1.727	2.192	13.94	5.89	9.06	63.57	86.07	190.84	340.48
359	45.43	15.08	3.913	1.736	2.177	13.85	2.29	12.79	63.16	121.51	190.12	374.79
1	31.26	10.38	2.723	1.206	1.517	9.65	3.07	7.31	44.00	69.45	130.82	244.27
564	31.10	10.37	2.709	1.177	1.532	9.74	2.78	7.59	44.41	72.11	130.15	246.67
Total	153.39	50.78	13.264	5.846	7.418	47.18	14.03	36.76	215.14	349.14	641.93	1206.21
Average	38.35	12.70	3.316	1.462	1.855	11.80	3.51	9.19	53.79	87.29	160.48	301.55
Grand Total	896.76	251.58	60.706	24.745	35.961	229.10	47.47	204.11	1047.25	1939.10	3752.93	6739.28
Grand Avg.	52.75	14.80	3.571	1.456	2.115	13.48	2.79	12.01	61.60	114.06	220.76	396.43

TABLE 37.—THE QUANTITIES OF MATERIALS GAINED.

Low Protein								High Protein							
Rat No.	Empty Weight grams	Water grams	Protein grams	Fat grams	Ash grams	Energy		Rat No.	Empty Weight grams	Water grams	Protein grams	Fat grams	Ash grams	Energy	
						Gross cals.	Metab. cals.							Gross cals.	Metab. cals.
Group 1															
9460	36.84	24.06	5.90	5.45	1.16	86.07	78.72	9458	52.74	37.77	10.04	2.61	1.87	84.92	72.41
9572	19.20	11.25	3.18	3.74	0.73	55.00	51.04	9573	27.03	18.99	5.12	1.57	1.14	44.70	38.32
9847	32.90	19.32	5.65	6.05	1.16	90.61	83.57	9848	49.35	33.68	10.34	2.89	2.38	86.49	73.61
9858	32.94	20.74	6.69	3.96	1.38	75.17	66.83	9857	52.43	34.93	11.83	2.91	2.19	97.87	83.13
Average	30.47	18.84	5.36	4.80	1.11	76.71	70.04		45.39	31.34	9.33	2.50	1.90	78.50	66.87
Group 2															
338	33.00	16.98	4.83	9.71	1.02	119.77	113.75	333	54.45	35.27	10.75	6.21	1.92	119.07	105.68
360	32.08	18.13	5.19	7.41	1.21	101.68	95.21	354	53.53	36.50	12.12	2.61	2.13	95.79	80.69
4	24.99	14.22	3.54	6.30	0.83	78.80	74.29	3	38.48	27.32	7.70	1.76	1.48	60.93	51.34
563	19.06	11.13	3.43	3.54	0.78	53.15	48.88	568	38.98	26.39	8.03	2.95	1.32	75.27	65.26
567	19.57	11.45	3.71	3.48	0.82	54.30	49.68	569	38.60	26.28	7.99	2.80	1.40	72.10	62.14
Average	25.74	14.38	4.14	6.09	0.93	81.52	76.36		44.81	30.35	9.32	3.27	1.65	84.63	73.02
Group 3															
9461	32.87	21.86	5.37	4.20	1.15	72.19	65.50	9466	50.67	37.09	9.36	2.07	2.00	73.25	61.59
9574	15.40	9.96	2.54	2.16	0.66	35.62	32.46	9570	25.27	18.00	4.46	1.61	1.14	41.08	35.52
9845	31.05	18.83	5.99	4.52	1.18	79.62	72.16	9846	46.71	32.64	10.41	1.65	1.86	76.04	63.07
9853	30.97	19.46	6.00	4.00	1.32	70.85	63.37	9854	42.90	28.97	9.98	1.80	1.96	75.00	62.56
Average	27.57	17.53	4.98	3.72	1.08	64.57	58.37		41.39	29.18	8.55	1.78	1.74	66.34	55.69
Group 4															
332	33.91	18.44	5.02	8.98	1.09	112.57	106.32	334	53.95	35.17	10.79	5.89	1.85	118.72	105.28
357	29.38	17.08	4.89	6.12	1.21	86.94	80.85	359	46.84	32.03	10.85	2.29	1.77	84.41	70.89
2	23.55	13.56	3.73	5.16	0.96	70.82	66.17	1	38.68	26.63	7.54	3.07	1.36	72.04	62.65
565	20.88	12.53	3.38	4.01	0.80	57.11	52.90	564	35.06	23.30	7.36	2.78	1.32	70.21	61.04
Average	26.93	15.40	4.26	6.07	1.02	81.86	76.56		43.63	29.28	9.14	3.51	1.58	86.35	74.97
Grand Average	27.56	16.41	4.65	5.22	1.03	76.48	70.69		43.86	30.06	9.10	2.79	1.71	79.29	67.95

TABLE 38.—STORAGE OF ENERGY AS DETERMINED FROM THE BALANCE OF ENERGY ON THE LIVE ANIMAL.

Low Protein					High Protein				
Animal No.	Absorbed Energy cals.	Urinary Energy cals.	Heat Production cals.	Stored Energy cals.	Animal No.	Absorbed Energy cals.	Urinary Energy cals.	Heat Producion cals.	Stored Energy cals.
Group 3									
9461	694.74	14.01	586.01	94.72	9466	722.76	19.35	596.09	107.32
9574	325.36	7.87	276.33	41.16	9570	336.61	8.83	267.39	60.39
9845	618.98	12.03	494.45	112.50	9846	635.17	16.71	517.96	100.50
9853	701.08	16.89	580.71	103.48	9854	723.44	19.91	599.61	103.92
Total	2340.16	50.80	1937.50	351.86		2417.98	64.80	1981.05	372.13
Average	585.04	12.70	484.38	87.96		604.50	16.20	495.26	93.03
Group 4									
332	451.32	8.51	322.05	120.76	334	481.87	15.18	343.00	123.69
357	449.18	7.98	350.11	91.09	359	481.34	15.33	373.12	92.89
2	308.86	6.60	225.10	77.16	1	332.70	10.37	245.12	77.21
565	307.53	5.51	243.25	58.77	564	329.55	11.10	249.91	68.54
Total	1516.89	28.60	1140.51	347.78		1625.46	51.98	1211.15	362.33
Average	379.22	7.15	285.13	86.94		406.37	13.00	302.79	90.58
Grand Total	3857.05	79.40	3078.01	699.64		4043.44	116.78	3192.20	734.46
Grand Average	482.13	9.93	384.75	87.45		505.43	14.60	399.03	91.81