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FOREWORD

Dr. Leroy S. Palmer, Chief of the Division of Agricultural Biochemistry, passed away on March 4, 1944, leaving some of his research in animal nutrition unfinished. His death is a serious loss to nutrition research in general and particularly to the Minnesota Experiment Station project on inheritance of food utilization in animals. It is especially regrettable that he had not completed the organization of this bulletin and aided in the interpretation of all the data. However, before his death he had edited Part I and a portion of Part II of this bulletin.

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ACKNOWLEDGMENT

The assistance of O. E. Mydland is gratefully acknowledged in caring for the breeding rats of the high and low strains.

GENETIC DIFFERENCES IN THE BIOCHEMISTRY AND PHYSIOLOGY INFLUENCING FOOD UTILIZATION FOR GROWTH IN RATS

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INTRODUCTION

THE INTERPRETATION of nutrition experiments is frequently, if not usually, inconclusive because of the variability of the response of individual animals to identical nutritional regimes. Many results of nutrition experiments that have been interpreted as measuring differences in the nutritive value of different foods or diets of like character probably represent merely normal variations between the animals employed. Although biometric principles are being employed with increasing frequency in the interpretation of such experiments, this does not, of course, establish the cause of the variation between animals. In our search for this cause, it was fully appreciated that the problem involved is extremely complicated but there also seemed to be reasonable assurance that if the major factors determining the differences in food utilization by different animals, particularly by individuals of different strains in the same species, can be established, greater uniformity might be effected.

Starting in 1928 from the thesis that genetic factors control food utilization efficiency, we were able to separate from the progeny of a single pair of somewhat related rats two strains having uniformly different indices of efficiency in food utilization (*39*)[1]. At the same time we were able to show a marked sex difference in food utilization in rats.

For several years our major effort was confined to building up these two strains, one of which we designated the high efficiency strain and the other the low efficiency strain. Sib matings were practiced exclusively within each strain, pairs being selected for carrying on the strain which were as uniform as possible and conformed as closely as possible to the mean efficiency index of

[1] Italic figures in parentheses refer to literature cited.

the strain. After testing about 1,600 animals of both sexes in the first 11 generations of the high efficiency strain, the exclusive sib mating system of inbreeding was modified for this strain beginning with a group of animals in F_{12} whereby a combination of sib matings followed by backcrossing of the daughters and granddaughters to the sire was practiced. From the granddaughters' offspring a new sire was selected and sib matings followed by backcrossing were again repeated. Later the same system was adopted for the low efficiency strain.

By 1933 the two strains of rats had become so well established that studies were begun on the physiological and biochemical differences which could reasonably be associated with the marked difference in their efficiency of food utilization. At the same time inbreeding and selection were continued to make the animals in each strain as uniform as possible.

The first study was concerned with the physiological effects of anterior pituitary growth hormone on growth and food utilization in male and female rats of both strains (*40*). The hormone promoted growth and increased efficiency of food utilization in both strains within limits set by an inherent level which appeared to be under control of heredity and sex. Female rats were stimulated toward a male level of growth and efficiency of dry matter utilization. Strains of rats with a lower order of efficiency were affected less definitely toward the attainment of a higher efficiency level. A decreasing order of effect was exerted on the low efficiency female, the high efficiency female, and the low efficiency male; no effect was exerted on the high efficiency male.

This study was followed by the work reported in this bulletin.

I. Balance of Nitrogen and Energy During Growth and Carcass Composition of Two Strains of Rats Differing in Efficiency of Food Utilization[2]

Charles E. Calverley, Leroy S. Palmer, and Cornelia Kennedy

IT IS COMMON practice to express the efficiency of growing or fattening animals in terms of the food required to secure a unit gain. The value of rations is also compared in the same manner. However, when applied to animals this does not take into consideration differences between their maintenance requirement and is therefore strictly valid for comparative purposes only when animals of the same species, sex, and initial weight are growing or gaining at the same rate.

Some years ago it seemed to us (*42*) that a better expression of the efficiency of growing animals is

$$100 \times \frac{\text{(dry matter consumed)}}{\text{(gain in weight)}} \div \text{mean weight during period}$$

and we proposed to use it as the efficiency index of the animal when applied to a six-week period beginning (in the case of rats) at 60 grams live weight. There is essentially a straight line relation between food intake and gain for this species (especially for males) during this period of their life.

We found (*42*) that the rats of our stock colony, though inbred for several years, when fed a constant diet varied widely in their efficiency of food utilization when calculated in this manner. Starting with one pair of tested rats from this colony, 18 of their F_1 progeny were also tested. We (*39*) then crossed the latter in various combinations, securing 158 animals for test in F_2. Two pairs of these, differing widely in "efficiency index," were selected for further breeding by *sib* matings from which two strains were developed which, by the fifth generation, showed an average difference of about 40 per cent in "efficiency index." In this study the females in each strain were found to be less efficient than the males. This seemed to be related, in part, to a higher percentage of dry matter and fat in the female body. Later we found (*40*) that the major physiological difference between the strains apparently was not due to differences in pituitary growth

[2] The material for this section was taken from a thesis submitted by Charles E. Calverley in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1938.

hormone, although some improvement in efficiency index of the low efficiency strain followed injection of pituitary growth hormone preparation.

Brody (*9*) has expressed the belief that these differences might be only apparent, because the animals of the low efficiency strain store less protein and water and more fat than the animals of the high efficiency strain. It was his belief that the strain differences would disappear if the efficiency of the animals could be expressed on the basis of gross energetic efficiency using the equation

$$\text{Gross energetic efficiency} = \frac{\text{net energy of gain}}{\text{available food energy}}$$

Kleiber (*28*), on the basis of a theoretical consideration of the formula for gross (or total) efficiency, has stated that total efficiency is equal to the difference between a fairly constant net efficiency and the quotient B/U, B being the basal metabolism and U the food consumption in energy units. He has stated (*30*) further that the reciprocal relation, U/B, can be used as a reliable index of an animal's efficiency.

This investigation was undertaken to determine whether the differences in efficiency of food utilization apparently existing between the strains of rats referred to above and between the sexes in these strains, when expressed as efficiency index, were actual or whether such differences would disappear when studied in the light of actual energy and nitrogen balances obtained during the efficiency index period. Further, it was hoped that this study would shed further light on the essential physiological differences between the two strains and between the sexes in the strains.

PROCEDURE

General Plan

The method was to carry out nitrogen and energy balances according to the procedure of Forbes and his co-workers (*50*). Fifteen to 18 rats of each sex and of each strain were used. Starting at the weight of 60 grams, the rats were carried through a six-week feeding test during which time weekly records of body weight and food consumption were kept, and the animals were also on a continuous nitrogen and energy balance trial. At the close of the test the rats were killed and analyzed for moisture, ash, ether extract, and protein (N x 6.25). These results were compared with similar analyses of 30 control rats of each sex

and of each strain killed at 60 grams body weight. In this manner the composition and amount of the gains were determined. Using the food and weight records, and the fecal and urine analyses, it was possible to determine the digestibility and metabolizability of the rations, and to calculate the gross efficiency, efficiency index, and U/B ratio of the animals.

Selection and Handling of Animals

The animals used, both for the check analyses and for the balance and feeding trials, were selected from the regular stock colonies of the high and low efficiency strains which have been maintained in the laboratory since 1928. The rats, when selected, were as close as possible to the prescribed weight of 60 grams. The rats were usually given the experimental diet for two to three days before they were either killed as check animals or placed on the balance and feeding test. The latter were housed in individual round cages, with wire bottoms of half-inch mesh set in galvanized iron funnels (coated with special varnish) for the collection of urine. A sheet of 12-mesh copper screen directly under the cage bottom separated the feces from the urine. A piece of muslin fastened over the mouth of the funnel collected the small amounts of wasted food.

The rations were fed in improvised feeding cups, similar to the cup described by Forbes (*50*), made as follows: A Fisher (Franke-type) porcelain cup was placed in the center of a holder prepared by cutting off a pound coffee can of conventional shape at an incline. The cup was retained in place by fastening it to a piece of quarter-inch wire mesh which was laid over the whole assembly and hinged to the coffee can on the highest side. A hole in the center of the mesh permitted access to the food, while practically all waste fell through the mesh into the coffee can. The sloping top of the screen discouraged the rat from sleeping on the feeding assembly.

Composition and Analysis of Foods Used

At the start of the experiment the ration in use in the laboratory for the "efficiency index" test was that given by Morris, Palmer, and Kennedy (*39*). The percentage composition was as follows: commercial casein 35.0, tapioca dextrin 36.6, leaf lard 15.0, rendered, filtered butterfat 9.0, salt mixture 4.4. Daily supplements of 0.25 gram of dry, whole yeast, 0.9 gram of dried lettuce, and 0.5 gram of fresh beef liver were given. This ration was used at first since all previous work had been done while using it.

Since refusals of yeast and, more particularly, lettuce had always been encountered, an effort was made with the first series of rats to obviate this by mixing the daily allotments of yeast and lettuce with that amount of basal ration the animals were expected to consume. However, many rats refused to eat an average amount of ration apparently because of the unpalatability imparted to it by the dried lettuce. In the second series only the basal diet was fed *ad libitum* and refusals of the definite amounts of supplements offered noted as in the work already published (*39*), (*40*).

After the second series was underway the percentage composition of laboratory ration was changed to: commercial casein 35.0, tapioca dextrin 30.85, leaf lard 11.1, rendered, filtered butterfat 9.0, cold-pressed wheat germ oil 3.4, U.S.P. cod liver oil 0.5, salt mixture 4.4, whole dry brewers' yeast 5.75. Five-tenths gram fresh beef liver was fed daily as before. This diet was adopted for the third and final series of rats comprising most of the rats tested (10 of each sex and of each strain). Variability of food intake and wastage was much less when using this ration.

The rations and other articles of food were analyzed for nitrogen by the Kjeldahl procedure; gross energy was determined by means of an Atwood bomb calorimeter, the thermometer being standardized by burning both pure benzoic acid and pure sucrose. Moisture determinations were made to permit the calculation of efficiency indices on the dry matter basis.

Collection and Analysis of Excreta

Feces were removed from the copper screen daily, air dried at room temperature for one or two days, and then stored, in stoppered bottles, in a refrigerator until analyzed. It was found that drying at 65°C. in a vacuum oven for eight hours resulted in a negligible loss of nitrogen and permitted grinding, mixing, and sampling of the feces. The ground material was kept tightly stoppered until samples were withdrawn for nitrogen and gross energy determinations.

The urine passed through the metal funnels into 400 milliliter beakers. The bottom of the cage, the feces screen (after removal of the feces), and the funnel were washed daily with hot water slightly acidified with sulfuric acid. The urine in the beakers was kept acidified by means of a 1 per cent solution of sulfuric acid. The beakers were emptied approximately twice weekly and the urine solutions stored in a refrigerator at 4°C. Nitrogen was determined on the samples, but attempts to dry the urine to

permit gross energy determinations were unsuccessful. We were forced to calculate this from the nitrogen content. For this we adopted the value used by Johnson, Hogan, and Ashworth (27) for the same purpose, namely, 7 Calories per gram of nitrogen. We found that this agreed with two determinations made by the bomb calorimeter in cases where drying was accomplished without the loss of ammonia.

Analysis of Carcasses

Essentially the same procedure was used in the analysis of the check groups of rats and those carried through the balance and feeding test. Immediately after slaughter the alimentary tract of the animals was emptied and the carcass reweighed, then frozen to await analysis. The carcass was chopped while frozen and transferred for drying to a 300 milliliter tared aluminum, covered dish lined with filter paper. Loss of moisture was determined after preliminary drying in a steam-heated cabinet followed by heating in a vacuum oven at 105°C. The carcass was then removed from the dish for preliminary ether extraction. After determining the weight lost by the extraction the highly hygroscopic carcass residue was ground as quickly as possible in a Quaker hand burr mill and stored in a tightly stoppered bottle. Aliquot samples of the residue were analyzed for residual ether extract, nitrogen, and ash. After removal of the extracted carcass the dish and paper liner were reweighed to determine the weight of the blood which had soaked into the liner during drying. This was considered as protein. The gross energy of composite samples of the ether extract and of the protein of the extracted residue was determined and used in computing the energy value of the gains.

EXPERIMENTAL DATA

Composition of Control Animals

The mean results of the analyses of the 60-gram check rats on the fresh and dry basis are given in table 1. These results were subjected to analysis of variance using the procedure of Fisher (*17*), the data also being shown in the table.

They indicate a highly significant difference of moisture percentage between the check males and females, while the difference between strains is also significant. The young check males have a higher moisture percentage than the young check females.

Differences in ash content on the fresh basis seem very

Table 1. Mean Chemical Composition[1] and Analysis of Variance of Check Rats

FRESH BASIS

Group	Weight	Moisture	Ash	Protein	Ether extract
High efficiency	Grams		Per cent		
Males (30)[2]	56.2	71.23	2.82	17.41	8.45
Females (30)	55.9	69.84	2.87	17.60	9.64
Males and females	56.1	70.54	2.85	17.51	9.05
Low efficiency					
Males (30)	55.7	71.63	2.85	17.86	7.59
Females (30)	55.2	70.67	2.96	18.14	8.36
Males and females	55.5	71.15	2.91	18.00	7.98
Males, high eff. + low eff.	56.0	71.43	2.84	17.64	8.02
Females, high eff. + low eff.	55.6	70.26	2.92	17.87	9.00

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Moisture	Ash	Protein	Ether extract
Strains	1	11.57*	0.13*	7.86**	34.40**
Sexes	1	41.81**	0.14**	1.67*	28.86**
Interaction	1	1.71	0.03	0.05	1.37
Error	116	2.18	0.02	0.42	1.33

DRY BASIS

Group	Dry matter	Ash	Protein	Ether extract
High efficiency	Grams		Per cent	
Males (30)	16.17	9.47	60.56	29.31
Females (30)	16.86	9.45	58.41	31.16
Males and females	16.52	9.46	59.49	30.24
Low efficiency				
Males (30)	15.80	10.07	63.05	26.67
Females (30)	16.17	10.14	62.04	28.41
Males and females	15.99	10.11	62.55	27.54
Males, high eff. + low eff.	15.99	9.77	61.81	27.99
Females, high eff. + low eff.	16.52	9.80	60.23	29.79

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Dry matter	Ash	Protein	Ether extract
Strains	1	9.29**	12.47*	280.03**	268.30**
Sexes	1	7.49**	0.01	74.66**	131.61**
Interaction	1	1.03	0.04	9.68	3.87
Error	116	0.74	2.11	10.69	10.50

[1] Empty carcass.
[2] Figures in parentheses indicate the number of rats used.
* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

significant, but these conclusions are not borne out on the dry basis. The data indicate that the females of the low efficiency strain probably differ significantly from all other check animals.

Highly significant differences in protein percentage of the check animals exist between the strains rather than between sexes. These differences are accentuated when calculated on the dry basis. The young high efficiency check rats have a lower protein percentage than the young low efficiency check rats.

Differences in percentage of ether extract are highly significant between all groups of check rats. The high efficiency check rats are fatter than the low efficiency check rats of the same sex, and in each strain the females are fatter than the males.

Composition of Test Rats

The results of the analyses of the rats carried through the feeding and balance test are given in table 2. These results were

Table 2. Mean Chemical Composition[1] and Analysis of Variance of Test Rats

FRESH BASIS

Group	Weight	Moisture	Ash	Protein	Ether extract
High efficiency	Grams		Per cent		
Males (15)[2]	255.7	61.72	2.73	19.88	15.70
Females (16)	183.1	58.69	3.12	18.64	19.66
Males and females	219.4	60.21	2.93	19.26	17.68
Low efficiency					
Males (17)	192.7	64.26	2.97	20.43	12.34
Females (18)	138.4	62.95	3.41	19.81	13.96
Males and females	165.6	63.61	3.19	20.12	13.15
Males, high eff. + low eff.	224.2	62.99	2.85	20.16	14.02
Females, high eff. + low eff.	160.75	60.82	3.27	19.23	16.81

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Moisture	Ash	Protein	Ether extract
Strains	1	193.37**	1.16**	12.36**	343.42**
Sexes	1	74.22**	2.88**	13.65**	122.92**
Interaction	1	11.28	0.05	1.31	21.32
Error	62	5.27	0.03	0.37	7.07

DRY BASIS

Group	Dry matter	Ash	Protein	Ether extract
High efficiency	Grams		Per cent	
Males (15)	97.88	7.18	52.28	40.61
Females (16)	74.90	7.58	45.27	47.42
Males and females	86.39	7.38	48.78	44.02
Low efficiency				
Males (17)	68.87	8.32	57.35	34.30
Females (18)	51.28	9.23	53.63	37.51
Males and females	60.08	8.78	55.49	35.91
Males, high eff. + low eff.	83.38	7.75	54.82	37.46
Females, high eff. + low eff.	63.09	8.41	49.45	42.47

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Dry matter	Ash	Protein	Ether extract
Strains	1	11764.95**	32.34**	754.11*	1098.37**
Sexes	1	6383.02**	7.37**	458.65**	397.26**
Interaction	1	129.78	1.81	38.21	50.17
Error	62	48.38	0.43	16.84	20.46

[1] Empty carcass.
[2] Figures in parentheses indicate the number of rats used.
* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

likewise subjected to the variance analysis, also shown in the table.

At the end of the feeding period the differences between the means of the moisture percentage of all classes are highly significant, showing that the strain difference has become more evident. Rats of the low efficiency strain have a higher moisture percentage than those of the high efficiency strain. Within each strain males have a higher moisture percentage than females.

On the other hand, highly significant differences between the mean protein percentage of all classes indicate that the sex difference has become more evident, in addition to the strain difference found in the 60-gram rats. On either the fresh or dry basis, rats of the low efficiency strain have a higher protein percentage, and within each strain males have a higher protein percentage than females. Protein declined in relation to total dry matter as the animals became mature, owing to the increase in ether extract.

The means of the ether extract of the test animals show the same order of significance as those of the check animals, i.e., there is a highly significant difference between all classes. It is evident that the greater initial fatness of the high efficiency animals increases with age, especially in the case of the females. On the dry basis these increases are considerably magnified and it is evident that the fat has been deposited almost exclusively at the expense of the protein and ash. Thus the indication obtained in the earlier study by Morris, Palmer, and Kennedy (*39*), that the sex difference in efficiency is related in part to the higher fat content of the females, is confirmed. It is evident, however, from the present data that this is a physiological characteristic which is fully manifested as early as the fourth week of life. From a statistical standpoint it does not become any greater with advancing age.

The differences of the means of the ash analyses of the test animals are also highly significant. In general the females have a higher ash percentage than the males, this being especially evident on the dry basis.

Thus as the rats grow they have a tendency to become fatter, the high efficiency much more so than the low efficiency rats, and the females of each strain more so than the males. The protein trend is almost exactly the opposite; on the fresh basis the percentage of protein increased slightly more in the low efficiency rats and more in the males than in the females; on the dry basis the percentage of protein decreased less in the low efficiency rats

and decreased less in the males than in the females. The bodies of all rats become drier with age, as would be expected, the high efficiency rats of both sexes increasing more in dry matter percentage than the low efficiency rats, the females of the two strains showing a greater difference than the males. There was no uniform sex difference in this respect within the strains.

Results of Balance Trials

In nitrogen and energy balances as carried out in this study, the percentage of nitrogen accounted for is the criterion of exactness of the balance. The average recovery of nitrogen for all rats tested was 97.95 per cent. The nitrogen of the shed hair was not determined. It is felt that the recoveries were high enough to insure the validity of the balances.

In studying the results of the balances it is immediately noticed that there is considerable divergence in the apparent digestibility of nitrogen and energy between the rats of the different feeding groups in the several series. This is shown in table 3. It can readily be seen that in the first series (fed the supplements admixed with basal ration) the apparent digestibility of both the energy and nitrogen is lower than in the second series when lettuce, yeast, and liver supplements were fed separately, and still lower than in the third series when the newer basal diet was fed and liver was the only supplement. For this reason the biometric study of the results of the balances was confined to the third series comprising 10 animals of each sex and of each strain.

The partition of food energy, as given by the data for the third series, is shown in table 4 together with the analysis of variance of the results. This indicates a high degree of significance between the four groups in regard to their digestion of the ration. Further study indicated that in reality the males of the

Table 3. Mean Apparent Digestibility of Energy and Nitrogen of Various Diets by the Several Groups

Group	First series[1]		Second series[2]		Third series[3]	
	Energy	Nitrogen	Energy	Nitrogen	Energy	Nitrogen
			Per cent			
High efficiency males	92.17	86.13	92.50	91.95	94.95	94.19
High efficiency females	92.15	83.08	93.80	92.45	95.40	93.77
Low efficiency males	91.77	88.43	93.08	92.25	95.53	94.30
Low efficiency females	91.78	86.33	94.68	93.10	95.43	93.75

[1] First series: Those fed basal diet admixed with supplements.
[2] Second series: Those fed supplements separately.
[3] Third series: Those fed modified basal diet with liver supplement only.

Table 4. Mean Partition and Analysis of Variance of Food Energy

Group	Proportion in feces	Calculated digestible	Proportion in urine	Calculated metabolizable	Proportion in body gain	Heat loss
			Per cent			
High efficiency						
Males (10)[1]	5.05	94.95	4.34	90.61	23.21	67.40
Females (10)	4.60	95.40	4.98	90.42	20.44	69.98
Males + females	4.83	95.18	4.66	90.52	21.83	68.69
Low efficiency						
Males (10)	4.47	95.53	4.64	90.89	17.08	73.81
Females (10)	4.57	95.43	5.25	90.18	13.87	76.31
Males + females	4.52	95.48	4.95	90.54	15.48	75.06
Males, high eff. + low eff.	4.76	95.24	4.49	90.75	20.15	70.61
Females, high eff. + low eff.	4.59	95.42	5.12	90.30	17.16	73.15

Analysis of variance

Variation due to	Degrees of freedom	Mean squares: Digestible fraction	Urinary fraction	Metabolizable fraction	Gain	Heat loss
Classes	3	0.66**	1.57**	0.90*	164.37**	156.78**
Strains	1	0.93**	0.81**	0.01	403.22**	405.79**
Sexes	1	0.31	3.91**	2.03**	89.40**	64.54**
Interaction	1	0.76	0.01	0.68	0.48	0.04
Error	36	0.08	0.02	0.09	4.80	4.98

[1] Figures in parentheses indicate the number of rats used.
* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

high efficiency strain differed significantly from all other groups in having a somewhat lower ability to digest the total energy of the ration employed.

So far as the urinary excretion of food energy is concerned, the calculations show that the difference between the sexes is highly significant, but that the difference between the strains within each sex is without significance. The females excreted a significantly higher amount of the energy intake by the urine.

While the statistical analysis points to some significance of the differences with regard to the proportion of the food energy which became available for metabolism, perusal of the data and further study indicate that in reality this significance is primarily between the males and females of the low efficiency group.

The analyses of the gain and of the heat loss (maintenance, voluntary activity, and heat increment) bear an almost reciprocal relation to each other inasmuch as the metabolizable fractions are so nearly identical. Results for both components indicate that all differences are highly significant. The high efficiency strain animals stored a larger proportion of their food energy and lost less heat than did the low strain animals. The males of each strain excelled the females in energy storage and lost less energy as heat.

To summarize, differences in proportion of digestible and metabolizable food energy and urinary excretion of energy are not large although statistically significant in some cases. The variations in gains are very much larger and highly significant. The variations in heat loss are undoubtedly the most important, and account for a part of the efficiency difference between the two strains.

The partition of food nitrogen and the analyses of the variances are given in table 5. The biometric study reveals that the males in each strain showed a small, but significant, superiority in their ability to digest food nitrogen. However, the differences between the classes with regard to the urinary excretion of nitrogen are highly significant. Further study shows this significance to be due both to a highly significant strain difference and especially to the difference between sexes. Thus the males of both strains eliminated a very significantly lower proportion of the ingested nitrogen in the urine than the females, and each sex of the high efficiency strain eliminated a highly significantly lower proportion of the food nitrogen in the urine than the corresponding sex of the low efficiency strain animals.

In so far as the nitrogen gains are concerned all differences between the groups are also highly significant. The males combined somewhat better digestibility with a highly significantly

Table 5. Mean Partition and Analysis of Variance of Food Nitrogen

Group		Proportion in feces	Calculated digestible	Proportion in urine	Proportion in gains
High efficiency			Per cent		
Males	(10)[1]	5.81	94.19	63.17	28.40
Females	(10)	6.23	93.77	71.46	20.29
Males + females		6.02	93.98	67.32	24.35
Low efficiency					
Males	(10)	5.70	94.30	66.67	25.34
Females	(10)	6.25	93.75	74.04	17.88
Males + females		5.98	94.03	70.36	21.61
Males, high eff. + low eff.		5.76	94.25	64.92	26.87
Females, high eff. + low eff.		6.24	93.76	72.75	19.09

Analysis of variance

Variation due to	Degrees of freedom	Mean squares: Digestible fraction	Urinary fraction	Gain
Classes	3	0.80	250.17**	227.31**
Strains	1	0.02	132.87**	74.80**
Sexes	1	2.35*	613.09**	606.06**
Interaction	1	0.05	4.61	1.06
Error	36	0.39	3.75	3.36

[1] Figures in parentheses indicate the number of rats used.
* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

lower percentage urinary excretion to yield higher percentages of gain than the females; and the same tendency for the high efficiency strain to eliminate less of the food nitrogen in the urine is reflected in a highly significantly better percentage of food nitrogen stored for the high efficiency animals within each sex.

Gross Efficiencies and Character of Gains

Inspection of individual data pertaining to the partition of metabolizable food energy revealed no constant or marked differences between comparable rats of the three series. Table 6 gives the partition of the metabolizable food energy for the entire group of animals. Biometric analyses were made on the data from all three series within each sex and strain group.

Differences in the percentage of metabolizable food energy found in the total gain (gross efficiency) are all highly significant, the gross efficiency of the "high" strain being much greater than that of the "low" strain. The gross efficiency of the males is significantly greater than that of the females within each strain.

As the data indicate, the differences between the means of ether extract stored by the strains are highly significant, but the differences between the sexes within each strain are without significance. This confirms a fact already pointed out, namely, that the sex differences in body content of ether-extractable substances are not accentuated during the period of growth of these

Table 6. Mean Partition and Analysis of Variance of Metabolizable Food Energy

Group	Total gain	Gained as ether extract	Gained as protein	Heat loss
		Per cent		
High efficiency				
Males (15)[1]	25.13	14.59	10.47	74.87
Females (16)	22.74	15.18	7.56	77.26
Males + females	23.94	14.89	9.02	76.07
Low efficiency				
Males (17)	19.56	10.07	9.49	80.44
Females (18)	16.06	9.18	6.86	83.94
Males + females	17.81	9.63	8.18	82.19
Males, high eff. + low eff.	22.35	12.33	9.98	77.66
Females, high eff. + low eff.	19.40	12.18	7.21	80.60

Analysis of variance

Variation due to	Degrees of freedom	Mean squares: Total gain	Gained as ether extract	Gained as protein	Heat loss
Classes	3	256.82**	156.10**	45.52**	256.82**
Strains	1	619.11**	458.74**	11.11**	619.11**
Sexes	1	145.31**	0.56	124.93**	145.31**
Interaction	1	6.05	9.00	0.52	6.05
Error	62	7.20	6.85	0.60	7.20

[1] Figures in parentheses indicate the number of rats used.
** Highly significant—exceeds the 1 per cent point.

strains of rats. The high efficiency strain rats show a much greater proportion of metabolizable food energy gained as ether extract than the low efficiency strain.

On the other hand, the proportion of metabolizable food energy gained as protein differs in a highly significant manner between the sexes and to a lesser degree, yet very significantly, between the strains. The greater percentage of energy gain as protein exhibited by the males follows logically from the greater percentage of nitrogen retention previously noted.

The high efficiency males, combining both the superior energy storage as ether extract of the high efficiency strain and the better energy storage as protein of the males, are the most efficient class. The females of the same strain have a lower efficiency owing to their poorer energy storage in the form of protein. Although the males of the low efficiency strain store energy as protein fairly well, they fall far behind the high efficiency group in the storage of energy as ether extract. The low efficiency females are the least efficient of all groups because of the combination of lesser storage of energy as ether extract characteristic of the strain and the poorer storage as protein characteristic of the sex.

In order to determine the reasons for the differences in gains existing between the strains and sexes a series of correlation coefficients was calculated. Table 7 gives the correlation found between the percentage distribution of the various components of metabolizable energy. Gross efficiency could not be correlated with heat loss since in the method employed for determining energy balances heat loss was computed by difference. The data represent animals of all series.

The correlations between percentage of metabolizable food energy gained in all forms and the percentage gained as ether extract are highly significant in all cases and indicate that a great deal of the variation in gross efficiency really represents

Table 7. Correlation Coefficients of the Percentage Distribution of the Components of Metabolizable Food Energy

Group	No.	$r_{TE.FE}$[1]	$r_{FE.HL}$[1]	$r_{PE.HL}$[1]
High efficiency males	15	+.985**	−.951**	−.011
High efficiency females	16	+.974**	−.973**	−.156
Low efficiency males	17	+.970**	−.879**	−.212
Low efficiency females	18	+.936**	−.934**	−.410
All animals	66	+.927**	−.922**	−.511**

[1] TE=Total energy gained.
PE=Gained as protein.
FE=Gained as fat ether extract.
HL=Lost as heat.
** Highly significant—exceeds the 1 per cent point.

differences in energy stored as ether extract. The correlation between percentage metabolizable energy gained as ether extract and the percentage lost as heat is also highly significant in all cases. This would indicate that one of the elements of heat loss (maintenance, voluntary activity, or heat increment) is also of great importance in the gross efficiency differences. The relation of the percentage metabolizable energy gained as protein to the percentage lost as heat is not of any importance although the value for all animals is highly significant.

The correlations between the actual amount of metabolizable energy stored as protein and the nitrogen excreted in the feces and urine are shown in table 8. Since no significant values were obtained, it appears that the only differences between either the strains or the sexes within the strains with respect to their efficiency of protein storage are those shown in tables 5 and 6. The ration furnished a surplus of protein, much above the amino acids required, so that all the animals eliminated two and one-half to three times as much nitrogen in the urine as was stored as new protein. Under such conditions significantly different correlations between nitrogen stored and nitrogen excreted would hardly be expected.

In another study Palmer and Kennedy tested the effect of the protein supply on the efficiency index of the two strains of rats by reducing the protein level of the standard diet from 35 per cent to 18 per cent. Data from 60 male rats of each strain were obtained. The usual regime was further modified in this experiment by allowing all the rats a standardized allotment of ration which was increased from week to week as the animals grew older, and which was based on the voluntary consumption of the ration by the large number of male rats of the lower efficiency strain. Only those animals that completely consumed the food offered were included in the statistical study. The experiment thus consisted of two sets of 30 pairs of animals, representing the two strains, all of which consumed the same total

Table 8. Correlation Coefficients of the Energy Gained as Protein with Nitrogen Lost in the Excreta

Group	No.	$r_{GP.FN}$[1]	$r_{GP.UN}$[1]
High efficiency males	10	+.004	+.340
High efficiency females	10	−.083	+.438
Low efficiency males	10	+.251	+.350
Low efficiency females	10	−.029	+.055

[1] GP=Energy gained as protein.
FN=Fecal nitrogen.
UN=Urinary nitrogen.

Table 9. Effect of Reducing Protein Consumption During Growth on Equalized Total Energy Intake Diets on Efficiency Index of Male Rats of High and Low Efficiency Index During Growth

Strain	Mean efficiency index 35 per cent protein	Mean efficiency index 18 per cent protein
High	1.28	1.59
Low	1.80	2.19

amount of energy, but one half of each set of pairs consumed approximately one half as much protein as their pair mates.

The results are shown in table 9. It is seen that not only was the controlled food intake without influence on the difference between the strains with respect to the efficiency index but that the reduced protein level increased the index value (reduced the efficiency) materially in both strains. Inasmuch as the mean difference in this effect seemed to indicate a slightly greater effect of the lowered protein on the "high" strain than on the "low" strain the differences were tested for significance. Students' t value was found to be 1.1785 and the corresponding probability (P) of the significance 0.2872. The odds are thus only 2.5 to 1 that the differences would be repeated in another similar experiment.

This experiment may not be considered as lending support to the balance data in table 5 showing that the strains do exhibit significant differences in efficiency of protein metabolism. Whether differences would be found in efficiency index due to differences in protein metabolism by further reduction in protein intake remains to be determined.

Validity of Certain Criteria as Indices of Gross Efficiency

Kleiber (*28*) proposed the U/B ratio, already referred to, as an index of gross efficiency. Later (*29*) he criticized the efficiency index employed in this laboratory. It seemed desirable to study the relation of these criteria in the case of our animals. The efficiency indices were computed by means of the equation already given. The U/B ratios were calculated from the total Calorie intake and the basal metabolism was computed from the average weight during the experimental period using Brody's (*9*) formula:

$$B\ M = 70.5\ W^{.734},$$

when W is expressed in kilograms. The several values together with the appropriate correlation coefficients are given in table 10.

Table 10. Correlation Between Gross Efficiency (G.E.) and Other Measures of Efficiency

Group	No.	Gross efficiency (G.E.)	Efficiency index (E.I.)	U/B ratio (U.B.)	$r_{GE.EI}$	$r_{GE.UB}$
High efficiency male	15	25.13	1.28	3.06	—.601*	—.115
High efficiency female	16	22.74	2.17	3.08	—.684**	—.010
Low efficiency male	17	19.56	1.92	2.83	—.066	—.305
Low efficiency male	15[1]	19.38	1.82	2.86	—.550*	—.475
Low efficiency female	18	16.06	3.36	2.87	—.693**	—.192

[1] Excepting two animals that made poor gains in body weight.
* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

Except for the low efficiency males, the correlation coefficients of gross efficiency with efficiency index are either significant or highly significant. In the case of the low efficiency males inspection of the data revealed that the low correlation was caused by animals 201-1 and 201-2 which made exceptionally small weight gains to give a low efficiency as judged by the high index values 2.46 and 2.80, while the gains made were sufficiently high in ether extract to give a normal gross efficiency. When these two animals are omitted from the correlation, the $r_{GE.EI}$ becomes significant and although $r_{GE.UB}$ increases it does not become significant. The correlations indicate that the efficiency index is a more reliable guide of energetic efficiency, at least for growing rats, than the ratio of the energy consumed to the basal metabolism. This is further borne out by the fact that the correlation coefficients of U/B ratio with gross efficiency are in no case statistically significant.

DISCUSSION

The study has demonstrated beyond question that fundamental differences exist between the two strains of rats. These are apparent in the initial and final analyses of body composition, the character of the gains made, and the gross efficiency of the animals.

The differences apparent in body composition at 60 grams, which are accentuated during the subsequent growth period on an entirely different diet, would seem to indicate that this trend toward a specific body composition is under genetic control. Brody's (9) supposition, that chemical analysis would show less storage of water and protein and more storage of fat by the low efficiency strain, is not supported by the results presented. Indeed, the low efficiency strain not only started the test period with a higher percentage of water and protein and a lower percentage

of fat but these differences became greater as the animals became more mature.

The outstanding difference between strains in gross efficiency is primarily due to differences in ether extract storage and relative gain in body protein. The failure to store ether extract is undoubtedly reflected in greater heat losses. Perhaps the moderately high fat diet is better adapted to the needs of the high efficiency strain with their tendency toward a greater content of ether extract. According to the views of Mitchell and Hamilton (*24, 38*) this would result in a lessened heat increment, a component of heat loss, thus yielding higher gross efficiencies. The greater relative gain in body protein of the high efficiency strain is accompanied by less nitrogen loss in the urine. The enhanced gross efficiency of males in comparison with the females within each strain is due in large part to better protein storage, and lesser excretion of urinary nitrogen. This may indicate a relatively higher metabolic rate for the females.

The efficiency index dry matter consumed per unit gain per 100 grams body weight was found to be satisfactory in judging the gross energetic efficiency of the animals employed in this study. In the two cases in which it failed, the animals made such small total body gains as to be regarded as quite unsatisfactory although their gross efficiency in converting food energy into body fat was not abnormal. From a practical standpoint classification of growing animals by this efficiency index is evidently to be preferred.

SUMMARY AND CONCLUSIONS

Two strains of rats differing in their efficiency of food utilization differ significantly in their chemical composition throughout the period of rapid growth. The high efficiency strain rats have a higher percentage of ether extract and a lower percentage of protein than do the low efficiency strain rats. This is apparent both on the fresh and on the dry matter basis. These differences are clearly of hereditary origin and probably would be manifested on any other uniform diet fed to the two strains.

The fresh as well as the dry carcass of the female rats of both strains has a higher percentage of ether extract and a lower percentage of protein than that of the males of the same strain.

The differences found in digestibility of the rations fed are not large and do not involve the strains. The high efficiency males exhibit a highly significantly lower apparent digestibility of

energy than the low efficiency males. These animals probably consumed more food in relation to the capacity of their digestive tract than the other groups.

The differences found in the percentage of food energy available for metabolism do not involve the strains. However, there is a small but significant sex difference in the low efficiency strain, the males showing a higher percentage of food energy available for metabolism than the females.

The high efficiency strain rats show considerably greater efficiency in the storage of food energy. Within each strain the males are more efficient in this respect than the females.

The high efficiency strain rats store more energy as ether extract and protein than the low efficiency strain. These variations seem to be due predominantly to differences in the heat increment of the strains, although individual variations may be due to other causes.

The males of each strain store relatively more food energy as protein than do the females. A higher percentage utilization of nitrogen seems responsible for this.

Our efficiency index is shown to have been satisfactory for predicting the gross energetic efficiency of the animals in this study.

The U/B ratio of Kleiber is shown not to be reliable for predicting the gross energetic efficiency of the strains of rats employed in this investigation.

ADDENDUM[3]

Calverley's studies on the chemical composition of rats of the high and low efficiency strains were made on animals fed *ad libitum*. Since the completion of his work we have extended his study to obtain body composition data of 30 male rats in each of the two strains, already described by him, which have eaten exactly the same quantity of our standard test ration. These rats were analyzed for moisture, protein, ether extract, and ash at the end of our standard six weeks efficiency period and a statistical study made to determine the significance of the difference between the two strains with respect to body composition, gains, body weight efficiency index, dry matter efficiency index, and energy efficiency index.

[3] These data were taken from a thesis submitted by Richard W. Luecke to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Master of Science, June, 1941. The paper was prepared by Cornelia Kennedy.

Results

The chemical composition of the body of each animal at the start of the test period was assumed to be the same as that determined for male animals of the two strains in Calverley's study. His experimental and analytical technique have been closely followed. The mean chemical composition of the rats at the beginning of the efficiency test period on both the fresh and dry matter basis as calculated from Calverley's data is given in table I, together with their biometric analysis. The data in this table are for male rats and therefore differ somewhat from that in the tables in Part I, pages 12 and 13, which include data for both male and female rats. The only significant differences in the percentage composition of the 60-gram male rats of the two strains on a fresh basis are in protein and ether extract, and on the dry matter basis, in ash, protein, and ether extract. Table II shows data of the same nature for male rats at the end of the six-week efficiency test period. The low efficiency strain rat on a fresh basis still has a highly significantly greater percentage of protein than the high efficiency strain rat, but the moisture is highly significantly greater in the high strain rat. Differences in percentage of ether extract are no longer significant. However,

Table I. Mean Chemical Composition of Male Rats at Beginning of Efficiency Test

FRESH BASIS

Group	Empty weight	Moisture	Ash	Protein	Ether extract
	Grams		Per cent		
High efficiency males	55.80	71.23	2.82	17.41	8.45
Low efficiency males	55.24	71.63	2.85	17.87	7.60

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Moisture	Ash	Protein	Ether extract
Strains	1	2.35	.0184	3.13**	11.03**
Error	58	0.72	.014	0.46	.23

DRY BASIS

Group	Dry matter	Ash	Protein	Ether extract
	Grams	Per cent		
High efficiency males	16.17	9.81	60.56	29.31
Low efficiency males	15.80	10.07	63.05	26.67

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Dry matter	Ash	Protein	Ether extract
Strains	1	2.06	1.02**	92.80**	93.95**
Error	58	0.83	0.14	9.81	9.92

** Highly significant—exceeds the 1 per cent point.

Table II. Mean Chemical Composition of Male Rats at End of Efficiency Test

FRESH BASIS

Group	Empty weight	Moisture	Ash	Protein	Ether extract
	Grams		Per cent		
High efficiency males (30)	253.63	66.06	2.60	18.93	12.44
Low efficiency males (30)	227.93	64.58	2.67	19.98	12.76

Analysis of variance

Variation due to	Degrees of freedom	Mean squares				
		Empty weight	Moisture	Ash	Protein	Ether extract
Strains	1	9907.35**	30.19**	.0403	16.88**	1.50
Error	58	69.77	1.47	.0302	0.74	2.22

DRY BASIS

Group	Dry matter	Ash	Protein	Ether extract
	Grams		Per cent	
High efficiency males (30)	86.20	7.62	55.76	36.53
Low efficiency males (30)	80.71	7.47	56.48	35.95

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Dry matter	Ash	Protein	Ether extract
Strains	1	450.89**	.3430	7.82	5.09
Error	58	26.29	.7927	11.29	11.62

** Highly significant—exceeds the 1 per cent point.

there is considerable variation within the strains in ether extract.

In calculating these data on a dry basis, there is no longer a significant difference between the two strains in the percentage amounts of protein, ash, or ether extract but there is a highly significant difference in dry weight. This is also true of the percentage composition of the moisture-free gain (table III). Although there is no significant difference in percentage composition of body or of gain, yet it is shown by the data in table IV that the high efficiency rats actually made highly significantly greater gains in total body weight, dry matter, protein, ash, and energy and significantly greater gains in ether extract on the same intake of dry matter of the same composition and energy. This clearly indicates a definite genetic difference in utilization of food by the two strains.

A comparison of body composition data of Calverley's test rats (table 2, page 13) and those of this experiment shows only a slight difference in composition between the low efficiency rats but a notable difference in that of the high efficiency rats. This difference is more likely due to the larger quantity of food ingested by the high efficiency animals of Calverley's group than

to changes in body composition due to genetic causes. The low efficiency animals in both experiments probably ate approximately the same quantity of food. The figure for the restricted amount of food allotted to all rats on controlled food intake was obtained by taking the mean food consumption, week by week, of 500 low efficiency strain rats. There is no reason to believe that the amount of food consumed by this strain has changed materially over a period of years.

Table III. Percentage Composition of Mean Gain

FRESH BASIS

Group	Empty weight	Moisture	Ash	Protein	Ether extract
	Grams		Per cent		
High efficiency males (30)	198	64.56	2.54	19.34	13.55
Low efficiency males (30)	173	62.55	2.56	20.63	14.40

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Moisture	Ash	Protein	Ether extract
Strains	1	60.64**	.0074	24.60**	36.08**
Error	58	3.67	.0621	1.30	3.79

DRY BASIS

Group	Dry matter	Ash	Protein	Ether extract
	Grams		Per cent	
High efficiency males (30)	70.19	7.15	52.91	38.05
Low efficiency males (30)	64.64	6.85	55.13	37.09

Analysis of variance

Variation due to	Degrees of freedom	Mean squares			
		Dry matter	Ash	Protein	Ether extract
Strains	1	461.93**	1.36	73.87	13.80
Error	58	14.39	0.57	68.68	37.28

** Highly significant—exceeds the 1 per cent point.

Table IV. Composition of the Mean Gain

Group	Empty weight	Ash	Protein	Ether extract	Dry matter	Energy gain
	Grams	Grams	Grams	Grams	Grams	Calories
High efficiency males (30)	198	5.02	38.27	26.74	70.19	405.94
Low efficiency males (30)	173	4.46	35.48	24.85	64.64	377.16

Analysis of variance

Variation due to	Degrees of freedom	Mean squares					
		Empty weight	Ash	Protein	Ether extract	Dry matter	Energy gain
Strains	1	9484.68**	4.61**	117.60**	53.71*	461.93**	12426.63**
Error	58	31.49	.24	7.74	13.12	14.39	877.88

* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

Table V. Mean Chemical Composition of Test Rats Fed Ad Libitum (Fat-Free Basis)

Group	Empty weight	Moisture	Ash	Protein
	Grams	Per cent		
High efficiency males	216	73.21	3.24	23.58
Low efficiency males	169	73.31	3.39	23.31

Table VI. Utilization Efficiencies

Group	Body weight efficiency index	Dry matter efficiency index	Energy efficiency index
High efficiency males	1.23[1]	10.89[1]	1.85[1]
Low efficiency males	1.55	12.61	2.14

Analysis of variance

Variation due to	Degrees of freedom	Mean squares		
		Efficiency index	Dry matter efficiency index	Energy efficiency index
Strains	1	1.49**	44.48**	1.26**
Error	58	.01	1.28	.07

[1] The smaller index denotes greater efficiency.
** Highly significant—exceeds the 1 per cent point.

When Calverley's data are calculated on a fat-free basis (table V) the percentage differences between the two strains with respect to moisture, ash, and protein become very small, but the difference in the fat-free empty carcass weights is still large, which shows that the difference in weight of the two strains is due not only to deposition of fat but also to the other components of gain.

Further evidence of better food utilization by the high efficiency strain is shown in their mean calculated efficiency indices (table VI). The same equation is used for all three indices, i.e., body weight, dry matter, and energy, by substituting the proper value in the equation shown in Part I, page 7 of this bulletin.

Conclusion

Despite the restrictions in food intake placed on the high efficiency strain rats, as a result of which they were not allowed to consume more dry matter than the low efficiency animals, they gained more ash, protein, ether extract, dry matter, and Calories. As a result of this, their mean calculated efficiency indices were each very significantly better than the corresponding indices for the low efficiency strain. These facts confirm our assertion that there is a genetic difference in efficiency of food utilization in the two strains of rats.

II. Efficiency of Metabolism for Maintenance of Mature Animals Differing in Efficiency of Food Utilization During Growth[4]

Cecil Lohn, Leroy S. Palmer, and Cornelia Kennedy

PART I of this bulletin, as well as previous publications from this division which describe the differences between rats in utilizing their food, has dealt entirely with differences manifested during the period of rapid growth. It has been demonstrated that the differences which were manifested by segregation of second generation hybrids could be fixed (within certain limits) at two widely different levels of efficiency. This was done by continuing to inbreed the two strains and selecting the breeding stock from among the animals whose actual efficiency performance was within the limits chosen as the "high" and "low" levels.

In this program of breeding and testing it has been assumed that the strain differences thus established, which are exhibited during the period of most rapid growth and repeated in the succeeding generations, are probably possessed in some degree after the animals attain maturity. However, it has not heretofore been convenient to test this assumption experimentally or to determine what physiological differences are demonstrable in the mature animals.

The investigation reported in this section was carried out to determine the efficiency of food metabolism for maintenance of male animals from the two strains of rats after they had reached maturity. The problem included the determination of the number of Calories required for maintenance, activity, basal metabolism, and rectal temperatures. The low efficiency strain rats employed were from the twenty-first and twenty-second generations of sib matings, while the high efficiency strain rats were from the twenty-third to the twenty-sixth generations.

REVIEW OF LITERATURE

Many studies have been made to determine the energy requirement for maintenance of farm animals. The usual procedure has been to feed a sufficient amount of ration, containing the necessary nutrients, over a long period of time so that the animal neither gains nor loses weight.

[4] The material for this section was taken from a thesis submitted by Cecil Lohn in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1938.

Mitchell and Carman (*37*) in an extended study determined the composition of the gain in weight and the utilization of food energy in growing rats. They found that the range of values for the percentage utilization of energy for maintenance was quite narrow, being 91.3 to 96.8 per cent for rats weighing 67 to 265 grams. Boas Fixsen (*6*) estimated that the daily Calorie intake, in terms of metabolizable food energy, must be above 12.5 Calories per 100 grams body weight for rats weighing less than 375 grams. Later, Boas Fixsen and Jackson (*7*) set the requirement at 12.0 Calories per 100 grams for rats of 375 grams or less and at 11.5 Calories per 100 grams for animals above this weight. Forbes, Kriss, and Miller (*18*) found it required about 20 Calories to supply the daily energy for maintenance of 100-gram animals. Jackson (*26*) maintained young male rats (average weight 49.8 grams) for 15 weeks on a daily energy intake which averaged 0.346 Calories per gram body weight. He regards this as slightly above the energy requirement for maintenance.

The basal respiratory quotient (R.Q.) has been found by a large number of investigators to be very close to 0.72 for rats in the post absorptive state. Benedict and associates have chiefly used the multiple-chamber apparatus as described by Benedict (*3*) for measuring the respiratory exchange of small animals. Wesson (*57*) described a similar closed system and later (*58*) described a more simplified apparatus. A great number of investigators have used an open circuit system patterned after the Haldane (*23*) apparatus. These workers have found the basal metabolism of the rat to vary from 600 to over 800 Calories per square meter body surface.

It is generally believed that the urinary nitrogen output on a diet of nearly zero protein content may be employed to calculate the minimum requirement of protein for maintaining the animal. Mitchell and Carman (*37*) found the excretion of urinary nitrogen to be about the same in rats on a ration containing from 0.6 to 0.75 per cent whole egg nitrogen as on a ration practically nitrogen free. Mitchell (*35*), Mitchell and Carman (*37*), and Mason and Palmer (*34*) reported that the preliminary feeding period necessary for the animal to come to the minimum nitrogen excretion level on the "nitrogen-free" diet was about three or four days. Ashworth and Brody (*2*) concluded that 15 days is required to reach the endogenous level. At the end of this time the average value of endogenous urinary nitrogen was about 140 milligrams per kilogram body weight, and the ratio to basal metabolism about 1.4. In a continuation of this study Ashworth

(*1*) concluded that the ratio is about 1.5. Data taken by Olson and Palmer (*41*) indicated that as rats remained on low protein diets the endogenous urinary nitrogen became more uniform and progressively lower. Seegers (*44*) also found that the endogenous urinary nitrogen excretion decreased considerably over a period of about 20 days, then much more slowly for another 30 days, and then increased slightly.

Smuts (*47*) concluded that the low nitrogen excretion accepted by Ashworth and Brody as endogenous probably does not represent the true endogenous level any more than heat production after prolonged fasting represents the basal metabolism. He found the endogenous level to be 213 milligrams nitrogen per kilogram body weight and the ratio to basal metabolism to be about 2.00. Terroine and Sorg-Mattir (*52*, *53*) found ratios averaging between 2.17 and 2.37.

The body temperature of adult rats is quite variable, but most investigators report values between 37° and 38° C. Pembrey (*43*) found the average rectal temperature of adult rats to be 37.5° C., while Macleod (*33*) found a range of 37.5° to 38.5° C. with an average of 37.9° C. Congdon (*13*) reported an average temperature of 37.9° C. for young rats and 37.2° C. for adult rats. Graham and Hutchison (*21*) found a range of 37.5° to 38.5° C., and also that body temperature increases with room temperature. Drummond (*15*) found a diurnal variation in body temperature, the temperature of male rats varying from 38.1° C. at 10:00 a.m. to 38.5° C. at 6:00 p.m. Gudjonsson (*22*) found the rat's temperature to be quite unstable, but usually between 37.0° and 38.0° C. Benedict, Horst, and Mendel (*4*) found the average rectal temperature to be about the same for rats of different weight, varying from 37.4° C. for very large rats to 37.6° C. for medium-sized rats. The effect of exercise was studied by Horst, Mendel, and Benedict (*25*), who reported that the rectal temperature increased from 37.4° C. before exercise to 39.0° C. after exercise.

PROCEDURE

The general plan was to determine the energy requirement for maintenance, the endogenous nitrogen excretion in order to find the protein requirement for maintenance, the normal activity of the rat, the basal metabolism, and the rectal temperature.

Diet and Care of the Animals

The rations employed are shown in table 1. The standard ration is the same as the one used by Palmer and Kennedy in

Table 1. Composition of Rations

	Standard ration	Low nitrogen ration	"Nitrogen free" ration
		Per cent	
Casein	35.00	6.25	.....
Tapioca dextrin	30.85	49.60	57.40
Dried yeast	5.75	5.75	.....
Sucrose	.....	10.00	10.00
Salt mixture	4.40	4.40	4.00
Dry whole egg[1]	.....	.....	6.60
B and G concentrate powder[2]	.....	.....	0.70
Lard	13.70	13.70	13.70
Butterfat	9.0	9.0	6.30
Wheat germ oil	0.80	0.80	0.80
Cod liver oil	0.50	0.50	0.50

[1] The dry whole egg supplied 2.73 per cent fat to the ration, so that the percentage of butterfat in this ration was reduced to 6.30 in order that all rations would have the same energy value per gram. The percentage of nitrogen in the dry whole egg was 6.77. Therefore each gram of egg ration contained 4.46 milligrams of nitrogen.

[2] Purchased from National Oil Products Company of Harrison, New Jersey.

their study of the efficiency during growth. However, they fed two grams fresh beef liver daily as a supplement to the ration.

This study was a comparison of two highly inbred strains, the segregation of which was reported by Morris, Palmer, and Kennedy (*39*) and continued since then by Palmer and Kennedy. All the rats had been subjected to the standard efficiency test employed in this laboratory for the six-week period beginning at 60 grams weight and were therefore about 10 weeks old at the end of the test period. In general, the growth curve of the male rats of the strains studied reached a plateau at four months so they were not used in this investigation until they had reached this age.

The rats were kept at a uniform temperature of about 27° C. in all experiments except those on basal metabolism, as it has been shown that the critical temperature of the rat is about 28° C. (Goto, *20*, and Benedict and MacLeod, 5). The rats were housed in individual cages of galvanized iron (12 by 12 by 9 inches) with a raised screen floor of one-fourth inch mesh. Heavy absorbent paper in a drawer below collected the urine, feces, and any scattered food. The food cup used was of the McCollum type with a cover having a circular opening one and three-sixteenths inches in diameter.

Experimental and Analytical Technique

The energy requirement for maintenance of the rats was determined by feeding daily a measured amount of food which was changed each day until the amount sufficient to keep the rats' weight constant was found. It took 30 to 40 days to determine this amount. The animals were then maintained for 15

days without changing the food intake. The maintenance weight was taken as the rats' average weight during the 15 days.

To obtain a minimum nitrogen excretion the animals were first placed on the low nitrogen diet and fed the weight of food required for maintenance. After 15 days on the low nitrogen ration the animals were placed on the "nitrogen-free" ration for three days before being put in metabolism cages.

For the first group of rats there were six consecutive urinary collection periods of three days each, but the excretion of nitrogen varied quite widely so the collection periods were increased to six days.

The nitrogen metabolism cages were those in use in this laboratory and are described on page 9. The urine was collected in beakers containing 5 milliliters of 2 per cent sulfuric acid solution plus a few crystals of thymol to prevent decomposition of the urine and loss of ammonia. Each day the cage bottom and funnel were washed with hot 0.05 *N* sulfuric acid solution, using a small brush and wash bottle. Every two days the washings were transferred to a sealed Mason jar. At the end of the six-day period the samples were made up to one liter in a volumetric flask. Nitrogen determinations were made on aliquots.

The voluntary activity was measured in a freely revolving cage of 1.1 meters inside circumference. The activity was measured for a 20-day period, readings of a number of quarter revolutions and food intake being taken every two days. Shirley (*45*, *46*) reported that the level of activity of rats is essentially constant from the fifth to the ninth month of age, and that they attain their activity stride after about 10 days in a revolving cage.

The chamber used in the metabolism studies was of simple construction. It consisted of a nine-inch desiccator, without cover, and a nine-inch bell jar with an opening in the top for a No. 8 rubber stopper. A small electric motor was mounted on top of the bell jar and connected to a shaft through a mercury seal to a fan blade inside the chamber. A capillary tube extended to just above the fan blade was used for withdrawing samples of gas for analysis. A thermometer was suspended from a side arm on the capillary tube so it hung near the side of the bell jar. The inside temperature was always maintained between 29° and 30° C. The assembled apparatus, with animal, is shown in figure 1.

All rats were fasted 18 to 24 hours before the metabolism test. To determine the basal metabolism, the rat was placed in the chamber for exactly 30 minutes (in the case of the rats weighing over 400 grams the time was reduced to 20 minutes). At least five

minutes before the end of the trial, the gas sampler, filled with mercury, was connected to the capillary tube and a sample of air was drawn in and out three or four times before the final sample was taken. The speed of the fan was increased so that the air would be more completely mixed.

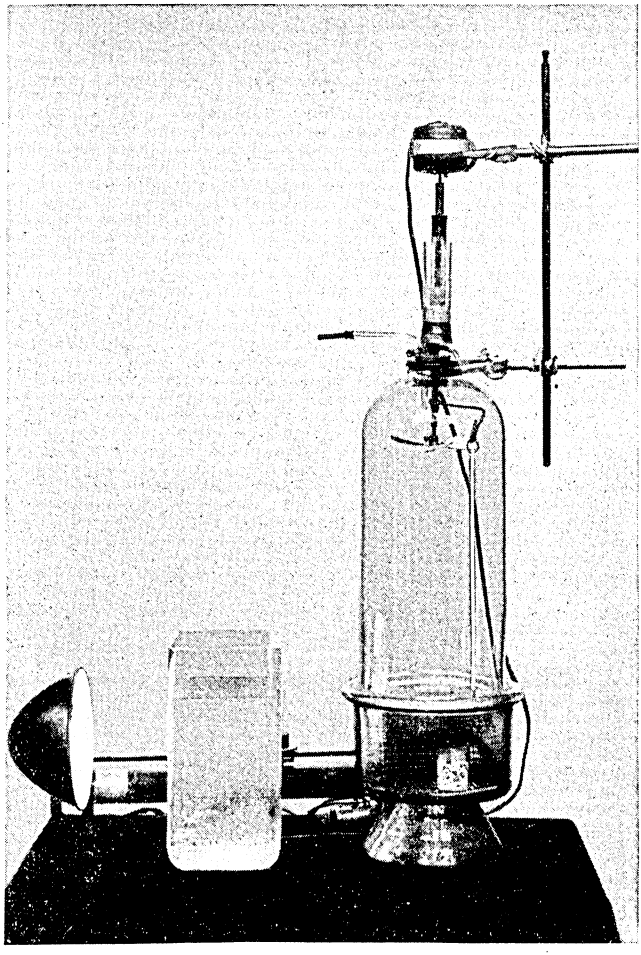

FIG. 1. Metabolism apparatus

The sample of gas was analyzed for carbon dioxide in the Haldane-Carpenter gas analysis apparatus according to directions of Carpenter, Fox, and Sereque (*12*). The chamber air was analyzed before the metabolism trial, and from the difference the percentage of carbon dioxide produced by the animal in 30 minutes was calculated.

The temperature of the rat was taken in most cases about 5:00 p.m. on the day the basal metabolism was measured. An ordinary clinical mercury thermometer was placed in the rat's rectum for at least two minutes.

Calculation of Data

Body surface area was calculated by Lee's (*31*) formula S (square centimeters) $= 12.62\ W^{0.60}$, W being the weight of the animal in grams. The statistics calculated followed the formulas given by Treloar (*55*).

The values used for calculating the metabolizable energy intake of the animals at maintenance weights were 4.1 Calories per gram of carbohydrate and protein and 9.3 Calories per gram of fat. These data were applied to the measured food consumption and the known compositions of the rations employed. The metabolizable energy value of the ration used for determining the maintenance requirement obtained by using these figures corresponds closely with the actual value found by Calverley and reported in Part I of this bulletin.

DISCUSSION

Comparisons of the mean results, standard deviations, coefficients of variation, and significance of differences are shown in table 2, and various coefficients of correlation in table 3.

Energy Requirement for Maintenance

The energy requirement for maintenance was determined for 47 high and 43 low efficiency strain male rats. The low efficiency strain animals had a very significantly higher maintenance requirement when expressed as Calories per kilogram body weight, but this statistical difference is greater than the actual because the high efficiency strain rats were heavier, the average weight being 349.1 grams as compared with 290.1 grams for the low efficiency strain. The problem was studied further by selecting from the data animals having essentially the same weight. The difference in energy requirement (table 2) is still highly significant. When calculated on the basis of Calories per square meter body surface the low efficiency strain averaged only about 2 per cent higher requirement, but the difference was, nevertheless, significant. This significance was reduced slightly for the animals of essentially like body weight. It is clear from the results of the present study that if the relationship between the strains regarding the energy required for maintenance per unit

body weight found at maturity holds also during growth, it indicates at least one of the factors responsible for the difference in the Efficiency Index of the two strains during growth.

Table 2 shows that there was somewhat more variation among the low efficiency strain males than the high efficiency strain males when the energy requirement was determined both on the body weight basis (except for the selected rats of equal body weight) and on the body surface basis. This would seem to be explained largely on the basis that the breeding program for the two strains has been directed for several years towards a reduction in variation of the efficiency index of male rats of both strains, and that this has been continued longer and with much

Table 2. Comparison of Differences in Means

Determinations	Efficiency strain		Mean	Standard deviation	C.V.	K[2]
Maintenance Cal. per sq. M. body surface	High	(47)[1]	1087.9	47.7	4.38	1.97*
	Low	(43)	1108.3	50.3	4.54	
Selected rats	High	(15)	1085.8	47.0	4.33	1.92
	Low	(15)	1097.7	52.8	4.81	
Maintenance Cal. per Kg. body weight	High		132.1	7.0	5.26	8.06**
	Low		145.6	8.8	6.02	
Selected rats	High	(15)	136.5	7.3	5.34	2.81**
	Low	(15)	139.1	7.4	5.31	
Endog. urinary nitrogen per sq. M. body surface	High		1244.7	114.0	9.15	2.97**
	Low		1167.4	127.2	10.9	
Endog. urinary nitrogen per Kg. body weight	High		154.5	15.2	9.8	0.53
	Low		156.4	18.6	11.9	
Rectal temperature degrees F.	High		100.7	0.54	5.33	5.11**
	Low		99.8	0.77	7.73	
Basal metabolism per sq. M. body surface	High		861.1	62.2	7.22	0.75
	Low		873.4	61.2	7.01	
Basal metabolism per Kg. body weight	High		100.7	7.7	7.60	4.02**
	Low		109.7	9.6	8.77	
Basal metabolism per Kg. body weight (selected rats)	High		103.4	4.8	4.63	0.60
	Low		105.0	7.1	6.76	
Efficiency index	High	(78)	1.28	0.08	6.25	14.5**
	Low	(60)	1.67	0.16	9.58	
Voluntary activity (meters per day)	High		50.0	41.0	81.95	0.47
	Low		46.1	29.7	64.4	
Energy intake in activity test (Cal. per 100 g. body weight)	High		15.6	1.42	9.12	1.06
	Low		16.0	2.07	12.9	
Mg. endog. urinary nitrogen per Cal. basal metabolism	High		1.45	0.19	13.10	1.72
	Low		1.37	0.18	13.14	
Maintenance Requirement						
Basal metabolism (in Cal. per sq. M. body surface)	High		1.28	0.089	7.0	0.19
	Low		1.27	0.107	8.4	

[1] Number of animals used.

[2] $K = \frac{\bar{x} - \bar{y}}{\sqrt{\frac{S.E.^2}{\bar{x}} + \frac{S.E.^2}{\bar{y}}}}$

* K equal to or exceeding 1.96 is significant.
** K equal to or exceeding 2.58 is highly significant.

Table 3. Correlation Coefficients Between Various Physiological Characteristics of the Strains

Strain	Characteristics correlated	No. animals	r
High	Cal. req. maint. per Kg. body wt. : effic. index	47	−.111
High	Cal. req. maint. per sq. M. body surface : effic. index	47	−.127
Low	Cal. req. maint. per Kg. body wt. : effic. index	43	+.140
Low	Cal. req. maint. per sq. M. body surface : effic. index	43	−.050
High	Endog. urinary nitrogen per Kg. body wt. : effic. index	47	−.015
High	Endog. urinary nitrogen per sq. M. body surface : effic. index	47	+.001
Low	Endog. urinary nitrogen per Kg. body wt. : effic. index	43	+.427**
Low	Endog. urinary nitrogen per sq. M. body surface : effic. index	43	+.354*
High	Cal. consumed per 100 g. body wt. : activity in M. per day	39	+.472**
Low	Cal. consumed per 100 g. body wt. : activity in M. per day	35	+.018
High	Basal Cal. per sq. M. body surface : effic. index	28	+.284
High	Basal Cal. per Kg. $wt.^{0.73}$: effic. index	28	+.478**
High	Effic. index : rectal temp.	28	−.231
High	Basal Cal. per sq. M. body surface : rectal temp.	28	−.149
High	Basal Cal. per Kg. $wt.^{0.73}$: rectal temp.	28	−.154
Low	Basal Cal. per sq. M. body surface : effic. index	33	+.207
Low	Basal Cal. per Kg. $wt.^{0.73}$: effic. index	33	+.269
Low	Effic. index : rectal temp.	33	−.119
Low	Basal Cal. per sq. M. body surface : rectal temp.	33	−.457**
Low	Basal Cal. per Kg. $wt.^{0.73}$: rectal temp.	33	−.501**
High	Basal Cal. per sq. M. surface : Cal. req. maint. per sq. M.	27	+.424*
Low	Basal Cal. per sq. M. surface : Cal. req. maint. per sq. M.	33	−.089

* Significant—exceeds the 5 per cent point.
** Highly significant—exceeds the 1 per cent point.

greater success with the high efficiency strain. As shown in table 3, no significant correlation coefficient was found in either strain between energy requirement for maintenance and the efficiency index determined during growth. However, the high efficiency animals showed a significant positive correlation between the basal metabolism per Kg. $wt.^{0.73}$ and the efficiency index.

No significant correlation was found between the basal metabolism and rectal temperature for the high efficiency strain rats, but there is a significant negative correlation between the basal metabolism, expressed either per square meter body surface or per Kg. $wt.^{0.73}$, and rectal temperature for the low efficiency strain. This means that the calorie requirement for maintenance of this strain increases with decrease in body temperature, and agrees with the fact that the low efficiency strain rats have a significantly lower average temperature and a higher energy requirement for maintenance. The lower body temperature and somewhat higher basal metabolism of the low efficiency strain rats and the significant negative correlation between body temperature and basal metabolism indicate that the temperature of the rat is one factor influencing basal metabolism.

Endogenous Urinary Nitrogen Excretion

The endogenous urinary nitrogen excretion per square meter body surface was found to be significantly higher for the high efficiency strain rats, as shown in table 2. The coefficient of variation shows the surface basis to be more reliable in expressing nitrogen excretion.

The ratio of milligrams endogenous urinary nitrogen excreted to Calories of basal heat produced is not significantly different between the two strains. Ashworth and Brody (2) found a much larger difference in their rats than was found between the high and low efficiency strain rats. The average value of this ratio for the high and low efficiency strain rats tested in this study was about the same as they found for all their rats.

No correlation (table 3) was found in the high efficiency strain rats between efficiency index obtained during growth and endogenous urinary nitrogen of the mature animal when expressed either on the weight basis or on the surface basis. There was, however, a significant positive correlation for the low efficiency strain rats. This means that rats in this strain which exhibited a lower total efficiency during growth excreted more endogenous nitrogen when mature. It would seem from this correlation that the high efficiency strain should have a lower endogenous nitrogen excretion, but this is not the case, except when measured on the weight basis. This difference shown by the correlations suggests a probable inherent physiological difference between the strains.

Voluntary Activity

No significant difference was found in the voluntary activity, expressed as meters per day, of the two strains (table 2). There was no significant difference in the energy intake per 100 grams body weight during the activity trials. The correlation between activity and food intake is significant for the high efficiency strain rats, but there is no correlation for the low efficiency strain rats. The high efficiency strain showed more variability in activity and less in food intake than the low efficiency strain.

Basal Heat Production

The high efficiency strain rats were found to have a highly significantly lower basal heat production, when calculated on a weight basis, than the low efficiency strain rats; but when calculated on the body surface basis or when animals having essen-

tially the same weight were compared, the difference was not significant. This is to be expected because, as shown in Part I of this bulletin, high strain rats have a higher fat content and a lower protein content, and, therefore, a lower protoplasmic mass to which basal metabolism is closely related. There is less variation in both strains when basal metabolism is expressed on the surface basis than when expressed on the weight basis, indicating basal metabolism is more closely related to surface area.

Formula for Basal Metabolism

Brody, Proctor, and Ashworth (*10*) developed a formula relating basal metabolism to body weight which they claim holds for all species of mature mammals from mice to elephants. This formula is Q (basal metabolism) $= 70.5\ W^{0.734}$, W being weight in kilograms. Q was calculated for the two strains studied in this investigation and compared with the basal metabolism found by experiment. The coefficient of correlation between the calculated value Q and the experimental value of basal metabolism was +0.57 for the high efficiency strain and was +0.39 for the low efficiency strain. The coefficient of correlation between the experimental value of basal metabolism in Calories per day and body weight in kilograms was +0.58 for the high efficiency strain and +0.43 for the low efficiency strain. The values for the high efficiency strain are highly significant and those for the low efficiency strain are significant. Basal metabolism as Calories per day was plotted against body weight in kilograms on log log graph paper, and a regression line was drawn (see figures 2 and 3). The slope of the line is the exponent of the body weight in kilograms, while the antilog of the y intercept is the coefficient. The final power formula represented by the regression line relates basal metabolism in Calories per day to body weight in kilograms. For the high efficiency strain rats the formula was B.M. $= 75.38\ W^{0.702}$ and for the low efficiency strain rats was B.M. $= 58.15\ W^{0.437}$. The formula of the high efficiency strain is very similar to that found by Brody *et al.* (*10*) but that of the low efficiency strain is quite different. There is no apparent reason why the low efficiency strain should not be represented by a formula similar to that of the high efficiency strain, as the coefficient of correlation between basal metabolism and body weight is significant (although not as significant as in the high efficiency strain) and the variation is less than that of the high efficiency strain. The difference obtained suggests some inherent differences between the two strains.

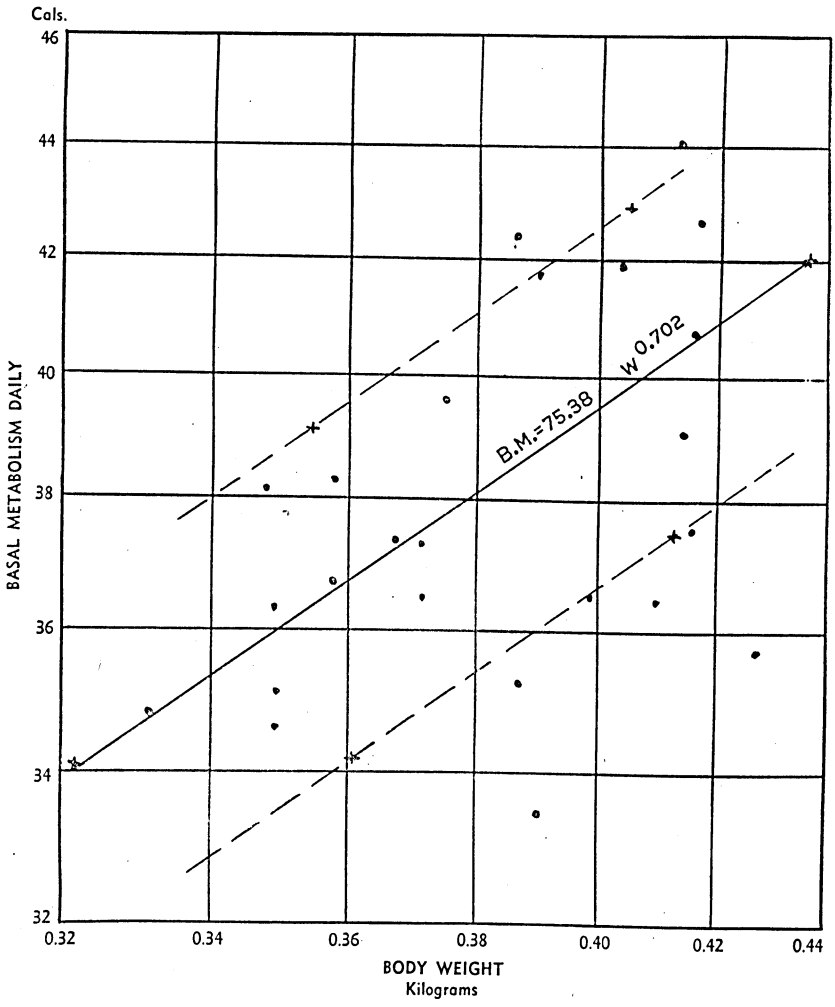

FIG. 2. Regression curve relating basal metabolism to body weight in kilograms (high efficiency strain males)

The difference between the rectal temperature of the two strains is quite significant. The average temperature for the high efficiency strain is 100.7° F. or 38.2° C., and for the low efficiency strain it is 99.8° F. or 37.7° C. There is considerably more variation in the low efficiency strain.

The ratio of maintenance requirement in Calories per square meter body surface to basal metabolism in Calories per square meter is very nearly the same for both strains. The ratio is 1.277 for the high efficiency strain and 1.273 for the low efficiency

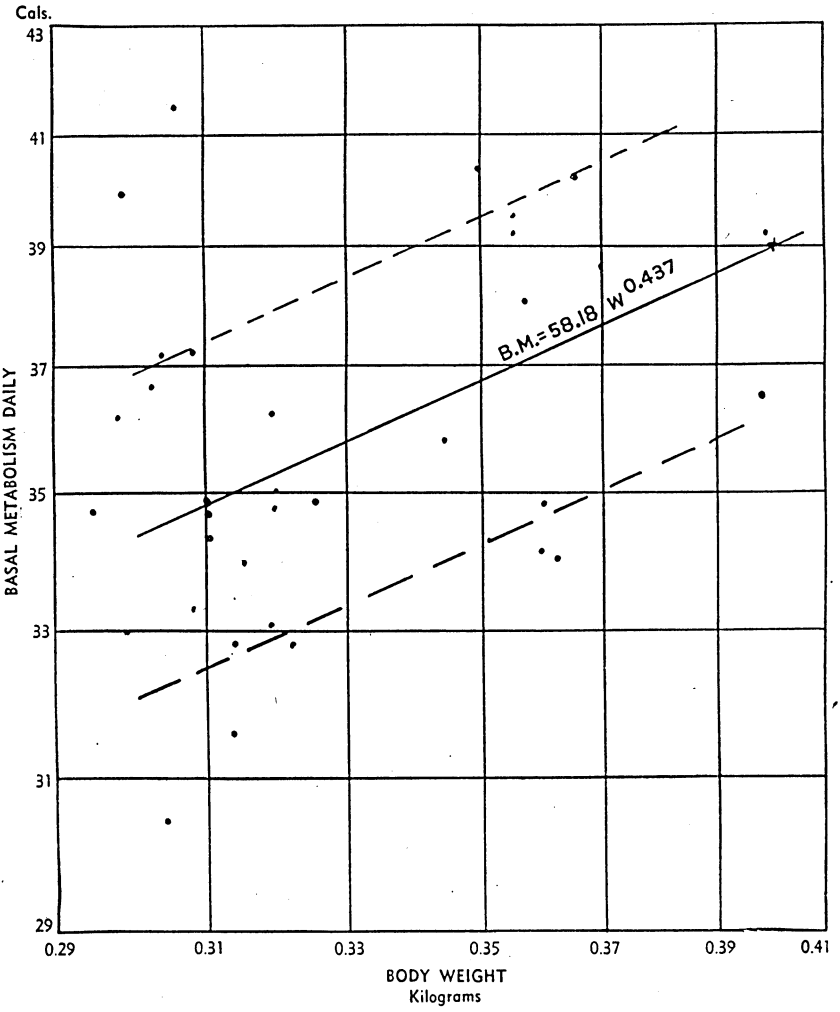

FIG. 3. Regression curve relating basal metabolism to body weight in kilograms (low efficiency strain males)

strain. This shows that the maintenance requirement in Calories per square meter is 27.7 per cent higher than the basal metabolism for the high efficiency strain animals and 27.3 per cent higher than the basal metabolism of the low efficiency strain rats. However, there is more variation in this ratio in the case of the low efficiency strain. A significant positive correlation between basal metabolism and maintenance requirement was found for the high efficiency strain rats, but no correlation was found for the low efficiency strain rats.

SUMMARY AND CONCLUSIONS

The energy requirement for maintenance was determined for 47 high efficiency strain male rats and 43 low efficiency strain male rats. The low efficiency strain had a significantly higher requirement when expressed as Calories per kilogram body weight, but this was due to a considerable extent to the difference in weight between the two strains. However, the difference was still significant when tested on animals of practically the same weight. When calculated on the basis of Calories per square meter body surface the low efficiency strain had only 2 per cent higher requirement, but the difference was, nevertheless, significant.

The endogenous urinary nitrogen excretion per square meter body surface was found to be somewhat higher for the high efficiency strain rats.

The milligrams endogenous urinary nitrogen per Calorie basal metabolism was found to be nearly the same for both strains.

There was no significant difference in the activity of the two strains, both being quite lazy.

The low efficiency strain had a slightly higher basal metabolism than the high efficiency strain.

The rectal temperature of the high efficiency strain was significantly higher than that of the low efficiency strain.

There was a tendency in the low efficiency strain rats for the basal metabolism to be higher with a lower body temperature.

The ratio of maintenance Calories to basal Calories per square meter was nearly the same for both strains, being slightly higher for the high efficiency strain.

A significant positive correlation was found for the high efficiency strain rats between basal metabolism and maintenance requirement when calculated on the body surface basis.

The formula relating basal metabolism to body weight is B.M. $= 75.38\ W^{0.702}$ for the high efficiency strain rats and B.M. $= 58.15\ W^{0.437}$ for the low efficiency strain rats.

Maintenance requirement, endogenous urinary nitrogen excretion, and basal metabolism show less variation when calculated on the body surface basis than when calculated on body weight basis.

In all the criteria studied the low efficiency strain rats showed greater variation.

The large differences in efficiency of food utilization observed in the two strains of rats during growth are explained only in a minor degree by differences observed at maturity.

III. Some Physiological Factors Related to Efficiency of Food Utilization[5]

Paul H. Weswig, Leroy S. Palmer, and Cornelia Kennedy

THE results of investigations already reported in this bulletin and elsewhere indicated that a study of physiological stimulants and inhibitors might give us important information in our quest for the factors determining the difference in the efficiency of food utilization between our two strains of rats. It seemed reasonable that possible differences in the normal control of metabolism in the two strains might be revealed if certain hormonal functions were increased or decreased. The importance of the thyroid gland in controlling the general metabolic rate of the organism is a well-established fact, and so we assumed that possible differences in the normal control of metabolism in the two strains by the thyroid might be revealed by imposing on the rats artificial hyperthyroidism. It is also known that castration affects certain organic and functional characteristics of animals. Removal of the gonads in the rabbit, dog, and rat leads to reduction in the size of the thyroid and depression of metabolic rate. If there is a strain difference in metabolic rate in our rats, castration might indicate one reason for unequal utilization of food by the two strains.

In an earlier study from this laboratory (*40*) it had been found that injections of anterior pituitary growth hormone promoted growth and increased efficiency of food utilization in both strains within limits set by an inherent genetic level. As a continuation of the study of endocrine regulation of growth and metabolism, it seemed profitable to extend our research to the effects of castration and artificially imposed hyperthyroidism.

REVIEW OF LITERATURE

From the very extensive literature on the effects of castration and artificial hyperthyroidism in the albino rat, only a very few citations will be given.

Many studies have been made to determine the effect of castration on weight and length of the albino rat and, although considerable variation is found in the data reported, the majority of workers have found that castration has an inhibiting

[5] The material for this section is a portion of the thesis submitted by Paul H. Weswig in partial fulfillment of the requirements for the Degree of Doctor of Philosophy, University of Minnesota, 1941.

effect on gain in weight and body length of rats. Stein (*48*) found the mean weight and body length of control animals to be significantly greater than that of castrated animals. His animals were castrated about the 22nd day of age and were killed 60 to 80 days later. He believes that the age at the time of castration and at the time of observation influences the result obtained. Donaldson and Hatai (*14*), Evans and Simpson (*16*), van Wagenen (*56*), and others have reported data showing that castrated rats do not attain the weight and body length of control rats. On the other hand, Stotsenburg (*49*) found that the growth of castrated and normal rats is similar. In some litters the castrated animals grew faster, while in others the controls grew faster. Freudenberger and Billeter (*19*) found no noticeable difference in growth of castrated rats until maturity, after which the spayed rats gained weight more rapidly than the controls. At 10 weeks of age the controls weighed 160 grams as compared with 190 grams for the spayed rats.

Tang (*51*) has studied changes in body length and the efficiency quotient of both male and female rats when gonadectomized, and found that control males were 7 per cent more efficient in food utilization than castrated males and that control females were 31 per cent less efficient than the spayed females. The length of the castrated rat was 2.6 per cent less than that of the control rat.

The feeding of desiccated thyroid gland has also been found to retard the rate of growth in body length and gain in weight. However, the reaction of the animal to thyroid feeding depends very much on the dosage and the age and weight of the animal. The decrease in growth rate is proportional to the amount of thyroid fed. MacKay and MacKay (*32*) fed rats from the 26th to the 75th day of age a diet containing 0.4 per cent desiccated thyroid and found 100 days later that the control rats weighed 186 grams and measured 196 millimeters in length, while the thyroid-fed rats, which ate far more than the control, weighed 138 grams and measured 190 millimeters in length. Bodansky and Duff (*8*) observed that young rats 22-26 days old continued to grow while receiving 1.0 milligram of thyroxine daily. This same dosage caused a rapid loss of weight and death in adult animals. Cameron and Carmichael (*11*) observed a distinct and marked decrease in growth rate in young rats 40-60 days old fed dried thyroid in the proportion of 1 milligram of gland to either 20, 10, 5, or 2 grams of body weight. The diet of these rats was bread and milk given *ad libitum*. They also found that with pro-

longed feeding of small doses the body weight tends to become normal again. They believe this is due to fat loss becoming balanced by hypertrophy of the organs concerned with increased metabolism. Large doses tended to inhibit growth completely. They noted an almost complete disappearance of fat tissue in animals fed thyroid.

PROCEDURE

The investigation reported in this section was planned to study the interrelations of castration and artificial hyperthyroidism on growth and the efficiency of food utilization in our high and low efficiency male rats. The study involved subjecting sufficient numbers, for statistical analysis, of male rats of both strains to (1) castration at weaning, (2) castration at weaning followed by thyroid administration, and (3) thyroid administration after weaning. Control groups of rats were used in each test. All animals were on experiment for our standard six-week efficiency test period, beginning when the rat reached 60 grams body weight.

The animals used in this investigation were descendants of the two strains of rats already described on pages 5 and 6 of this bulletin. Male rats weighing 60 grams were fed increasing equalized amounts of our standardized ration and 2.0 grams daily of fresh beef liver during the experimental period. On a dry matter basis the total amount of food consumed by each rat during the six-week period was 387.9 grams. The ration and its preparation have already been described on page 10 of this bulletin. The gross energy value of the ration is 5.5 Calories per gram.

All of the animals were housed in individual cages measuring 12 by 12 by 12 inches with a one-third inch mesh screen floor three inches from a tray which was covered with absorbent cardboard. The food was weighed out daily on a torsion balance accurate to 0.01 gram and the rats were weighed at weekly intervals on a Toledo balance accurate to 1.0 gram. The temperature of the laboratory was maintained at 27°-28° C. This temperature has been shown by a number of investigators to be about the critical temperature of the rat.

The rats that were castrated were operated on under the influence of nembutal when they weighed approximately 50 grams. After castration they remained with the mother rat until they reached the weight of 60 grams.

In a preliminary experiment to determine the amount of thyroxine to be given, a solution containing 0.5 milligram of

synthetic *dl*-thyroxine was injected subcutaneously into different groups of rats daily, every second day, or every fourth day. Because of the rapid loss in weight of the rats dosed daily or every second day it seemed advisable to change to desiccated thyroid gland and feed with the ration an amount equivalent in activity to 0.5 milligram of synthetic *dl*-thyroxine every fourth day throughout the six-week experimental period.

The measurements made to determine rate of growth were gain in weight and length of skeleton. The latter measurement was from the tip of the nose to the anus and was made after the rat had been killed. The gain-in-weight data were used in calculating the efficiency of food utilization index. The equation for this calculation is given on page 7. The biometric analyses of the data were calculated from the formulas of Treloar (*55*).

DISCUSSION

Turning to the data on skeletal length, it will be observed from table 1 that the length of the high efficiency line rat was definitely affected by the treatments imposed. Castration caused a greater decrease in body length than thyroid feeding. Castration also retarded the growth of the low efficiency line rat but thyroid feeding had no effect. The joint action of castration and thyroid feeding on either strain of rats was but little greater than castration alone.

The mean efficiency indices for the normal rats of both strains and for those of the treated rats are given in table 2. The efficiency with which the high line rat utilizes its food is definitely decreased by either treatment and by their combination, and although the efficiency indices of these rats approach that of the normal low line rat, they are still all significantly better.

Table 1. Mean Length in Centimeters of High and Low Efficiency Strain Rats

Group	Control	Castrated	Thyroid-fed	Castrated and thyroid-fed
High efficiency males	22.16±.09[1] (24)[2]	21.12±.07 (31)	21.76±.09 (20)	21.01±.06 (17)
Low efficiency males	20.51±.10 (32)	19.98±.08 (29)	20.51±.08 (26)	19.93±.09 (15)

Significance of Difference of Mean Lengths of Differently Treated Rats

Treatments	K[3]
Controls (high efficiency) vs. castrated (high efficiency)	9.41**
Controls (high efficiency) vs. thyroid-fed (high efficiency)	3.25**
Controls (low efficiency) vs. castrated (low efficiency)	3.74**

[1] Standard error.
[2] Figures in parentheses indicate the number of rats used.
[3] See page 36.
** Highly significant—exceeds the 1 per cent point.

Table 2. Mean Efficiency Indices of High and Low Efficiency Strain Rats

Group	Control	Castrated	Thyroid-fed	Castrated and thyroid-fed
High efficiency males	1.18[1]±.03[2] (25)	1.27±.02 (31)	1.29±.02 (20)	1.48±.03 (17)
Low efficiency males	1.74 ±.03 (33)	1.82±.05 (29)	1.82±.03 (29)	2.12±.07 (15)

Significance of Difference of Mean Indices of Differently Treated Rats

Treatment	K[3]
Control (high efficiency) vs. castrated (high efficiency)	2.42*
Control (high efficiency) vs. thyroid-fed (high efficiency)	3.11**
Control (low efficiency) vs. castrated (low efficiency)	1.24
Control (low efficiency) vs. thyroid-fed (low efficiency)	1.85
Castrated and thyroid-fed (high efficiency) vs. control (low efficiency)	5.83**

[1] The figure of the efficiency index becomes larger with decreasing efficiency and smaller with increasing efficiency of food utilization because the unit of food per unit of gain is divided by the mean weight of the animal for the test period.

[2] Standard error.

[3] See page 36.

* Significant—exceeds the 5 per cent point.

** Highly significant—exceeds the 1 per cent point.

Statistically neither castration nor thyroid feeding significantly increased the index number in the low line rat. However, as the test of significance is arbitrarily set at a value of K equaling 1.96 (one chance in 20 that its value would be exceeded by error in random sampling), significance should not be interpreted too strictly, especially when the number of items is small.

It is apparent from tables 1 and 2 that the effects of the different treatment on skeletal length and efficiency quotients do not run parallel in either line. In the high efficiency line, thyroid feeding reduced the efficiency of food utilization more and decreased body length less than castration, which produced a shorter rat, but one of somewhat greater efficiency in food utilization. In the low efficiency line, castration also produced a shorter rat, but thyroid feeding had no effect on length, and neither treatment changed very greatly the efficiency of food utilization.

The effect of castration on growth, which is reflected in the poorer utilization of food, may be due to the removal of a growth stimulus of the testes which Winters and his associates (*59*) have shown to be an important growth factor in young pigs. These investigators, in comparing the weights of boars and barrows under like experimental conditions, have shown that this stimulus accelerates the rate of growth of the boars over that of the barrows up to the time of puberty, when other factors come into play which offset the growth stimulus from the testes and the barrows begin to become significantly heavier than the boars. Our rats were approximately 10 weeks old at the end of the six-

Table 3. Data from Tables 1 and 2 Calculated on Percentage Basis

Group	Reduction in length			Reduction in efficiency of food utilization		
	Castration	Thyroid	Castration + thyroid	Castration	Thyroid	Castration + thyroid
	Per cent					
High efficiency	4.7	1.8	5.2	7.6	9.3	25.4
Low efficiency	2.6	0.0	2.8	4.6	4.6	21.8

week test period and were just maturing. We did not follow the growth rate after this period and so do not have comparable data on maturity.

Table 3, which is a part of the data in tables 1 and 2 calculated on a percentage basis, clearly shows a strain difference in response to both castration and thyroid feeding. Castration does not affect either the body length or the efficiency index of the low line rat to the same extent as it does these values in the high line rat. Thyroid feeding is not a serious inhibitor of skeletal growth in either strain but it does bear an important relation to efficiency of food utilization, especially in the high efficiency strain. The same amount of desiccated thyroid was fed to both the high and low strain rats, yet the efficiency of food utilization of the former was reduced 9.3 per cent and that of the latter only one half this amount. If the results of the thyroid feeding could be interpreted as meaning that the thyroid gland of the high efficiency rat normally secretes less thyroxine than that of the low efficiency rat, it would be one factor in explaining why the high line rat makes the more rapid gains in weight on the same food intake as the low line rat. This assumption is given support by the work of Lohn (Part II), who showed that the low efficiency rat has a higher basal metabolism than the high efficiency rat. By adding thyroid to the diet of the low efficiency rat oxidation processes are not greatly stimulated as they are probably near the maximum rate, and a small addition of thyroxine does not materially accelerate oxidation as it does in the high line rat whose metabolic rate is slower. The work of Luecke (Addendum Part I), which shows that the high efficiency rat stores more fat and protein than the low efficiency rat, also indicates a lesser destruction of food energy by the high efficiency rat than by the low efficiency rat. The total food intake of his rats for the six-week period was also 387.9 grams.

The results produced by the combined action of castration and thyroid feeding on the efficiency of food utilization in both strains support the evidence of a growth stimulus from the

gonads which is removed with castration, thus imposing a two-fold hardship on the thyroid-fed rat. There is also the possibility that there is an interrelation between the gonads and the thyroid that is not understood.

SUMMARY AND CONCLUSIONS

Periodic administration of thyroid during the test period markedly reduced the efficiency of male rats of the more efficient strain and also their skeletal length but did not significantly affect these characteristics in the less efficient strain. The efficiency of the males of both strains was not affected as significantly as one would expect by castration performed prior to the test period, the only effect evident being decreased efficiency in the high efficiency strain. The skeletal length of the rats in both strains was significantly reduced by castration.

The results of the thyroid feeding suggested that the less efficient rat secreted more thyroxine than the more efficient rat and that therefore there was greater wastage of food energy and consequently a lessened efficiency of food utilization.

GENERAL SUMMARY AND CONCLUSIONS

Over-all efficiency of food utilization in growing rats is controlled by inheritance factors.

The difference between the two strains is not due to difference in ability to digest and metabolize the test ration.

Although the less efficient strain animals consume less dry matter and grow at a slower rate when fed the test ration *ad libitum,* a decreased rate of growth and lower efficiency is still very marked when the amount of food consumed by the more efficient animals is restricted so that both strains are compelled to consume the same amount of the same ration.

The chemical composition of the bodies of the animals in the two strains is greatly influenced by the amount of test ration consumed, but, even when the food consumption is equalized, the more efficient strain makes greater gains in protein, ash, ether extract, dry matter, and Calories than the less efficient strain.

The calculated increment of heat lost in metabolism during growth is proportionately higher in the less efficient strain.

Mature males of the low efficiency strain show a higher energy requirement for maintenance both per unit surface area and per unit body weight, have a higher basal metabolism per unit body weight, a lower excretion of endogenous urinary nitrogen per unit surface area, and a lower body temperature than the more efficient strain.

Periodic administration of thyroid during the test period markedly reduces the efficiency of male rats of the more efficient strain and also reduces growth in length of skeleton, but does not significantly affect these characteristics in the less efficient strain.

Castration reduces the efficiency of food utilization in the more efficient strain but does not significantly affect that of the less efficient strain and reduces growth in length of skeleton of both strains.

The comparative biochemical and physiological studies made to determine the cause of the difference between the strains have shown that a number of physiological differences exist, some of which have been shown to influence strain differences in efficiency of food utilization. Further study is necessary to determine all of the factors involved and their interrelations.

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