# Growth and Reproduction in the Rat on Diets of Rice and Beans[1]

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PUERTO RICAN DIETS, characterized by a high content of polished rice and dried beans,[2] were shown by Cook and Rivera,[3] Axtmayer,[4] and Cook, Axtmayer, and Dalmau[5] to be deficient in multiple nutritional factors: calories, vitamin A, protein, calcium and, probably, the vitamin B complex. Rats grew poorly on the native diets, but an increase in their growth rate appeared when these diets were supplemented with casein, yeast, or cod-liver oil.

Malnutrition is prevalent among the population of Puerto Rico. The inadequate diet has been shown to be related to the slender physique and short stature of the Puerto Rican of the lower socio-economic class,[6] to the high infant mortality,[7] to the common occurrence of ocular manifestations associated with underconsumption of vitamins[8] and of lowered plasma ascorbic acid concentration values, attributed to vitamin C undernutrition.[9] Recent clinical studies of Dr. Ramón M. Suárez give evidence that "multiple deficiencies, in-

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1. Received for publication May, 1945. This work was aided by a grant from the Carnegie Corporation of New York City.

2. B. K. Ashford, Observations on the conception that sprue is a mycosis superimposed upon a state of deficiency in certain essential food elements. Am.J.Trop.Med., 2:139–150, 1922.

D. H. Cook, Some aspects of the food problem in Porto Rico. Porto Rico Rev.Pub.Health & Trop.Med., 3:59–67, 1927.

S. L. Descartes, S. Díaz Pacheco, and J. P. Nogueras, Food consumption studies in Puerto Rico. Bul. No. 59, Agricultural Experiment Station, Río Piedras, P. R., 1941.

3. D. H. Cook and T. Rivera, Rice and beans as an adequate diet. Porto Rico Rev.Pub. Health & Trop.Med., 5:3–7, 1929.

4. J. H. Axtmayer, A study of the vitamin B complex of the red kidney bean and polished rice. J.Nutrition, 2:353–357, 1929–1930.

5. D. H. Cook, J. H. Axtmayer, and L. M. Dalmau, A comparative study of the nutritive value of three diets of frequent use in Puerto Rico. Puerto Rico J.Pub.Health & Trop.Med., 16:3–13, 1940.

6. H. H. Mitchell, A study of factors associated with the growth and nutrition of Puerto Rican children. Human Biol., 4:469–508, 1932.

P. Morales Otero and M. A. Pérez, Health and socio-economic studies in Puerto Rico. III. Physical measurements of agricultural workers. Puerto Rico J.Pub.Health & Trop.Med., 12:450–474, 1936.

7. M. E. Wegman, R. Fernández Marchante, and M. Kramer, Infant mortality and infant feeding. Puerto Rico J.Pub.Health & Trop.Med., 17:228–245, 1942.

8. R. M. Suárez, Studies of the nutritional problem of Puerto Rico. I. Vitamin A deficiency in relation to dark adaptation and ocular manifestations. Puerto Rico J.Pub.Health & Trop. Med., 19:62–80, 1943.

9. H. E. Munsell, A. M. Cuadros, and R. M. Suárez, A study of plasma ascorbic acid values with relation to the type of diet used in Puerto Rico by groups of individuals of widely varied economic status. J.Nutrition, 28:383–393, 1944.

cluding inadequacy in proteins and the various food factors, are not rare among children belonging to the low-income groups of the Island."[10]

With the purpose of learning more about the nutritive qualities of the staples in the Puerto Rican diets and how to supplement them efficiently, the investigation of the effects of the rice and beans diets upon rats was continued.

One diet consisting of polished rice and red kidney beans only was compared with another containing polished rice, soybeans, casein, yeast, minerals, lard, and cod-liver oil, because the constituents required for one of the standard dietaries of the rat, such as the Sherman B diet,[11] were unavailable in Puerto Rico during 1942–1943. Observations were made upon growth, the time of sexual maturity of the females, estrus cycles, fertility of males and females, gestation, lactation, and the amount and percentage of bone ash in the femur. The capacity of the rats to recover from the effects of the rice and beans diet was also studied.

## EXPERIMENTAL METHODS

*Diets.* The composition and calculated nutritive values of the diets, prepared every week, are given in Table 1. The beans were soaked, cooked, dried, and ground. Proximate analyses of the prepared beans were then made. The rice was ground but not cooked. Table 1 shows clearly that Diet II is low in protein, fat, and calcium, the calculated calcium phosphorus ratio being 0.2 only.

*Procedure.* The rats were descendants of Wistar albino rats brought to the School of Tropical Medicine by Dr. D. H. Cook in 1927. Three groups were observed: (a) 29 males and 63 females receiving Diet I. Four generations were reared and bred on this diet. (b) 47 males and 45 females receiving Diet II. These rats were the young of females fed on Diet II from the day of birth of the young and throughout the period of lactation. After separation from the females, these young continued to receive Diet II. (c) 19 males and 24 females receiving Diet I after a period of retardation on Diet II. Males and females were reared separately from the age of 28 days. During their early growth period they were weighed twice a week.

10. R. M. Suárez, Studies of the nutritional problem of Puerto Rico. II. Appraisal of vitamin deficiency based on physical and biomicroscopic examination and X-ray studies of the long bones of a hospital population of 310 infants and children. Puerto Rico J.Pub.Health & Trop.Med., 21:61–73, 1945.

11. H. C. Sherman and M. Muhlfeld, Growth and reproduction upon simplified food supply. II. Influence of food upon mother and young during the lactation period. J.Biol.Chem., 53:41–47, 1922.



TABLE 1

*Composition and Calculated Nutritive Values of the Rice and Beans Diets*

| *Ingredients* | *Diet I Percent* | *Diet I Perce* |
|---|---|---|
| Polished rice | 43.0 | 67.0 |
| Soybean | 25.0 | — |
| Red kidney bean | — | 33.0 |
| Dried brewers' yeast | 10.0 | — |
| Casein (technical) | 7.5 | — |
| Lard | 12.0 | — |
| Cod-liver oil | 1.0 | — |
| Salt mixture[a] | 1.5 | — |
| *Percentage composition* (calculated) | | |
| Protein | 24.8 | 11.6 |
| Carbohydrate | 43.1 | 75.4 |
| Fat | 17.8 | 0.8 |
| Moisture | 7.9 | 8.9 |
| Crude fiber | 2.2 | 1.7 |
| Ash | 3.8 | 1.6 |
| Calcium | 0.487 | 0.04 |
| Phosphorus | 0.585 | 0.2 |
| *Nutritive values* | *Number* | *Numb* |
| Calories per 100 g. diet | 430 | 355 |
| Protein Calories per 100 Calories | 23 | 13 |
| Fat Calories per 100 Calories | 37 | 2 |
| Mg. calcium per 100 Calories | 113 | 13 |
| Mg. phosphorus per 100 Calories | 135 | 66 |
| Calcium/phosphorus | 0.84 | 0.2 |

[a]Calcium carbonate, 1 kg.; sodium chloride, 500 g.; ferric citrate, 50 g.; traces of sodiu fluoride, manganese sulfate, potassium alum, and potassium iodide.

Since maturity and estrus cycle data are most valuable chec upon growth,[12] estrus cycles were followed by daily examinatio of the vaginal contents according to the method of Long and Evans. The age and body weight at the time of the first estrus and t length of subsequent estrus cycles were also noted. At about 9 days of age, the females were mated during estrus with norm males,[14] and the morning after mating, examined for the presenc of the vaginal plug and the occurrence of spermatozoa in the vagin contents. During the 13th and 14th days of gestation, the "placent

12. H. M. Evans and K. S. Bishop, On the relation between fertility and nutrition. I. T ovulation rhythm in the rat on a standard nutritional regime. J.Med.Res., 1:319–331, 192

13. J. A. Long and H. M. Evans, The estrus cycle in the rat and its associated phenome Experimental studies in the physiological anatomy of reproduction. Memoirs U. Californ Berkeley, Vol. 6, 1922.

14. *Ibid.*

sign," a blood clot in the vagina that is an infallible sign of implantation of fetuses in the uterus, was looked for.

During pregnancy, the females were weighed at its beginning, on the 14th day, and daily after the 19th day. Normally, parturition occurred on the 22nd day, when litters were examined for size, sex ratio, number living, and birth weight. In cases of abnormal parturition, the females were killed. All rats, which died or were killed, were examined for dead fetuses and for the number of implantations and of resorption sites in the uterus.

Litters were not reduced in size during lactation. Observations were made on the number of young surviving the period and on the increase in body weight of mother and young. Females and young were weighed twice a week, on the 17th day, at which time the young began to eat the maternal diet, and on the 21st. The females were permitted a recuperative period of two weeks between pregnancies.

After the 90th day, males were mated with normal females in estrus. Inseminated females were examined for the "placental sign" on the 13th and 14th days of pregnancy. If three different females failed to present the sign of implantation after insemination, the male was killed, and the contents of the vas deferens in Locke's solution were examined for mature motile spermatozoa.

Growing rats were killed at different ages for bone ash studies. At all autopsies, the right femur was removed, cleaned of adhering soft tissues, air-dried, extracted with alcohol and ether, dried again at 100 degrees C, and ashed at low red heat. Determinations were then made of the amount of bone ash and of the percentage of ash on a moisture and fat-free basis.

Food consumption of some of the rats was measured. As biological variation among animals is known to be great, the data were compiled statistically whenever possible and expressed as the mean, standard deviation, coefficient of variation, and their probable errors.

## DISCUSSION

Since there was no significant difference in growth or reproduction between them, the data of the four generations of rats reared and bred on Diet I were combined.

*Growth.* The growth data are presented in Table 2 and in Chart I. Table 2 shows the mean body weight, standard deviation, coefficient of variation, and their probable errors at 28, 56, and 84 days, respectively. For purposes of comparison, the growth data cited by



Smith and Bing[15] have been included. Their rats, which were reared on a Sherman B diet supplemented with calcium, grew as well as, or better than, other rats reported in the literature.

TABLE 2

*Growth of Rats on Diets of Rice and Beans*

| *Diet and Sex* | *Age (Days)* | *Number of Rats* | *Mean Body Weight* | *Probable Error of Mean* | *Standard Deviation* | *Probable Error of Standard Deviation* | *Coefficient of Variation* |
|---|---|---|---|---|---|---|---|
| | | | G. | G. | G. | G. | |
| Wheat and milk[a] | | | | | | | |
| *Males* | 27 | 53 | 63 | | ± 9.39 | | 14.95 |
| | 55 | 104 | 188 | | ±35.27 | | 13.44 |
| | 85 | 46 | 285 | | ±31.62 | | 11.10 |
| *Females* | 27 | 11 | 60 | | ±11.49 | | 19.19 |
| | 55 | 38 | 150 | | ±23.07 | | 15.38 |
| | 85 | 28 | 189 | | ±20.90 | | 11.06 |
| Diet I | | | | | | | |
| *Males* | 28 | 97 | 60 | ±0.64 | ± 9.40 | ±0.46 | 15.7 ±0.78 |
| | 56 | 30 | 185 | ±2.40 | ±19.51 | ±1.70 | 10.5 ±0.92 |
| | 84 | 29 | 269 | ±3.39 | ±27.09 | ±2.40 | 10.0 ±0.89 |
| *Females* | 28 | 75 | 55 | ±0.74 | ± 9.46 | ±0.52 | 17.1 ±0.94 |
| | 56 | 47 | 141 | ±1.27 | ±12.94 | ±0.90 | 9.2 ±0.65 |
| | 84 | 47 | 183 | ±1.55 | ±15.75 | ±1.09 | 8.6 ±0.62 |
| Diet II | | | | | | | |
| *Males* | 28 | 47 | 19 | ±0.47 | ± 4.81 | ±0.33 | 24.8 |
| | 56 | 28 | 29 | ±0.82 | ± 6.47 | ±0.58 | 22.6 |
| *Females* | 28 | 45 | 20 | ±0.49 | ± 4.83 | ±0.34 | 24.2 |
| | 56 | 21 | 26 | ±0.87 | ± 5.91 | ±0.62 | 22.5 |

[a]A. H. Smith and F. C. Bing. J.Nutrition, 1:179–189, 1928–29.

It is evident from Table 2 that the rate of growth of rats on Diet I does not differ significantly from that observed by Smith and Bing.

Composite growth curves are shown in Chart I. The extent of biological variation is illustrated by the vertical lines representing the standard deviation of the mean body weight. A is the composite curve of 29 males and B, that of 47 females on Diet I from the time of birth to the age of 84 days.

Curves C and D represent 28 males and 21 females, respectively, fed on Diet II from the time of birth until 65 days of age, at which time they began to lose weight. The growth rate of these rats is seen to differ greatly from those on the control diet, though they gradually attained an average body weight of 28 to 32 g. However, with the exception of their small size, they appeared normal and remained active up to the age of 65 days, after which they began

15. A. H. Smith and F. C. Bing, Improved rate of growth on stock albino rats. J.Nutrition, 1:179–189, 1928–29.

losing weight and developed xerophthalmia, dying in about two weeks unless their diet was changed. Abscesses of the base of the tongue, a characteristic finding of vitamin A deficiency in the rat, were noted at autopsy of those that died.

The poor growth of rats on Diet II is in agreement with Cook and Rivera's[16] observations, though the latter did not note xerophthalmia in the rats fed typical Puerto Rican diets, presumably because those diets had been prepared with condiments such as *achiote*, tomatoes and peppers, all of which contain vitamin A. Cook and Rivera, however, reported the occurrence of rickets in their rats. In the present experiments, carried out in a sunlit laboratory with large open windows, rats on Diet II never developed rickets, as judged by the line-test and bone ash determinations, although the calculated calcium-phosphorus ratio of the diet was 0.2. Mild rickets invariably occurred if the rats were kept in a dark room with sunlight excluded.

The capacity of the rats to recover from the effects of Diet II was observed in the following manner. One member of a litter was given Diet I after 28 days of subsistence on Diet II, and a second, after 42 days. The rest of the litter were fed Diet II until they developed xerophthalmia and became moribund but, as soon as one of them died, the survivors were transferred to Diet I. This change in diet brought about immediate and rapid growth in all the animals. Those with xerophthalmia recovered, although in a few instances the cornea remained permanently opaque. As the rate of growth of the rats was similar during recovery, regardless of the time at which the diet had been changed, the growth data were combined. In Chart I curves E and F represent the growth rate of 19 males and 24 females, respectively, on Diet I after a period of stunting on Diet II. Chart I also demonstrates that after 84 days of resumed feeding on Diet I, the rats had not apparently attained the body weight of the control rats at 84 days of age.

In view of the great biological variation in growth, this difference is not statistically significant. Further work is in progress to determine whether rats, severely stunted on Diet II, eventually attain the full size characteristic of their species, as reported by Osborne and Mendel,[17] after stunting on low-calorie and low-protein diets. McCay, Sperling, and Barnes[18] noted that, during resumption of

16. D. H. Cook and T. Rivera, *op. cit.*
17. T. B. Osborne and L. B. Mendel, The resumption of growth after long continued failure to grow. J.Biol.Chem., 23:439–454, 1915.
18. C. M. McCay, G. Sperling, and L. L. Barnes, Growth, ageing, chronic diseases, and life span in rats. Arch.of Biochem., 2:469–479, 1943.



growth, rats subjected to severe stunting over a prolonged period by low-calories are unable to attain the same body size as controls, not subjected to retardation.

*Food consumption during growth.* Curves M and F, in Chart I, indicate the average amount of Diet I consumed daily by 12 males and 12 females, respectively, during the early growth period from 21 to 84 days of age. During the first three weeks, the food consumption increased rapidly from about 5 g. to 10 g. for males and 8 g. for females; during the rest of the period the increase was less and averaged 11.3 g. for males and 8.5 g. for females. The average daily food consumption for the entire period was 9.7 g. (calculated to supply 41 Calories) for males and 7.6 g. (calculated to supply 32 Calories) for females.

The amount of Diet II consumed daily by rats from 28 to 56 days of age did not vary greatly and was less than one third as great as that of Diet I. Twelve males consumed an average of 3.3 g. (calculated to supply 12 Calories) of Diet II daily.

*Estrus.* The data on the age and body weight of females at the time of first estrus and on the length of estrus cycles during the period from the first estrus until the age of 90 days are presented in Table 3. It will be seen that the first estrus occurred at a mean age of 38 days and at a mean body weight of 92 g., values which are in general agreement with the data cited by Anderson[19] for Wistar rats on a wheat and milk diet. The estrus cycles in rats fed Diet I were regular and of 4 to 6 days' duration, as described by Long and Evans[20] for the normal rat. Frequently, the first cycle was longer than 6 days, but not one of the rats had long cycles regularly.

Rats reared on Diet II from the time of birth failed to mature sexually. When they were permitted to resume growth on Diet I, estrus was observed to occur about two weeks after the change in diet, regardless of the length of time of retardation on Diet II. Table 3 shows that the first estrus occurred at a mean weight of 66 g., a value significantly lower than that of the controls on Diet I at the time of their first estrus. Presumably, maturation of the ovum continued during the period of retarded growth; subsequently, estrus appeared at 4 to 6 day intervals.

Although Diet II was inadequate for sexual development, it permitted the maintenance of regular estrus cycles in mature animals.

19. D. H. Anderson, Studies on the physiology of reproduction. I. The effect of thymectomy and of season on the age and weight at puberty in the female rat. J.Physiol., 74:49–64, 1932.
20. J. A. Long and H. M. Evans, *op. cit.*

TABLE 3

*Estrus Cycles of Rats on Rice and Beans Diets*

| Diet | Number of Determinations | Mean and Probable Error | | Standard Deviation and Probable Error | | Coefficient of Variation and Probable Error | |
|---|---|---|---|---|---|---|---|
| *Age in days at time of first estrus* | | | | | | | |
| Wheat and milk[a] | 11 | 42.0 | ±0.7 | | | | |
| Diet I | 47 | 37.9 | ±0.45 | ± 4.57 | ±0.39 | 12.1 | ±0.88 |
| Diet II | 21 | No estrus | | | | | |
| *Body weight in g. at time of first estrus* | | | | | | | |
| Wheat and milk[a] | 11 | 87.8 | ±1.6 | | | | |
| Diet I | 47 | 90.7 | ±1.24 | ±12.63 | ±0.88 | 13.9 | ±1.01 |
| Resumption of growth Diet I | 25 | 66.0 | ±3.96 | ±29.34 | ±2.80 | 44.4 | |
| *Length in days of estrus cycles* | | | | | | | |
| Diet I | 492 | 4.79 | ±0.03 | ± 1.09 | ±0.02 | 22.8 | ±0.47 |
| Resumption of growth Diet I | 343 | 4.75 | ±0.04 | ± 1.23 | ±0.03 | 26.0 | ±0.71 |

[a]D. H. Anderson, J.Physiol., 74:49–64, 1932.

Female rats, which had been reared on Diet I until the occurrence of estrus, were given Diet II. The rate of growth was altered, but there was no cessation of the 4 to 6 day cycles for about two months. After this time, the vaginal contents consisted largely of cornified cells, a finding characteristic of vitamin A deficiency.[21]

*Fertility.* The breeding and fertility data of 63 females and 29 males, reared on Diet I, may be summarized as follows: at the first pairing with a male during estrus, 52 of the females were inseminated, as proved by the presence of the vaginal plug and the occurrence of spermatozoa in the vaginal contents. Ten females were inseminated on the second pairing; one female failed to become inseminated by 3 different males and was subsequently discarded. Implatation of fetuses occurred in 59 of the inseminated females, as shown by the appearance of a vaginal blood clot on the 13th to 14th days of gestation. The 3 females, in which the blood clot did not appear, were bred again and in each case, the implantation sign followed insemination. All of the females presenting the implantation sign cast litters.

At the first pairing with a female in estrus, 20 males inseminated females; 4 inseminated females at the second pairing, and 2 at the third. Three males failed consistently to inseminate females and were killed. At autopsy, they presented large, firm testes and many

21. H. M. Evans and K. S. Bishop, On the relation between fertility and nutrition. II. The ovulation rhythm in the rat on inadequate nutritional regimes. J.Med.Res., 1:335–356, 1922.





motile spermatozoa in the contents of the vas deferens, but the cause of their impotence was not determined.

Of the 26 inseminated females, 20 presented the implantation sign and cast litters. The 6 males that failed to sire litters were bred again. Three of them were fertile, and the remaining 3 proved sterile. Though they retained the ability to inseminate females, insemination was never followed by implantation. These 3 males were litter-mates. They were killed and at autopsy, the testes were found small and watery. The contents of the vas deferens showed a few, non-motile spermatozoa, which findings are typical of vitamin E deficiency.[22]

Twelve of the fertile males were used for stock breeding. Their fertility data may be summed up as follows: in a total of 282 pairings with females, there were 263 (94 percent) inseminations, 247 (94 percent) of which were fertile.

The fertility of rats, which had been permitted to resume growth on Diet I after a period of stunting on Diet II, was observed to be similar to that of the control rats on Diet I. Twenty-three females and 14 males were bred. All the females were inseminated the first time of pairing; all copulations were fertile. One of the males failed to inseminate females but presented no abnormalities in the reproductive tract. Another was sterile, presenting findings typical of vitamin E deficiency. The rest were fertile.

In view of Mason's[23] report to the effect that the minimum daily requirement of vitamin E for preventing sterility in E-deficient rats, either as wheat germ-oil concentrate or as A-tocopherol, is essentially the same for both sexes, it was of interest to note that all females on Diet I were fertile, while 9 percent (4 out of 43) of males were functionally sterile and presented gross alterations, characteristic of vitamin E deficiency, in the reproductive tract. A detailed study of the vitamin E content of rice and beans diets will appear elsewhere.

*Gestation.* Gestation data are presented in Tables 4 and 5. Three groups of rats received Diet I during gestation: 62 were reared on Diet I; 23 resumed growth on Diet I after having been subjected to the effects of Diet II in early life, and 11 were permitted to recover on Diet I after having received Diet II during the previous

22. K. E. Mason, Testicular degeneration in albino rats fed a purified food ration. J.Exper. Zool., 45:159–229, 1926.
H. M. Evans and G. O. Burr, The antisterility vitamin fat soluble E. Memoirs U. California, Berkeley, Vol. 8, 1927.

23. K. E. Mason, Minimal requirements of male and female rats for vitamin E. Am.J. Physiol., 131:268–280, 1940.

gestation. All of the gestations on Diet I terminated on the 22nd day after insemination with the birth of litters containing living young.

The fourth group consisted of 24 females that received Diet II during gestation. They had been reared and bred on Diet I and were given Diet II during the second gestation from the day of insemination. Fifteen females cast litters: 11 contained living young; in the remaining 4, the young were dead at the time that the litters were found. Parturition occurred on the 23rd day in 7 of the gestations. Five of the rats not casting litters appeared to be ill at the time of parturition; 3 died and 2 were killed. As the 4 remaining rats did not cast litters, they were killed after the 22nd day.

At autopsy, 2 of the latter presented resorption sites in the uterus, such as are typical of vitamin E deficiency in the female rat;[24] all of the others presented dead fetuses. No gross abnormalities were noted in the young, the placenta, nor in the viscera. The difficulty during parturition may have been caused by a lack of vitamin A,[25] as it has already been shown that this vitamin is the limiting factor in a diet of rice and red kidney beans. However, these rats received Diet I until the day of insemination and presumably, the cod-liver oil content (1 percent) of Diet I would be sufficient to enable stores of vitamin A to accumulate in the liver.

It will be seen in Tables 4 and 5 that the data of the three groups of rats on Diet I are similar. These observations are in accord with the reports of Osborne and Mendel[26] and of Sherman, Rouse, Allen, and Woods[27] that female rats, which have been seriously stunted on inadequate diets and then permitted to recover on better diets, are able to produce healthy offspring and to suckle them successfully.

The litters that were born of females on Diet II were smaller in size, averaging 5.5 young instead of 8.7. They contained fewer living young, 57 percent as compared with 98; the birth weight was also less, 3.6 g. instead of 5.5 g. No difference was observed in birth weight between males and females nor in the percentage of living young.

24. H. M. Evans and K. S. Bishop, On the relation between fertility and nutrition. IV. The production of sterility with nutritional regimes adequate to growth. J.Med.Res., 3:233–283, 1923.

25. K. E. Mason, Foetal death, prolonged gestation, and difficult parturition in the rat as a result of vitamin A deficiency. Am.J.Anat., 57:303–349, 1935.

26. T. B. Osborne and L. B. Mendel, *op. cit.*

27. H. C. Sherman, M. E. Rouse, B. Allen, and E. Woods, Growth and reproduction upon simplified food supply. J.Biol.Chem., 46:503–519, 1921.



TABLE 4

*Data of Litters of Rats on Rice and Beans Diets*

| *Diet* | *Number of* | | | *Number of Young* | | | | *Mean Body Weight in g.* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Living* | | *Dead* | | | |
| | *Litters* | *Young* | *Young per Litter Average* | *Males* | *Females* | *Males* | *Females* | *Males* | *Females* |
| Diet I | 62 | 542 | 8.7 | 279 | 256 | 2 | 5 | 5.6 | 5.3 |
| Resumption of growth Diet I | 23 | 196 | 8.5 | 95 | 98 | 1 | 2 | 5.5 | 5.2 |
| Recovery after gestation on Diet II | | | | | | | | | |
| Diet I | 11 | 88 | 8.0 | 43 | 40 | 2 | 3 | 5.7 | 5.4 |
| Diet II | 15 | 82 | 5.5 | 24 | 23 | 18 | 17 | 3.6 | 3.7 |

TABLE 5

*Gain in Body Weight During Reproduction of Rats on Rice and Beans Diets*

| Diet | Number of Rats | Mean Gain in Body Weight and Probable Error | | Standard Deviation and Probable Error | | Coefficient of Variation |
|---|---|---|---|---|---|---|
| *21 days of gestation* | | | | | | |
| *Body weight of female and young* | | | | | | |
| Diet I | 62 | 90.6 | ±1.43 | ±16.7 | ±1.01 | 18.4 |
| Resumption of growth | | | | | | |
| Diet I | 23 | 86.2 | ±2.8 | ±20.2 | ±2.0 | 23.4 |
| Recovery after gestation on Diet II | | | | | | |
| Diet I | 11 | 90.1 | ±3.4 | ±16.0 | ±2.5 | 17.8 |
| Diet II | 24 | 4.9 | ±0.26 | ±19.0 | ±0.18 | |
| *Body weight of female* | | | | | | |
| Diet I | 62 | 36.3 | ±0.99 | ±11.6 | ±0.70 | 31.9 |
| Resumption of growth | | | | | | |
| Diet I | 23 | 28.8 | ±1.57 | ±11.2 | ±1.11 | 38.8 |
| Recovery after gestation on Diet II | | | | | | |
| Diet I | 11 | 32.3 | ±2.5 | ±11.6 | ±1.8 | 35.9 |
| Diet II | 15 | —27.3 | ±3.17 | ±17.8 | ±2.21 | 65.3 |
| *17 days of lactation* | | | | | | |
| *Body weight of female and young* | | | | | | |
| Diet I | 54 | 156.1 | ±3.63 | ±39.6 | ±2.57 | 25.4 |
| Resumption of growth | | | | | | |
| Diet I | 19 | 146.0 | ±5.26 | ±34.0 | ±3.73 | 23.3 |
| Diet II | 23 | —12.9 | ±2.61 | ±18.6 | ±1.85 | |
| *Body weight of female* | | | | | | |
| Diet I | 54 | —10.2 | ±1.20 | ±13.1 | ±0.85 | |
| Resumption of growth | | | | | | |
| Diet I | 19 | —1.7 | ±2.98 | ±19.1 | ±2.11 | |
| Diet II | 23 | —64.6 | ±2.82 | ±20.1 | ±1.99 | 31.1 |

Table 5 shows that, regardless of the previous history of the female, there was a mean gain in body weight of 90 g. on Diet I during 21 days of gestation. Since the mean body weight at the beginning of gestation was 200 g., there was an increase in body weight of 45 percent. About 35 g. (17 percent) were retained by the female after the birth of the litter. Although the variability was very great among females on Diet II, there was a gain of about 5 g. Those that cast litters lost an average of 25 g. Apparently the fetuses developed at the expense of maternal tissue.

*Food consumption during gestation.* The average amount of Diet I consumed by 28 females during 21 days of gestation was 273 g. There was no increase in food consumption during the last week of gestation when the body weight increased greatly; very little was eaten on the day of parturition (22nd day). The average daily consumption of Diet I was 13.0 g., an amount calculated as equivalent to 50 Calories.

The mean total amount of Diet II consumed by 17 females during



21 days of gestation was 217 g. During the first two weeks, the amount consumed daily averaged 12.1 g.; during the third week, it dropped to 6.5 g.; usually there was no food at all eaten after the 19th day. The rats, which spontaneously ate more food, were the ones better able to undergo gestation. The average daily consumption of Diet II was 10.3 g., an amount calculated as equivalent to 36.5 Calories.

*Lactation.* Two groups of rats on Diet I were observed during lactation: 57 females, reared and bred on Diet I, and 20 females that were permitted to resume growth on Diet I after having been subjected to the effects of Diet II in early life. The young of 3 litters, among the first group, and of one litter, in the second, did not survive the period of lactation. A third group of 23 females received Diet II during lactation. They had been reared and bred on Diet I and were given Diet II during the second period of lactation from the day of birth of the young. There were survivors in all of the litters of the third group. The rest of the lactation data are presented in Tables 5 and 6.

Data of litters born on Diet II were not included in the tables because 83 percent (39 out of 47) of the living young, in litters of females on Diet II during gestation, died before the 5th day of lactation. The 8 survivors were distributed among 3 litters. It was noted that they were members of small litters with individual birth weights exceeding 5 g. The birth weight of those that died averaged 3.3 g. The lactation data of the females with surviving young were similar to those of the females on Diet II from the day of birth of their young.

The data of the two groups of rats in Tables 5 and 6, receiving Diet I during lactation, are similar yet significantly different from those of the females receiving Diet II. The number of young surviving Diet II (see Table 6) was less, and the body weight at 21 days was less than half as great as that of the controls on Diet I.

During the first 17 days of lactation on Diet I (see Table 5), there was a mean gain in total body weight of female and young of 156 g. This represented a gain of 56 percent, as the initial weight of female and young was 280 g. Although the extent of biological variation was great, the females themselves usually lost about 10 g. (4 percent) in body weight during the first 17 days of lactation. On Diet II, the combined weight of female and young decreased about 13 g. during the first 17 days of lactation, and the females invariably lost about 65 g. (23 percent). It is evident that during the first 17 days of lactation the offspring developed at the cost of maternal tissue.

TABLE 6

*Lactation Data of Rats on Rice and Beans Diets*

| Diet | Number of Litters | Number of Young | | | | | | Mean Body Weight at 21 Days in g. | |
|---|---|---|---|---|---|---|---|---|---|
| | | Males | | | Females | | | | |
| | | At Beginning | At 21 Days | Percent | At Beginning | At 21 Days | Percent | Males | Females |
| Diet I | 54 | 250 | 237 | 94.8 | 212 | 194 | 91.4 | 35.9 | 34.2 |
| Resumption of growth | | | | | | | | | |
| Diet I | 19 | 83 | 81 | 97.6 | 80 | 74 | 92.5 | 33.5 | 30.9 |
| Diet II | 23 | 95 | 83 | 87.2 | 95 | 78 | 82.2 | 15.8 | 14.7 |



*Food consumption during lactation.* During the first 17 days of lactation, the average amount of Diet I consumed by 19 females was 374 g. The daily amount was 22 g., calculated as equivalent to 90 Calories. The amount of Diet II consumed by 14 females for the same period was 222 g., a daily average of 13 g., calculated as equivalent to 46 Calories.

*Bone Ash.* The bone ash matter, presented in Charts II and III, are self-explanatory. As sex was found to be without significant influence, the records of males and females are not treated separately.

When the bone ash data were plotted according to the age of the rat, it was evident that the degree of calcification of the femur of the male rats, as indicated by the percentage of ash, exceeded that of the females and was greater not only in rats reared on Diet I than in those receiving Diet II but also in those resuming growth. However, it will be noted in Charts II and III that, though there is considerable variation, the amount and percentage of bone ash in the femur is related to the body weight of the rat, regardless of sex, diet, or subjection to stunting on early life. For reference, data cited by Hammett[28] are included in Chart II and by Bethke, Steenbock, and Nelson[29] in Chart III.

## SUMMARY

Growth and reproduction in the rat on diets of rice and beans have been described in detail. One of the diets, consisting of polished rice, soybean, casein, yeast, minerals, lard, and cod-liver oil is apparently adequate except for its lack of ample vitamin E to prevent sterility in all male rats. The other diet, composed of polished rice and red kidney beans, is inadequate as the following results bear out:

1. Young rats grow very slowly and never attain sexual maturity, dying after about 70 days with symptoms of vitamin A deficiency.

2. If the diet is given during gestation from the day of insemination, fetuses develop at the expense of maternal tissue, and there is a high maternal and infant mortality.

3. If the diet is given during lactation from the day of birth of the litter, the young grow slowly at the cost of maternal tissue, and the body weight of those that survive is less than half of that of the controls.

4. If young rats subjected to retardation on Diet II are permitted

28. F. S. Hammett, A biochemical study of bone growth. I. Changes in the ash, organic matter and water during growth (*mus norvegicus albinus*). J.Biol.Chem., 64:409–428, 1925.

29. R. M. Bethke, H. Steenbock, and M. T. Nelson, Fat-soluble vitamins. XV. Calcium and phosphorus relations to growth and composition of blood and bone, with varying vitamin intake. J.Biol.Chem., 58:71–103, 1923–1924.

to recover on Diet I, their rate of growth and reproduction compares favorably with that of the controls on Diet I.

Under the conditions of this experiment, the amount and percentage of bone ash in the femur is related to the body weight of the rat, regardless of sex, diet, or subjection to a period of retardation in early life.

ACKNOWLEDGMENT

The writer wishes to acknowledge the assistance of Miss Hilda García de la Noceda in making the proximate analyses mentioned in this study.



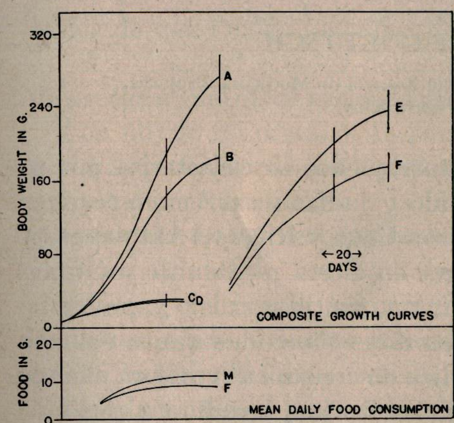


CHART I

Composite growth curves of rats. A, 29 males and B, 47 females on Diet I; C, 24 males and D, 14 females on Diet II; E, 19 males and F, 24 females, resuming growth on Diet I after having been stunted on Diet II. M, mean daily consumption of Diet I of 12 males and F, that of 12 females. Vertical lines represent standard deviations.

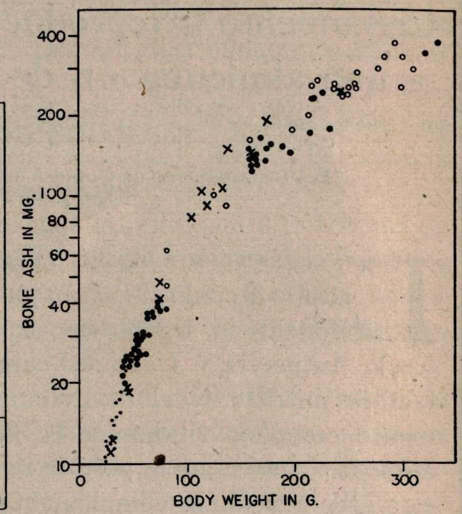


CHART II

Relationship between the amount of bone ash in the femur and the body weight of the rat. Large dots represent rats on Diet I; small dots, rats on Diet II; circles, rats resuming growth on Diet I after having been stunted on Diet II; X, data cited by Hammett (1925).

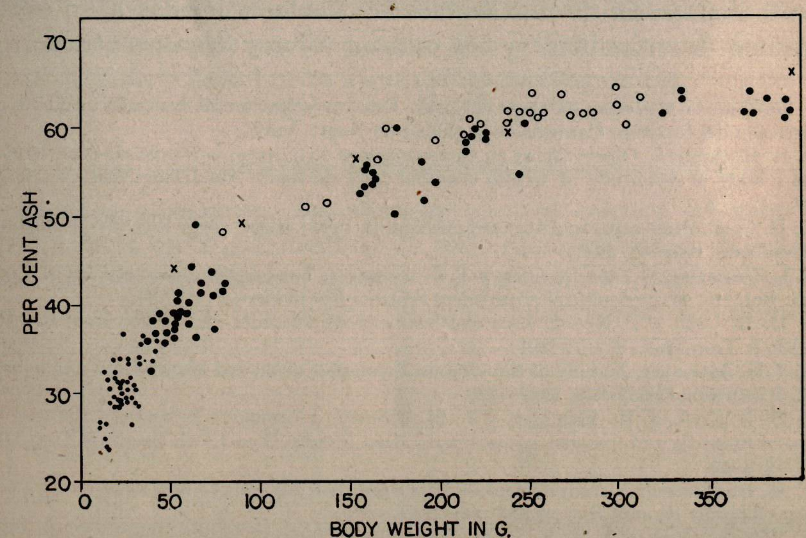


CHART III

Relationship between the percentage of bone ash in the extracted dry femur and the body weight of the rat. Large dots represent rats on Diet I; small dots, rats on Diet II; circles, rats resuming growth on Diet I after having been stunted on Diet II; X, data cited by Bethke, Steenbock, and Nelson (1923–24).