Influence of Dietary Protein Depletion and Repletion on Sex Organ Weight of Male Rats in Relation to Age

Takatoshi ESASHI,1 Ryokuero SUZUE,1 and James H. LEATEM2 (deceased)

1 The National Institute of Nutrition, Toyama, Shinjuku-ku, Tokyo 162, Japan
2 Rutgers University, New Brunswick, N. J. 08903 U.S.A.

(Received September 11, 1981)

Summary Studies were made on the effect of protein-free diet (PFD) feeding and on recovery from PFD feeding on sex organ weight, serum testosterone, pituitary prolactin and pituitary growth hormone content of young (4 months old) and old (18 months old) male rats. After 20 days of protein depletion, the testis weight remained almost the same, but the ratio of testis weight to body weight was higher in the lower age group, demonstrating a greater resistibility to protein deficiency in young rats. The weight of the seminal vesicle and prostate decreased on ingestion of PFD in both age groups, but a more marked increase was shown in refed young rats. Feeding of PFD for 20 days significantly reduced pituitary prolactin, pituitary growth hormone and serum testosterone in both age groups. Refeeding of protein increased these hormones, but a more marked increase was also observed in the younger group. These results seem to support the hypothesis that one of the important characteristics of an aging organism is its reduced capacity to adapt to environmental changes.

Key Words protein depletion, protein repletion, age, testosterone, male sex organ, pituitary prolactin, pituitary growth hormone

The functional integrity of the endocrine system is dependent upon adequate nutrition (1, 2). The detrimental effects of under-feeding on reproductive processes in male rats are well documented (3, 4). The absence of dietary protein is also known to have adverse effects on the reproductive system of male rats (5, 6). Feeding a protein-free diet to sexually immature rats results in marked regression of the reproductive tract and prevents the onset of spermatogenesis. In older rats, testes

1 江指隆年, 鈴江錦衣郎
and accessory sex organs are affected less severely and the spermatogenic function is usually maintained (6).

Sex organ protein and serum testosterone are reduced by under-feeding and are restored during refeeding in young rats (6–8). Organs protein and serum testosterone may also decline on aging (9–12), but the influence of protein depletion and repletion in relation to age has received little attention (13).

Our studies were made on the effects of protein-free diet (PFD), and of recovery from protein deficiency, on sex organ weight, serum testosterone, pituitary prolactin and pituitary growth hormone content of young (4 months old) and old (18 months old) rats.

MATERIALS AND METHODS

Animals. Adult Long-Evans male rats, 4 and 18 months of age from the Rutgers University inbred colony, were used. All animals were of known date of birth and had never been used as breeders. They were fed on Purina cereal basal diet supplemented weekly with cod liver oil on bread and sliced carrot and were kept under controlled lighting (12 hr of light and 12 hr of darkness with overhead fluorescent bulbs turned on at 06:00 hours), humidity (50–65%), and temperature (24±1°C) until 4 or 18 months of age.

During the experiments, the rats were housed individually in wire-mesh cages and were given feed and water ad libitum. On autopsy, they were examined grossly and the animals bearing tumors were discarded.

Table 1. Composition of the diets.

| Ingredients         | Protein-free diet | 18% casein diet |
|---------------------|-------------------|-----------------|
| Milk casein         | 0 g               | 180 g           |
| Sucrose             | 134               | 134             |
| Dextrose            | 382               | 202             |
| Dextrin             | 182               | 182             |
| Lard                | 222               | 222             |
| Salt mix. a         | 18                | 18              |
| Agar                | 32                | 32              |
| Vitamin E b         | 10                | 10              |
| Cod liver oil       | 20                | 20              |
| Vitamin mix. c      | 25 ml             | 25 ml           |
| Water               | 1,375             | 1,375           |

a Wesson salt mixture (14). b 100 mg in 10 g Crisco. c Vitamin content (per 100 ml mixture): vitamin B₁ 80 mg, V. B₂ 80 mg, V. B₆ 80 mg, V. B₁₂ 0.12 mg, V. C. 80 mg, V. K. 16 mg, V. A. palmitate 120,000 U. S. P. U., V. D₂ 20,000 U. S. P. U., calcium pantothenate 400 mg, para-aminobenzoic acid 400 mg, niacin 400 mg, biotin 2 mg, folic acid 8 mg, inositol 1.2 g, choline chloride 12 g.

J. Nutr. Sci. Vitaminol.
Experimental design. All rats were fed on protein-free diet (PFD) ad libitum for 20 days. At the end of the 20-day period, half of them were killed by decapitation between 1 and 3 P.M. and the remainder were fed 18% casein diet for 20 days and used as refeeding rats.

In the experiment for nitrogen balance testing, rats were kept in individual wire-mesh metabolic cages and feces and urine were collected during the last four days of the protein-depletion period and every four days of the protein-repletion period.

Diets. The composition of the protein-free diet and 18% casein diet is shown in Table 1. These diets include all known dietary essentials except for the presence or absence of protein. They are also isocaloric.

Assay. Serum testosterone content was estimated by radioimmunoassay. Briefly, the procedure included diethylether extraction, separation by Sephadex LH-20 column chromatography, and radioimmunoassay using antibody to testosterone-3-oxime-bovine serum albumin obtained from New England Nuclear.

Pituitary prolactin and growth hormone were measured by polyacrylamide gel electrophoresis (15). Protein was determined by the method of Lowry et al. (16). Nitrogen was determined by the micro-Kjeldahl method.

RESULTS AND DISCUSSION

Body weight and organ weight

As shown in Table 2, protein depletion brought about a significant loss in body weight and protein repletion a significant body weight gain in both age groups. The relative organ weight of the liver, kidney and heart is also shown in Table 2. Protein depletion did not demonstrate a significant effect on the ratio in relation to age. Refeeding protein for 20 days caused a significant gain in organ weight but did not show any age effect. The weight of the gonads, however, showed an age difference due to feeding PFD for 20 days (Table 3). The testis weight is almost the same in both age groups, but the ratio of testis weight to body weight is greater in the younger group, demonstrating a greater resistibility to protein deficiency in young rats. Refeeding of protein did not change the tendency, but the ratio decreased depending upon the increase in body weight. The two accessory organs, seminal vesicle and prostate, also demonstrated an age effect. The weight of both the seminal vesicle and prostate increased due to refeeding in both age groups, but a more marked increase was shown in young rats. The relative organ weight also showed a greater increase in refeed young rats.

The importance of dietary components for biosynthesis, secretion or clearance of hormones related to maintenance and development of sex organ weight, and sensitivity to hormone receptor has been noted by Mann (17) and others (18). The results obtained here may suggest that dietary protein contributes in some way to the functioning of these sex hormones. Age difference in response to dietary change is also suggested (19).
Table 2. Effects of protein depletion and repletion on body weight and organ weight in 4- and 18-month-old male rats.

| Age in months | Initial control | Protein-free diet for 20 days | Refed 18% casein diet for 20 days |
|---------------|----------------|-----------------------------|----------------------------------|
| Number of rats| 4              | 18                          | 4                                |
|               | 12             | 11                          | 4                                |
|               | 11             | 11                          | 11                               |
| Body weight   | 412 ± 17.3*    | 488 ± 23.2                  | 392 ± 11.4                       |
| Final         |                |                             | 530 ± 17.1                       |
|               |                |                             | 334 ± 9.7                        |
|               |                |                             | 467 ± 14.2                       |
|               |                |                             | 328 ± 10.7                       |
|               |                |                             | 523 ± 11.8                       |
|               |                |                             | 397 ± 16.6                       |
|               |                |                             | 567 ± 21.7                       |
| Kidney (g)    | 2.94 ± 0.10^a,b,** | 3.50 ± 0.23^a,c          | 2.10 ± 0.06^b,d,e               |
| (per 100 g B.W.) | 0.71 ± 0.02^a  | 0.72 ± 0.04^b,c          | 0.63 ± 0.01^a                  |
|               |                |                             | 0.63 ± 0.03^c,d                 |
|               |                |                             | 2.67 ± 0.12^e,j                |
|               |                |                             | 3.56 ± 0.12^f,j                |
| Heart (g)     | 1.07 ± 0.03^a,b | 1.16 ± 0.05                  | 0.88 ± 0.02^b,c               |
| (per 100 g B.W.) | 0.26 ± 0.11       | 0.24 ± 0.01^a           | 0.27 ± 0.01^b                  |
|               |                |                             | 0.24 ± 0.01^c                  |
|               |                |                             | 0.98 ± 0.03^a,c                 |
|               |                |                             | 1.24 ± 0.04^d,e                |
| Liver (g)     | 13.3 ± 0.73    | 15.4 ± 1.01*             | 11.7 ± 0.50^b                 |
| (per 100 g B.W.) | 3.15 ± 0.09         | 3.15 ± 0.09               | 3.50 ± 0.10^a                 |
|               |                |                             | 3.30 ± 0.10^b                 |
|               |                |                             | 3.30 ± 0.10^c                 |
|               |                |                             | 3.00 ± 0.10^d,e                |

* Mean ± SE. ** The results were compared by the t-test. Having the same superscript means significantly different to at least a 95% probability in each horizontal row.
Table 3. Effects of protein depletion and repletion on sex organ weight in 4- and 18-month-old male rats.

| Age in months | Number of rats | Initial control | Protein-free diet for 20 days | Refed 18% casein diet for 20 days |
|---------------|---------------|-----------------|-------------------------------|-----------------------------------|
|               | 4             | 18              | 4                             | 18                                |
| 4             | 12            | 11              | 11                            | 11                                |
| Testes (g)    | 2.99 ± 0.07^a*** | 3.09 ± 0.09     | 2.77 ± 0.07^a                 | 3.06 ± 0.09                       |
| (per 100 g B.W.) | 0.73 ± 0.07^a,b | 0.63 ± 0.03^a,c,d | 0.83 ± 0.04^b,c,f              | 0.66 ± 0.02^d,e,j                |
| Epididymis (g) | 0.98 ± 0.03^a,b | 1.07 ± 0.03^a,c,e | 0.84 ± 0.02^b,d                | 0.95 ± 0.03^d                     |
| (per 100 g B.W.) | 0.24 ± 0.01   | 0.22 ± 0.01^a   | 0.25 ± 0.01^b                 | 0.20 ± 0.01^b,c,e                |
| Seminal vesicle (mg) | 375 ± 14.8^a | 420 ± 19.2^b | 272 ± 12.9^a,c,d              | 326 ± 19.9^b,c,e                 |
| (per 100 g B.W.) | 91.0 ± 4.6    | 86.1 ± 5.5^a,b  | 81.4 ± 4.5                    | 69.8 ± 3.4^b                      |
| Ventral prostate (mg) | 499 ± 46.5^a,b,c | 354 ± 21.6^a,d | 251 ± 20.5^c,e                | 253 ± 18.8^d,f                   |
| (per 100 g B.W.) | 121 ± 8.6^a,b,c | 72.5 ± 6.1^a,d  | 75.1 ± 6.8^c,e,f              | 54.2 ± 3.8^d,e,g                 |
|                |               |                 | 377 ± 57.4^b,e                | 357 ± 27.6^f                      |
|                |               |                 | 95.0 ± 11.9^b,f,h             | 63.0 ± 3.9^e,h                    |

* Mean ± SE. ** Same as ** in Table 2.
Digestibility of protein

True digestibility of protein was not significantly different between the two groups during both the first four days and the 20 days of the refeeding period (Table 4). Therefore, a factor other than digestibility of protein seems to contribute to the greater gain in gonadal weight in refed young rats. Indeed, higher urinary nitrogen excretion of old rats has been shown by Everitt (20), demonstrating a decreased anabolic action.

Pituitary prolactin and growth hormone

Prolactin augments the action of lutenizing hormone in testosterone synthesis and increases the response of the seminal vesicle to testosterone (21). The augmenting effect of growth hormone on accessory gland growth has been observed, although the effect is generally accepted to be less than with prolactin (22, 23). The two hormones act synergistically with androgen to promote prostate growth (24).

As shown in Table 5, prior to protein depletion, pituitary prolactin was not significantly different between young and old rats. Feeding the protein-free diet for 20 days resulted in significantly reduced pituitary prolactin, but did not show up any difference by age. Refeeding of protein resulted in an increase in pituitary prolactin content from 6.5 μg to 25.3 μg per pituitary in refed young rats and from 7.2 μg to 30.9 μg in old rats. No significant difference between the two age groups

Table 4. Excretion of nitrogen during the first four days and the 20 days of refeeding periods.

| Age in months | Refed first four days | Refed 20 days |
|---------------|-----------------------|--------------|
| Number of rats| 4 18                  | 4 18         |
| Intake nitrogen (mg) | 1,707.4±65.8***      | 7,913.5±284b | 10,432.2±533.0b |
| Feces nitrogen (mg) | 196.7±8.4             | 1,107.8±51.1 | 1,275.1±69.7 |
| Refed          | 112.9±5.3             | 564.4±26.5   | 656.6±49.3 |
| PFD***         | 86.8±6.8              | 543.4±51.1   | 618.8±59.4 |
| Difference     | 90.3±12.1             | 618.8±59.4   | 813.6±517.0b |
| Absorbed nitrogen (mg) | 1,623.6±63.3a        | 7,370.0±260.0b | 9,813.6±517.0b |
| Digestibility (%) | 95.1±0.40            | 93.2±0.58    | 94.0±0.54 |
| Urinary nitrogen (mg) | 537.0±31.2a          | 3,081.8±164.9b | 5,442.1±312.4b |
| Refed          | 145.6±10.8a           | 729.8±54.2b  | 1,126.2±102.2b |
| PFD***         | 392.4±33.3a           | 2,352.0±166.0b | 4,315.9±286.9b |
| Difference     | 919.9±31.9a           | 5,018.0±277.9 | 5,497.8±390.7 |

* Mean ± SE. ** Same as ** in Table 2. *** Calculated from the average nitrogen excretion during the last four days of protein-free diet feeding periods. ' Intake N—(fecal N—metabolic fecal N)—(urinary N—endogenous N).

J. Nutr. Sci. Vitaminol.
Table 5. Effects of protein depletion and repletion on pituitary prolactin and growth hormone in 4- and 18-month-male rats.

| Age in months | Initial control | Protein-free diet for 20 days | Refed 18% casein diet for 20 days |
|---------------|----------------|-----------------------------|----------------------------------|
|               | 4 18           | 4 18                        | 4 18                             |
| Number of rats| 16 17          | 11 11                       | 11 11                            |
| Pituitary weight |                |                             |                                  |
| (mg)          | 10.7 ± 0.3a,***| 9.1 ± 0.3d                 | 10.6 ± 0.8                       |
| (per 100 g B.W.) | 2.6 ± 0.1     | 2.7 ± 0.1                   | 2.7 ± 0.1                        |
| Pituitary protein |              |                             |                                  |
| (µg/mg pituitary) | 140.6 ± 3.2a,b | 124.3 ± 2.7b                | 125.6 ± 4.7a                     |
| (µg/pituitary)   | 1,504 ± 50a,b  | 1,134 ± 49b,e               | 1,365 ± 134                      |
| Pituitary prolactin*** | |                             |                                  |
| (µg/mg pituitary) | 3.3 ± 0.4a    | 0.73 ± 0.1a,c,e            | 2.43 ± 0.47a                     |
| (µg/mg protein)  | 22.3 ± 2.2a   | 5.94 ± 0.80a,e,d           | 16.6 ± 4.2a                      |
| (µg/pituitary)   | 35.3 ± 1.9a   | 6.64 ± 0.79a,d,e           | 25.8 ± 4.5a                      |
| Pituitary growth hormone*** | |                             |                                  |
| (µg/mg pituitary) | 100.3 ± 7.6a,b | 64.7 ± 6.2b,d,e,f         | 106.7 ± 8.9c,e,g                |
| (µg/mg protein)  | 706.3 ± 44.2a,b | 520.9 ± 48.1b,c,d,e       | 857.3 ± 78.0d,f                 |
| (µg/pituitary)   | 1,073 ± 83.1a,b | 589 ± 58b,d,e       | 1,131 ± 126d,f                   |

* Mean ± SE. ** Same as ** in Table 2. *** Standard materials were obtained from the Institute of Arthritis and Metabolic Diseases of the National Institute of Health, United States Public Health Service.
Table 6. Effect of protein depletion and repletion on serum testosterone in 4- and 18-month-old male rats.

| Age in months | Initial control | Protein-free diet for 20 days | Refed 18% casein diet for 20 days |
|---------------|----------------|-------------------------------|----------------------------------|
| Number of rats| 4              | 18                            | 4                                |
|               | 9              | 10                            | 11                               |
| ng/ml         | 2.60 ± 0.36* *** | 1.22 ± 0.14*                | 0.72 ± 0.09<sup>c</sup>          |
|               |                |                                | 0.77 ± 0.09<sup>b</sup>          |
|               |                |                                | 1.58 ± 0.19<sup>c,d</sup>        |
|               |                |                                | 0.95 ± 0.10<sup>d</sup>          |

* Mean ± SE. ** Same as ** in Table 2.
was observed. Prior to protein depletion, pituitary growth hormone (GH) was significantly higher in young rats even though the absolute pituitary weight was significantly greater in old ones. Twenty-day PFD feeding reduced the GH level to one-half of the initial control level in young rats, whereas in old rats the level was 63%. Refeeding of protein increased the level to the initial control level in both age groups.

These results suggest that the two hormones contribute in part, if at all, to the higher gain in gonadal weight of refed young rats. Estimation of circulating prolactin and GH is necessary for further discussion of this matter.

**Serum testosterone**

The greater increase in weight of the seminal vesicle and prostate suggests a difference of circulating testosterone in both refed age groups, and the experiment produced expected results (Table 6). Prior to protein depletion, serum testosterone was significantly higher in young rats. Following 20 days of protein-free diet feeding, serum testosterone was reduced in both age groups to 0.7 ng/ml. Refeeding of protein resulted in a modest increase of androgen to 0.95 ng/ml in old rats and a greater increase to 1.6 ng/ml in young rats.

All the results obtained in the present study support the heretofore-mentioned hypothesis on the biological aging of organisms that one of the important characteristics of an aging organism is its reduced capacity to adapt to environmental changes (25).

Thus an effect of age on sex organ weight and hormonal levels is reflected in nutritionally modified circumstances.

The authors wish to thank Dr. S. W. C. Chan (State University of New York, Brockport, New York, U.S.A.) for his advice.

**REFERENCES**

1) Leathem, J. H. (1961): Nutritional effects on endocrine secretions, in Sex and Internal Secretions, Vol. 1, ed. by Young, W. C., Williams and Wilkins Co., Baltimore, Maryland, pp. 666–704.
2) Leathem, J. H. (1966): Nutritional effects on hormone production. *J. Anim. Sci.*, 25, suppl., 68–82.
3) Widdowson, E., Mavor, W. O., and McCance, R. A. (1964): The effect of undernutrition and rehabilitation on the development of the reproductive organs: Rats. *J. Endocrinol.*, 29, 119–126.
4) Glass, A. R., and Swerdloff, R. S. (1980): Nutritional influences on sexual maturation in the rat. *Fed. Proc.*, 39, 2360–2364.
5) Horn, E. H. (1955): Nutritional and hormonal influences upon reproductive maturation, organ weights and histochemistry of the immature male rat. *Endocrinology*, 57, 399–408.
6) Leathem, J. H. (1970): Nutrition, in *The Testis*, Vol. 3, ed. by Johnson, A. O., Gomes, W. R., and Vandemark, N. L., Academic Press, New York, pp. 169–205.

Vol. 28, No. 2, 1982
7) Leathem, J. H. (1958): Hormones and protein nutrition. *Recent Progr. Hormone Res.*, 14, 141–182.
8) Wolf, R. C., and Leathem, J. H. (1955): Hormonal and nutritional influences on the biochemical composition of the rat testis. *Endocrinology*, 51, 286–290.
9) Ewing, L. L., Means, A. R., Beames, C. G., Jr. and Montgomery, R. D. (1966): Biochemical changes in rat testis during postnatal maturation. *J. Reprod. Fertil.*, 12, 295–307.
10) Gray, G. D. (1978): Changes in the level of LH and testosterone in the circulation of aging male rats. *J. Endocrinol.*, 76, 551–552.
11) Chan, S. W. C., Leathem, J. H., and Esashi, T. (1977): Testicular metabolism and serum testosterone in aging male rats. *Endocrinology*, 101, 128–133.
12) Kaler, L. W., and Neaves, W. B. (1981): The androgen status of aging male rats. *Endocrinology*, 108, 712–719.
13) Howland, B. E. (1975): The influence of food restriction and subsequent refeeding on gonadotropin secretion and serum testosterone levels in male rats. *J. Reprod. Fertil.*, 44, 429–436.
14) Wesson, L. G. (1932): A modification of the Osborne-Mendel salt mixture containing only inorganic constituents. *Science*, 75, 339–340.
15) Zanini, A., and Giannattasio, G. (1972): Polyacrylamide gel electrophoresis of rat anterior pituitary gland after different extraction procedures. *J. Endocrinol.*, 53, 177–178.
16) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951): Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265–275.
17) Mann, T. (1974): Effect of nutrition on male accessory organs, in *Male Accessory Sex Organs*, ed. by Brundes, D., Academic Press, New York, pp. 173–181.
18) Leathem, J. H. (1958): Male reproductive system and protein nutrition, in *Reproductive Physiology and Protein Nutrition*, ed. by Leathem, J. H., Rutgers Univ. Press, New Brunswick, New Jersey, pp. 12–22.
19) Harmans, S. M., Darner, R. L., and Roth, G. S. (1978): Testosterone secretion in the rat in response to chorionic gonadotrophin; Alterations with age. *Endocrinology*, 102, 540–544.
20) Everitt, A. V. (1958): The urinary excretion of protein, non-protein nitrogen, creatinine and uric acid in aging male rats. *Gerontology*, 2, 33–46.
21) Price, D., and Williams-Ashman, H. G. (1961): The accessory reproductive glands of mammals, in *Sex and Internal Secretions*, ed. by Young, W. C., Williams and Wilkins Co., Baltimore, Maryland, Vol. 1, pp. 366–448.
22) Grayhack, J. T., Bunce, P. L., Kearns, J. W., and Scott, W. W. (1955): Influence of the pituitary on prostatic response to androgen in the rat. *Bull. Johns Hopkins Hosp.*, 96, 154–160.
23) Grayhack, J. T., and Lebowitz, J. (1967): Effect of prolactin on citric acid of lateral lobe of prostate of Sprague-Dawley rat. *Invest. Urol.*, 5, 87–94.
24) Grayhack, J. T. (1963): Pituitary factors influencing growth of the prostate. *Nat. Cancer Inst. Monogr.*, 12, 189–199.
25) Nakano, K., and Sidransky, H. (1978): Age-related changes in ribosomal profiles and *in vitro* protein synthesis in skeletal muscle during fasting and subsequent refeeding. *J. Nutr.*, 108, 399–409.

*J. Nutr. Sci. Vitaminol.*