PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 165, 349–353 (1980)

Effect of LH and FSH on Vanadium Distribution in Hypophysectomized Rats (40984)

RICHARD A. PEABODY, STANLEY WALLACH[1], R. LAURENCE VERCH, AND MERVYN LIFSCHITZ

Medical Research and Medical Services, Veterans Administration Medical Center, and the Departments of Biochemistry and Medicine, Albany Medical College, Albany, New York 12208

Abstract. The effect of LH and FSH on radiovanadium (^{48}V) metabolism in testis was explored by administering LH and/or FSH daily to hypophysectomized (HYPOX) rats prior to and after ^{48}V injection. Seven and fourteen days after ^{48}V injection, the animals were counted in a whole body gamma counter, sacrificed, and serum and tissue samples taken for weighing and counting. Whole body, serum, and tissue contents of ^{48}V of HYPOX rats were not affected by LH or FSH except in testis. In testis, there were both independent and additive effects of LH and FSH on testicular weight and ^{48}V content, expressed as either content per gram or per organ. LH was more active than FSH. The usual fourfold increase in ^{48}V content per gram seen in HYPOX testis was reduced 50% by LH + FSH. On an organ basis, ^{48}V content was increased 50–100% by LH + FSH compared to either HYPOX or intact rats. These data demonstrate that gonadotropins affect the ^{48}V content of the testes in HYPOX rats in the absence of growth hormone, adrenal steroids, and thyroxine. Whether these data indicate a direct effect of vanadium in reproductive physiology or are secondary to nonspecific trophic or other effects on the testis remain to be determined.

Vanadium (V) is widely distributed in nature and is present in trace amounts, 1–20 ng/g, in animal tissues (1, 2). V is essential for growth in rats and chicks and V deficiency also affects lipid metabolism (3). Radiovanadium (^{48}V) is taken up readily by bone and soft tissues (4, 5) but quantitative analyses of V distribution and transport have been hampered by a lack of sensitive methods for chemical analysis. In previous studies in this laboratory, an increased uptake of ^{48}V was found in almost all tissues of hypophysectomized (HYPOX) rats (6) with restoration to or toward normal by growth hormone (GH) and/or thyroxine (T_4) replacement (7). In HYPOX testis, an increased ^{48}V per gram also occurred and GH and T_4 replacement resulted in incomplete restoration to baseline levels. When testis ^{48}V was expressed as percentage ^{48}V per organ, HYPOX rats had decreased ^{48}V content, and as above, showed incomplete restoration by GH and T_4. With the working hypothesis that gonatrophins exert an influence on testicular ^{48}V, the effects of LH and FSH on the testis content of ^{48}V in HYPOX rats were studied.

Materials and methods. Male HYPOX Long–Evans rats, 6 weeks old and 125–135 g in weight, were obtained from Charles River Breeding Laboratories 4 days after surgery. Unoperated rats of the same age were also obtained from this source. There were four groups: HYPOX controls, HYPOX + LH, HYPOX + FSH, and HYPOX + LH + FSH. To guard against undetected systematic errors in the performance of the experiments, and to compare with earlier experiments (6, 7), a group of intact rats was studied concurrently with HYPOX groups. All rats were fed Wayne rat chow and had 5% (w/v) dextrose added to their drinking water. "Carrier-free" $^{48}VOCl_3$ (V^{5+}), half-life 16 days, was obtained from Amersham/Searle, Inc. Although "carrier-free" (no added V by commercial designation) this radioisotope contains a small amount of unlabeled V as an impurity in the cyclotron target used in its production.

Two days after receipt of the animals (6

[1] Requests for reprints should be addressed to: Medical Service, Veterans Administration Medical Center, Albany, N.Y. 12208.

0037-9727/80/100349-05$01.00/0

TABLE I. ^{48}V CONTENT OF RAT BODY, SERUM, AND TISSUE AT 14 DAYS

	HYPOX	HYPOX + LH	HYPOX + FSH	HYPOX + LH + FSH	Intact rats
A. Percentage dose/whole animal	42.6 ± 0.12	43.7 ± 2.3	43.9 ± 1.8	39.6 ± 2.9	36.1 ± 1.1
B. Percentage dose/ml or g					
Serum	0.06 ± 0.00″	0.05 ± 0.00″	0.05 ± 0.00″	0.05 ± 0.00″	0.02 ± 0.00″
Liver	0.46 ± 0.08	0.44 ± 0.07	0.47 ± 0.07	0.47 ± 0.03	0.20 ± 0.02
Pancreas	0.20 ± 0.02	0.20 ± 0.03	0.19 ± 0.02	0.22 ± 0.02	0.06 ± 0.00″
Kidney	3.15 ± 0.22	2.97 ± 0.24	3.61 ± 0.02	2.91 ± 0.17	0.74 ± 0.06
Lung	0.19 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.08 ± 0.00″
Heart	0.13 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.06 ± 0.00″
Bone	2.79 ± 0.15	2.51 ± 0.13	2.68 ± 0.11	2.32 ± 0.09	1.44 ± 0.05

Note. Values are means ± SEM. There are five to eight animals per group.
″ SEM of 0.004 or less.

days after surgery), 40μg of ovine LH and/or FSH[2] was administered daily subcutaneously to HYPOX rats for 10 days before ^{48}V was injected. The gonadotropins were continued until sacrifice an additional 7 or 14 days later. The hormonal injections were started before ^{48}V injection so that their effect on ^{48}V distribution could be evaluated during partial recovery of the testis from the endocrine deficiency created by HYPOX surgery. After 10 days of LH and/or FSH administration, 3 μCi ^{48}V (containing approximately 4 ng unlabeled V) was injected into the tail vein. Sacrifice was performed 7 and 14 days after injection of ^{48}V by aortic exsanguination during pentobarbital anesthesia.

Daily doses of LH and FSH employed in selected studies (8–14) vary widely, 10–200 μg LH equivalent to NIH-LH-S1 and 45–220 μg FSH equivalent to NIH-FSH-S1. The doses used in the present study were selected empirically since the dose required for restoration of testicular mass and function could not be predicted from earlier studies. The HYPOX rats weighed 133 ± 9 g at the initiation of the experiment and 136 ± 10 g at sacrifice. The lack of weight gain and the presence of small testes were taken as indicators of the completeness of HYPOX. The HYPOX rats had excellent survival and did not manifest diarrhea or rhinorrhea during the period of study.

Body content of ^{48}V immediately prior to sacrifice was determined in a whole body gamma counter consisting of two shielded, opposed 3-in. scintillation probes. The outputs of the detectors were combined and analyzed in a Baird-Atomic Model 530 spectrometer. Serum and tissue samples were weighed and then counted in an automatic gamma counter. Comparison of data from untreated HYPOX rats was made by an analysis of variance combined with Duncan's multiple range test (15).

Results. Table I shows the whole body, serum, and tissue contents of ^{48}V in all groups of animals. The data were similar to previous data showing that HYPOX rats have normal body content of ^{48}V but up to four fold increases in serum and tissue content of ^{48}V compared to intact rats (6, 7). Neither LH nor FSH had any effect on body, serum, or tissue ^{48}V contents other than testis.

The testicular data are shown in Table II. LH and FSH each increased testis weight one- to threefold over untreated HYPOX rats. Inexplicably, LH increased testicular weight more than FSH, although FSH presumably has a greater effect on seminiferous tubular development, the major component of testicular mass. The gonadotropins also appeared to have additive effects on testicular weight. The ^{48}V content, per gram of testis, was increased fourfold in

[2] NIH-LH-S20 (equivalent to 1.19 NIH-LH-S1 u/mg and NIH-FSH-S12 (equivalent to 1.25 NIH-FSH-S1 u/mg) were obtained from the NIAMDD Hormone Distribution Program. Contamination of LH with FSH was less than 0.05 NIH-FSH-S1 u/mg and contamination of FSH with LH was less than 0.01 NIH-LH-S1 u/mg.

TABLE II. ^{48}V CONTENT OF RAT TESTIS

	HYPOX	HYPOX + LH	HYPOX + FSH	HYPOX + LH + FSH	Intact rats
Day 7					
Testicular weight (g)	0.40 ± 0.03	1.24 ± 0.04[a]	1.00 ± 0.12[a]	1.35 ± 0.18[a]	2.54 ± 0.07
dpm/g	19,051 ± 1641	13,249 ± 789[a]	11,226 ± 751[a]	12,727 ± 2095[a]	4,204 ± 172
	(0.47 ± .04)	(0.33 ± .02)	(0.28 ± .02)	(0.32 ± .05)	(0.10 ± .00[c])
dpm/organ	7,443 ± 200	16,363 ± 923[a]	10,820 ± 640	15,803 ± 1487[a,b]	10,640 ± 373
(both testes)	(0.18 ± .01)	(0.41 ± .02)	(0.27 ± .02)	(0.39 ± .04)	(0.26 ± .01)
Day 14					
Testicular weight (g)	0.47 ± 0.10	1.06 ± 0.14[a,b]	0.67 ± 0.05	1.49 ± 0.08[a,b]	2.75 ± 0.07
dpm/g	14,374 ± 2058	8,406 ± 806[a]	10,014 ± 810[a]	6,578 ± 393[a,b]	2,624 ± 128
	(0.46 ± .07)	(0.27 ± .03)	(0.32 ± .03)	(0.21 ± .01)	(0.08 ± .00[c])
dpm/organ	6,059 ± 372	8,589 ± 981[a,b]	6,590 ± 474	10,007 ± 479[a,b]	7,215 ± 351
(both testes)	(0.19 ± .01)	(0.28 ± .03)	(0.21 ± .02)	(0.32 ± .02)	(0.23 ± .01)

Note. Values are means ± SEM. There are three to six animals per group for Day 7 and five to eight animals per group for Day 14. Data in parentheses indicate percent dose per g or per organ (both testes).

[a] $P < 0.05$ vs untreated HYPOX group.

[b] $P < 0.05$ vs HYPOX + FSH group.

[c] SEM of 0.004 or less.

HYPOX rats. LH or FSH administration decreased this parameter 30 to 40% compared to unsupplemented HYPOX rats. There was no additive or differential effect of either gonadotropin on Day 7 but by Day 14, combined administration of LH and FSH had an additive effect, although ^{48}V content (per g) was still more than twice normal (7).

In contrast to an elevated ^{48}V content per gram, the total ^{48}V content of both testes was decreased by 15–30% in HYPOX rats (6, 7). LH increased this parameter above normal whereas FSH increased total ^{48}V content to normal. The combination of FSH + LH did not yield additive effects to increase total ^{48}V content above that seen with LH alone.

In previous studies we have observed that neither moderate reduction of food intake nor weight differences influence ^{48}V distribution (5) in intact rats. The intact rats in the present experiment had moderate food restriction and increased their weight from 150 ± 4 to 190 ± 11 g during the course of the experiment. Their serum and tissue contents of ^{48}V did not differ from those observed previously in intact rats (6, 7), supporting the validity of the experiments (Tables I and II).

Discussion. These data demonstrate that the gonadotropin doses used have a significant restorative effect on testicular weight. Restorative effects on the content of ^{48}V, regardless of whether ^{48}V content is expressed as content per gram or content per organ is also seen and LH alone is capable of increasing ^{48}V content per organ to normal or above. Although the doses of LH and FSH used did not restore testicular weight or ^{48}V content per gram to normal, replacement therapy with other deficient hormones was not included and it is possible that a fuller effect would have been observed if GH, T_4, and adrenal steroids had also been administered. Previous work in this laboratory has shown that GH and T_4 replacement have partial restorative effects on testis ^{48}V content in the absence of gonadotropins (7). These hormones were specifically deleted to determine whether LH and FSH alone are capable of influencing ^{48}V content in testis.

The data were essentially similar at 7 and 14 days although there were some irregular responses, especially when FSH was given alone. These findings complement the findings of increased testis weight and changes in testicular components reported by Woods and Simpson during 15 days of treatment of HYPOX animals with similar doses of LH and/or FSH (8). Their data indicated that seminiferous tubule maturation, sperm production, and Leydig cell number all increased. We have no explanation for the anomalous finding of greater testicular weight gain with LH than with FSH.

Rats on low V diets manifest reduced reproductive capacity and the survival of their young is impaired. Female rats maintained on a diet containing less than 10 ng V/g have a decreased rate of pregnancies per mating period as well as litter size. The effect is noted after three generations on the deficient diet but is most marked after four generations (3). Whether such data indicate a direct involvement of V in reproduction is unclear since adverse systemic effects secondary to V deficiency could reduce reproductive capacity. Although the present data provide additional evidence in support of such a role for V, it is also possible that the effects of gonadotropin replacement on testicular ^{48}V content are secondary to nonspecific trophic effects of LH and FSH on the testis or specific cell types within the testis (8).

An additional problem relates to the continuing difficulty of obtaining valid stable V analyses in biologic fluids and tissues. Flameless atomic absorption spectroscopy (FAAS) may eventually become an acceptable method as instruments of greater sensitivity are developed. Myron and his associates are now able to measure foodstuffs with reasonable accuracy by FAAS (16, 17). Neutron activation analysis, although having sufficient sensitivity, is not generally available and also has unsolved technical problems. The implication of abnormal testicular V metabolism in the hypogonadotrophic state by the ^{48}V results will require confirmatory studies using stable V analyses when they are validated.

Further studies are needed to clarify these issues, especially whether direct effects of LH and/or FSH on the V content of the cellular components of the testis can be demonstrated. Since the effects of LH and FSH in testis appear to be mediated via the generation of cAMP, and in turn, stimulation of protein and RNA synthesis in Leydig, Sertoli, and other testicular cells (18–20), a relation between V and cyclic nucleotide metabolism should also be sought.

The authors gratefully acknowledge the help of Robert Kohberger, Ph.D, Albany Medical College Computer Center, for suggesting and programming the statistical methods used. This work was supported by the Medical Research Service of the Veterans Administration and by a grant from the Armour Pharmaceutical Company.

1. Hopkins, L. L., and Mohr, H. E., *in* "Newer Trace Elements in Nutrition"(W. Mertz and W. E. Cornatzer, eds.), p. 195. Dekker, New York (1971).
2. Welch, R., and Allaway, W. H., Anal. Chem. **44**, 1644 (1972).
3. Hopkins, L. L., and Mohr, H. E., Fed. Proc. **33**, 1773 (1974).
4. Hopkins, L. L., and Tilton, B. E., Amer. J. Physiol. **211**, 169 (1966).
5. Soremark, R., Ullberg, S., and Applegren, L., Acta Ondontol. Scand. **20**, 225 (1962).
6. Peabody, R. A., Wallach, S., Verch, R. L., and Kraszeski, J., *in* "Trace Substances in Environmental Health" (D. D. Hemphill, ed.), Vol. X, p. 441. University of Missouri Press, Columbia, (1976).
7. Peabody, R. A., Wallach, S., Verch, R. L., and Lifschitz, M. L., *in* "Trace Substances in Environmental Health" (D. D. Hemphill, ed.), Vol. XI, p. 297. University of Missouri, Columbia, (1977).
8. Woods, M. C., and Simpson, M. E., Endocrinology **69**, 91 (1961).
9. Lostroh, A. J., Endocrinology **85**, 438 (1969).
10. Ogasa, A., and Yokoki, Y., Nat. Inst. Anim. Health Quart. **14**, 199 (1974).
11. El Safoury, S., and Bartke, A., J. Endocrinol. **61**, 193 (1974).
12. Sanborn, B. M., Elkington, J. S., Chowdhury, M., Tcholakian, R. K., and Steinberger, E., Endocrinology **96**, 304 (1975).
13. Ghraf, R., Lax, E. R., Wagner, W., and Schriefers, H., J. Endocrinol. **73**, 289 (1977).
14. Sivelle, P. C., McNeilly, A. S., and Collins, P. M., Biol. Reprod. **18**, 878 (1978).

15. Duncan, D. B., Biometrics **13**, 164 (1957).

16. Myron, D. R., Givand, S. H., and Nielson, F. H., J. Agr. Food Chem. **25**, 297 (1977).

17. Myron, D. R., Zimmerman, T. J., Shuler, T. R., Klevay, L. M., Lee, D. E., and Nielsen, F. H., Amer. J. Clin. Nutr. **31**, 527 (1978).

18. Braun, T., and Sepsenwohl, S., Endocrinology **94**, 1028 (1974).

19. Irby, D. C., and Hall, P. F., Endocrinology **89**, 1367 (1971).

20. Means, A. R., Endocrinology **89**, 981 (1971).

Received March 1, 1979. P.S.E.B.M. 1980, Vol. 165.