volving the bridging and the nonbridging oxygens. The average bond lengths, as established by x-ray diffraction studies, are, for the bridging oxygens, 1.67 and 1.65 Å for chains A and B in orthopyroxene (*6*), and 1.68 Å in Ca-pyroxene (*7*). For the nonbridging oxygens the average bond lengths are 1.60 Å for both chains A and B in orthopyroxene (*6*), and 1.59 Å in pyroxene ($CaMgSi_2O_6$) (*7*). Thus the Si–O bond lengths for the bridging oxygens are roughly 0.07 Å longer than those for the nonbridging oxygens. The population ratio of the nonbridging to bridging oxygens is 2 : 1. As can be seen from Fig. 1D, the oxygen 1*s* spectrum of orthopyroxene does indeed consist of two components with an intensity ratio of about 2 : 1. The same intensity ratio was also observed in the deconvoluted oxygen spectrum from Ca-pyroxene. Furthermore, since longer bond lengths indicate in general a more ionic bonding character, one expects the bridging oxygens to have lower binding energy than the nonbridging oxygens. This result is also clearly demonstrated in Figs. 1D and 2.

4) The 1*s* level of the oxygen atoms in Ca-pyroxene and Ca-olivine have lower binding energies than those in the Fe-Mg set. This effect is most likely due to the influence of the cations in the crystal structure.

The results presented here show that photoelectron spectroscopy is a useful technique for the systematic study of the correlation between bond lengths and core binding energies among given pairs of atoms, such as Si–O in the silicate chains.

LO I YIN
SUBRATA GHOSE
ISIDORE ADLER

*National Aeronautics and Space Administration, Goddard Space Flight Center, Greenbelt, Maryland 20771*

**References**

1. K. Siegbahn *et al., ESCA Atomic, Molecular, and Solid State Structure Studied by Means of Electron Spectroscopy* (Almqvist & Wiksell, Stockholm, 1967); C. S. Fadley, D. A. Shirley, A. J. Freeman, P. S. Bagus, J. V. Mallow, *Phys. Rev. Lett.* **23**, 1397 (1969).
2. B. J. Lindberg, K. Hamrin, G. Johansson, U. Gelius, A. Fahlman, C. Nordling, K. Siegbahn, *Phys. Scripta* **1**, 286 (1970).
3. R. Nordberg, R. G. Albridge, T. Bergmark, U. Ericson, J. Hedman, C. Nordling, K. Siegbahn, B. J. Lindberg, *Ark. Kemi* **28**, 257 (1968); U. Gelius, P. F. Hedén, J. Hedman, B. J. Lindberg, R. Manne, R. Nordberg, C. Nordling, K. Siegbahn, *Phys. Scripta* **2**, 70 (1970).
4. J. I. Trombka, R. Schmadebeck, G. A. Osswald, in preparation.
5. J. D. Birle, G. V. Gibbs, P. B. Moore, J. V. Smith, *Amer. Mineral.* **53**, 807 (1968); H. Onken, *Mineral. Petrogr. Mitt.* **10**, 34 (1965).
6. S. Ghose, *Z. Kristallogr.* **122**, 81 (1965).
7. J. R. Clark, D. E. Appleman, J. J. Papike, *Mineral. Soc. Amer. Spec. Pap. 2* (1969), p. 31.

29 April 1971

## Follicle-Stimulating Hormone and the Regulation of Testosterone Secretion in Rabbit Testes

Abstract. *Regulation of testosterone secretion is presumably mediated by interstitial cell–stimulating hormone (ICSH). However, there is little information on the actions of other chemical messengers in regulating testosterone secretion. We have shown that follicle-stimulating hormone augments testosterone secretion stimuated by ICSH in rabbit testes perfused in vitro with an artificial medium.*

Alterations in testosterone secretion are presumably mediated by interstitial cell–stimulating hormone (ICSH), and testosterone is thought to act on the hypothalamo-hypophyseal complex to regulate ICSH release. The fact that testosterone is influenced by a wide variety of signals (*1*) suggests that this mechanism cannot be the sole regulator of testosterone secretion. Similarly, mechanisms discovered in other organ systems imply by extension a multiplicity of controls for regulation of testosterone.

For instance, insulin, hydrocortisone, and prolactin are required to stimulate synthesis of milk protein in mouse mammary glands (*2*); and prolactin, luteinizing hormone, and follicle-stimulating hormone (FSH) are needed to maintain functional corpora lutea in pregnant hamsters (*3*).

Determination of the hormonal factors that regulate testosterone secretion in mammals has been hampered by the lack of a suitable experimental preparation that could be used in examining the effects of chemical messengers on testosterone secretion without interference from extrinsic signals. The continued development of the perfused rabbit testis in vitro (*4, 5*) suggests that this may be the best experimental system in which to examine the effects of a variety of hormones on testosterone secretion. Secretion of testosterone by testes perfused with defibrinated rabbit blood is approximately 500 ng per gram of testis per hour throughout a 6-hour perfusion (*5*). In contrast, secretion by testes perfused with an artificial medium declines during the first 2 hours of perfusion to a basal amount of 50 ng of testosterone per gram of testis per hour (*6*). Addition of ICSH to the artificial medium restores secretion. These observations demonstrate that an artificial medium lacks factors responsible for maintaining testosterone secretion in rabbit testes.

It has been suggested that FSH augments testosterone secretion (*7*), but quantitative measures of such an effect on secretion stimulated by ICSH have not been described. Such studies have

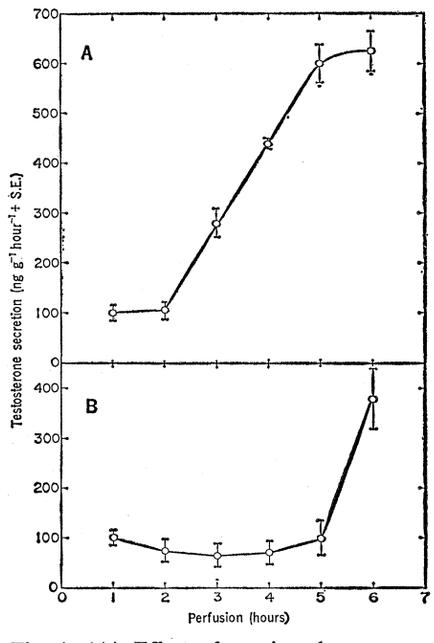


Fig. 1. (A) Effect of varying the concentration of ICSH in artificial medium on hourly rate of testosterone secretion ($n = 4$). The concentrations at successive hours were: 1 hour, 0.0 μg/ml; 2 hours, 0.01 μg/ml; 3 hours, 0.025 μg/ml; 4 hours, 0.05 μg/ml; 5 hours, 0.1 μg/ml; and 6 hours, 1.0 μg/ml. (B) Effect of varying the concentration of FSH in artificial medium on hourly rate of testosterone secretion ($n = 4$). The concentrations at successive hours were: 1 hour, 0.0 μg/ml; 2 hours, 0.01 μg/ml; 3 hours, 0.025 μg/ml; 4 hours, 0.05 μg/ml; 5 hours, 0.1 μg/ml; and 6 hours, 1.0 μg/ml. Gonadotrophins (GTH) were infused directly into the arterial cannula by means of a Sage Micro Pump equipped with a 500-μl Hamilton gas-tight syringe. The concentration of GTH in the syringe was altered at the end of each hour of perfusion. Thus, the same volume of GTH solution was perfused each hour. The volume of fluid added to the arterial cannula never exceeded 120 μl/hour.

been hindered by the fact that ICSH contaminates most FSH preparations. We circumvented this problem in two ways; we used concentrations of FSH that failed to stimulate secretion of testosterone when administered alone, and we tested FSH in the presence of ICSH concentrations that produced maximum secretion of testosterone in the perfused testis.

Testes of mature New Zealand White rabbits were perfused for 6 hours (*4, 5*) with an artificial medium consisting of Krebs-Ringer bicarbonate buffer (*p*H 7.4), bovine albumin powder (3 percent, weight per volume), glucose (1 mg/ml), washed rabbit erythrocytes added to a hematocrit of 25 percent, penicillin, and dihydrostreptomycin (*8*). Testosterone in venous effluent was collected at hourly intervals and measured by gas-liquid chromatography (*9*). The chemical purity of testosterone heptafluorobutyrate isolated from venous effluent of the perfused testis was determined by comparison of its mass spectrum with that of authentic testosterone heptafluorobutyrate. Spectra were obtained by injecting the samples by way of the direct probe into the prototype of the LKB-9000 gas chromatograph–mass spectrometer (*10*). The molecular weights and mass spectra of the standard and the isolated heptafluorobutyrate derivatives of testosterone were similar.

The concentration of ICSH which produced maximum secretion of testosterone was determined by varying the concentration of ICSH in the perfusion medium (Fig. 1A). The ICSH was infused into the arterial cannula, by means of the Sage Micro Pump, at a rate sufficient to maintain its concentration at 0.01, 0.025, 0.05, 0.1, and 1.0 $\mu$g/ml for 2, 3, 4, 5, and 6 hours of perfusion. The concentration of ICSH in the infusion pump was such that the volume of fluid added to the arterial cannula never exceeded 120 $\mu$l/hour. Secretion of testosterone increased linearly between the 2nd and 5th hours of perfusion and then reached a plateau. Thus secretion was maximum when ICSH was maintained at a concentration above 0.1 $\mu$g per milliliter of artificial medium, an indication that those cellular elements producing testosterone were saturated with ICSH. A concentration of FSH of 0.1 $\mu$g/ml in the medium contained enough ICSH (1.9 ng of NIH-LH-S1) to increase testosterone secretion by perfused testes (Fig. 1B). Testosterone secretion was not stimulated by 0.01 or 0.25 $\mu$g of FSH per milliliter.

We next tested the effect of FSH upon ICSH-stimulated secretion of testosterone at concentrations of FSH that failed to stimulate testosterone secretion when administered alone or at ICSH concentrations that produced maximum secretion. We used a saturating concentration of 8.0 $\mu$g of ICSH per milliliter and a nonstimulatory concentration of 15 ng of FSH per milliliter of medium. The ratio of ICSH to FSH was arbitrarily set at 2 : 1; thus, 4.0 $\mu$g of FSH per milliliter of medium was tested with 8 $\mu$g of ICSH per milliliter and 30 ng of ICSH per milliliter was tested with 15 ng of FSH per milliliter of medium.

In the absence of exogenous gonadotrophic hormone (GTH), secretion of testosterone declined during the first 2 hours of perfusion to a basal amount (Fig. 2A). When administered alone, FSH (15 ng/ml) did not significantly alter secretion from this basal amount. Secretion was increased in the presence of 30 ng of ICSH per milliliter by the 5th and 6th hour of perfusion. The same concentration of ICSH combined with 15 ng of FSH per milliliter increased the amount of testosterone secreted beyond that obtained with 30 ng of ICSH per milliliter of medium alone. The total amount of testosterone secreted during the 5th and 6th hours by testes treated with ICSH and FSH was 625 $\pm$ 318 ng per gram of testis per hour compared to 325 $\pm$ 144 ng for testes treated with ICSH only. This statistically significant difference ($P < .05$) supports the hypothesis that FSH augments ICSH-stimulated secretion of testosterone. In similar experiments with ovine growth hormone (STH) (NIH-GH-S9), STH failed to augment ICSH-stimulated testosterone secretion.

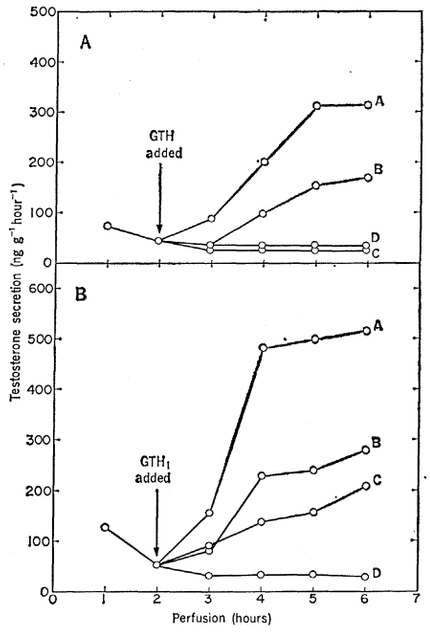


Fig. 2. (A) Testosterone secretion by testes perfused with (*A*) artificial medium containing 15 ng of FSH and 30 ng of ICSH per milliliter ($n = 8$); (*B*) artificial medium containing 30 ng of ICSH per milliliter ($n = 8$); (*C*) artificial medium containing 15 ng of FSH per milliliter ($n = 3$); and (*D*) artificial medium ($n = 5$). (B) Testosterone secretion by testes perfused with (*A*) artificial medium containing 4.0 $\mu$g of FSH and 8.0 $\mu$g of ICSH per milliliter ($n = 8$); (*B*) artificial medium containing 8.0 $\mu$g of ICSH per milliliter ($n = 7$); (*C*) artificial medium containing 4.0 $\mu$g of FSH per milliliter ($n = 3$); and (*D*) artificial medium ($n = 5$). Gonadotrophins (GTH) were added to the medium reservoir at the end of the 2nd hour of perfusion.

The possibility exists that the FSH-dependent stimulation resulted from ICSH present as a contaminant in the FSH preparation (NIH-FSH-S7, ovine). Therefore, an experiment was designed to determine the response of testes exposed simultaneously to FSH (4 $\mu$g/ml) and saturating amounts of ICSH (8 $\mu$g/ml). Secretion of testosterone declined to a basal level in the absence of exogenous GTH in the artificial medium (Fig. 2B). Secretion of testosterone by testes perfused with medium containing 4 ng of FSH per milliliter was significantly higher ($P < .01$) than control values by the 5th and 6th hours of perfusion. This response probably reflects the effect of FSH and its ICSH contaminant (76 ng of NIH-LH-S1 per 4.0 $\mu$g of NIH-FSH-S7). Testes treated with medium containing 8.0 $\mu$g of ICSH or 8.0 $\mu$g of ICSH and 4.0 $\mu$g of FSH per milliliter secreted higher ($P < .01$) amounts of testosterone than either control testes or those receiving only 4.0 $\mu$g of FSH per milliliter by the 5th and 6th hours of perfusion. The total amount of testosterone secreted during the 5th and 6th hours was 1022 $\pm$ 400 ng per gram of testis per hour for testes stimulated with medium containing 4.0 $\mu$g of FSH and 8.0 $\mu$g of ICSH per milliter. This rate of secretion was significantly higher ($P < .05$) than the 521 $\pm$ 260 ng obtained with testes stimulated by medium containing 8.0 $\mu$g of ICSH alone. This experiment shows that the synergistic effect of FSH upon ICSH-stimulated testosterone secretion could not be due to contamina-

tion by ICSH because testes were maximally stimulated by saturating levels of ICSH.

Taken together these results demonstrate that FSH augments testosterone secretion in perfused rabbit testes stimulated with ICSH and thus provides direct evidence that FSH acts synergistically with ICSH to control testosterone secretion. The fact that FSH stimulates secretion of testosterone in the presence of saturating concentrations of ICSH suggests that the gonadotrophic hormones act at different sites or by different mechanisms. This suggests a degree of control of testosterone secretion in rabbit testes separate from ICSH.

BRYAN H. JOHNSON*
LARRY L. EWING
*Department of Physiological Sciences, Oklahoma State University, Stillwater 74074*

### References and Notes

1. C. Desjardins, L. L. Ewing, B. H. Johnson, *Endocrinology*, in press; K. B. Eik-Nes, *J. Reprod. Fert.* **14** (Suppl. 2), 125 (1967); C. Desjardins and L. L. Ewing, *Fed. Proc.* **27**, 548 (1968); E. Steinberger and M. Ficher, *Biol. Reprod. Supp.* **1**, 119 (1969); K. B. Eik-Nes, *Am. J. Physiol.* **217**, 1764 (1969).
2. D. H. Lockwood, R. W. Turkington, Y. J. Topper, *Biochem. Biophys. Acta* **130**, 493 (1966).
3. G. S. Greenwald, *Endocrinology* **80**, 118 (1967).
4. N. L. VanDemark and L. L. Ewing, *J. Reprod. Fert.* **6**, 1 (1968).
5. L. L. Ewing and K. B. Eik-Nes, *Can. J. Biochem.* **44**, 1327 (1966).
6. B. H. Johnson and L. L. Ewing, *Proc. Ann. Meet. Soc. Study Reprod. 3rd* (1970), p. 28.
7. A. J. Lostroh, *Endocrinology* **76**, 438 (1969); G. M. Connell and K. B. Eik-Nes, *Steroids* **12**, 507 (1968).
8. J. H. Exton and C. R. Park, *J. Biol. Chem.* **242**, 2622 (1967).
9. A. C. Brownie, H. J. van der Molen, E. E. Nishizawa, K. B. Eik-Nes, *J. Clin. Endocrinol.* **24**, 1091 (1964); M. A. Kirschner and G. D. Coffman, *ibid.* **28**, 1347 (1968).
10. R. Ryhage, *Ark. Kemi* **26**, 305 (1967); G. R. Waller, *Proc. Okla. Acad. Sci.* **47**, 271 (1967).
11. We thank the National Institute of Arthritis and Metabolic Diseases for gifts of NIH-FSH-S7 and NIH-ICSH-S16; R. Amey for technical assistance; and Dr. G. R. Waller and K. Kinneberg of the Department of Biochemistry, Oklahoma State University, for providing mass spectral data (NSF research grant GB-7731). Supported in part by PHS grants HD-00636, HD 05795-01, and FR-5569. B.H.J. was supported by NIH postdoctoral research fellowship FO 2 HD-39957-01.
* Present address: Department of Animal Science, North Carolina State University, Raleigh 27607.

5 April 1971

## Transplanted Precursors of Nerve Cells: Their Fate in the Cerebellums of Young Rats

Abstract. *The multiplying cells of the external granular layer in 7-day-old rats were labeled with [$^3H$]thymidine. Slabs of the cerebellum were transplanted into the same region of uninjected hosts of the same age. The transplanted, undifferentiated cells of the donors migrated actively in the cerebellar cortex of the hosts and apparently differentiated there into basket and granule cells.*

A few investigators have attempted to transplant neural tissue from one adult mammal into the brain of another, but they found that the transplants degenerated (*1*). Others who worked with young mammals observed that the transplants grew and differentiated to some extent (*2*). Recently, Wenzel *et al.* (*3*) reported that outgrowing processes of transplanted nerve cells of adult mice penetrated into the cerebral cortex of the host. However, to our knowledge there are no reports available to indicate that transplanted nerve cells can be structurally incorporated into the brains of recipients.

Instead of working with mature neural tissue of adult mammals, we used undifferentiated portions of the cerebellar cortex of young mammals (rats and rabbits) and transplanted this tissue into immature but developing brain regions (cerebellar cortex, hippocampus, and olfactory bulb) of recipients. For easy identification, the donor cells were tagged radioactively. Here we report briefly the results of transplantation of slabs of the cerebellar cortex of infant rats into the cerebellar cortex of rats of the same age.

Eight laboratory-bred Long-Evans hooded rats from one litter were injected intraperitoneally with [$^3$H]thymidine when they were 7 days old (*4*). A large proportion of the proliferating cells of the germinal zone of the cerebellar cortex in the external granular layer, and a smaller proportion of cells in the internal granular layer became labeled, and this material served as the transplant. One hour after injection slabs of the cerebellar cortex of the donors were surgically removed, washed twice in Ringer solution, and transplanted into the cerebellums of rats of the same age. Two or more animals were killed 3 hours or 1, 2, 4, 10, or 16 days after surgery by transcardiac perfusion with 10 percent neutral formalin. The cerebellums of these animals were processed for histology and autoradiography in the usual manner (*5*).

In the cerebellums of the hosts that were killed 3 hours after surgery the transplants with their labeled cells were easily identifiable (Fig. 1). A few

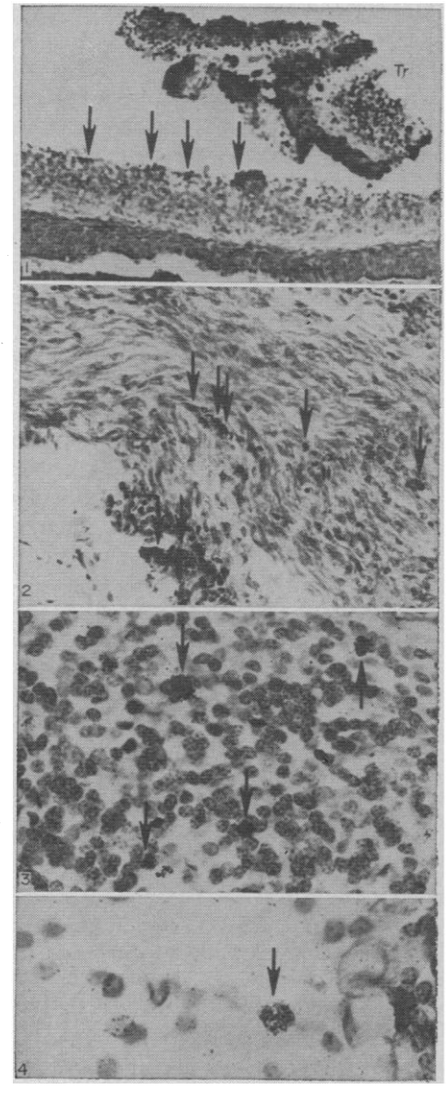


Fig. 1. Transplant (*Tr*) in the host cerebellum, 3 hours after surgery. Note extensive labeling of cells in the external granular layer and sparse labeling in the internal granular layer. Arrows indicate a few labeled cells attached to the exposed surface of the host cerebellum (× 300). Fig. 2. Stream of migrating cells in the transplant 4 days atfer surgery. Arrows indicate labeled cells (× 325). Fig. 3. Labeled cells (arrows) in the internal granular layer of the host cerebellum 16 days after the transplantation (× 500). Fig. 4. Labeled cell, presumably a basket cell (arrow), in the molecular layer of the host cerebellum, 16 days after the transplantation (× 800).