primarily from the diet which may contain antigens from killed bacteria as well as certain plant and animal antigens, and possibly occult viruses which are doubtlessly disseminated by congenital routes.

Our data correlate very well with those of Ball(12), who found that the number of small lymphoid cells in the thymus increased by a factor of 10 between birth and 14 days post-partum and that the maximum number of cells was achieved around 50 days of age. The embryonic rate of increase, however, is substantially greater than that after birth; embryonic growth was not a part of this study.

A number of factors cause variation in size of the thymus: stress conditions such as ether anesthesia, exposure to cold or extreme heat, lack of food, and crowded cage conditions may cause shrinkage of the organ. Pregnant females beyond the age at which regression normally occurs have enlarged thymuses. The time that elapsed between removal of germfree animals from the isolators and autopsy also contributed to variation in thymus size.

Summary. Qualitatively, the growth patterns of the thymus of germfree and conventional mice are very similar, and the gross appearance is the same. In both groups of animals the period of thymus growth extends from birth to about 50 days, with accelerated growth during the first 2 weeks after birth, and peaks at about 15 days. Growth continues at a decelerated rate and regression of the thymus begins at about 50 days of age. A plateau is reached at about 200 days of age, but the thymus does not disappear completely. However, the thymus of conventional mice grows more rapidly and attains greater size than that of the germfree mice.

1. Finstad, J., Papermaster, B. W., Good, R. A., Lab. Invest., 1964, v13, 490.
2. Miller, J. F. A. P., Brit. Med. J., 1963, v2, 459.
3. Wilson, R., Sjodin, K., Bealmear, M., Proc. Soc. Exp. Biol. and Med., 1964, v117, 237.
4. Wostmann, B. S., Ann. N. Y. Acad. Sci., 1959, v78, 254.
5. Bauer, H., Horowitz, R. E., Watkins, K. C., Popper, H., J. Am. Med. Assn., 1964, v187, 715.
6. Thorbecke, G. J., Ann. N. Y. Acad. Sci., 1959, v78, 237.
7. Trexler, P. C., Reynolds, L. I., Appl. Microbiol., 1957, v5, 406.
8. Reyniers, J. A., Ann. N. Y. Acad. Sci., 1959, v78, 3.
9. Trexler, P. C., Bio-Medical Purview, 1961, v1, 47.
10. Pollard, M., Salomon, J. C., Proc. Soc. Exp. Biol. and Med., 1963, v112, 256.
11. Pollard, M., Matsuzawa, T., ibid., 1964, v116, 967.
12. Ball, W. D., Exp. Cell Res., 1963, v31, 82.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Direct Luteinizing Activity of FSH-NIH.* (29767)

HENRY C. BROWNING AND GERALDINE A. LARKE
University of Texas, Dental Branch, Texas Medical Center, Houston

FSH-NIH-S1 and S2 have luteinizing potencies, in relation to LH-NIH-B1, of 0.006(1) and 0.00028†, respectively, by the ovarian ascorbic acid depletion (OAAD) test(2). LH activity may also be determined by other methods, including that of production of corpora lutea in ovarian isografts in male mice(3). The present report concerns this type of response to the 2 FSH preparations.

Materials and methods. Groups of 8-15 male mice (BALB/c strain) 3-6 months of age, received bilateral intraocular ovarian isografts from females of the same age. Two to 4 weeks later, each animal in a group received a single specific dose of FSH-NIH-

* Supported by USPHS Research Grant CA-02880.

† FSH-NIH-S1 and S2 and LH-NIH-B1 were generously supplied by the Endocrinology Study Section, NIH. In the absence of a specific reference in the text, potency estimates were taken from the protocols accompanying these gonadotropins.

S1 or S2, or of LH-NIH-B1, given i.p. in 0.1 cc of buffered sterile saline (BSS). In certain groups, animals were anesthetized with pentobarbital sodium (0.075 mg/g body wt) ½-1 hour after FSH administration, and again 24 and 48 hours later. In certain other groups, animals received atropine sulfate (0.35 mg/g body wt, s.c. in 0.06-0.08 cc BSS) 2½ hours after FSH or LH administration.

Two groups of male mice received 2.5 mg of testosterone propionate (s.c. in 0.05 cc of sesame oil) 4 to 5 days after birth. They received the usual grafts at 3 months of age and FSH-NIH-S1 or LH-NIH-B1 4 weeks later. Two groups of females received the same treatment, except that they were ovariectomized at 5 months of age and received FSH-NIH-S1 or S2 8 weeks later.

Grafts were examined daily from the time of hormone administration until 6 days later (3). The luteal response was taken as the percentage of grafts in a group that showed development of corpora lutea during this period.

Results. Untreated males; FSH-NIH-S1, and S2, LH-NIH-B1. The luteal response to 5, 10, 20, or 40 μg of FSH-NIH-S1 was 9%, 54%, 77% and 90%, respectively, in different groups of mice, each group bearing 21-22 grafts. It was 7%, 35% and 71% to 5, 10 or 20 μg of FSH-NIH-S1, and 14%, 34% and 78% to the same doses of FSH-NIH-S2, in different groups of mice, each bearing 18 to 21 grafts and tested simultaneously. To 0.75, 1.5, or 2.25 μg of LH-NIH-B1, the response was 19%, 45% and 75% in other groups, each bearing 20 to 21 grafts. Calculated regression lines, with empirical points, for probit-response to log-dose(4) are shown in Fig. 1. The slopes of the 4 regression lines did not depart significantly from linearity or parallelism (chi^2 test), being (with S.E.) 2.80 ± 0.52 or 3.32 ± 0.85 for FSH-NIH-S1, 3.04 ± 0.79 for FSH-NIH-S2, and 3.15 ± 0.90 for LH-NIH-B1. The ED^{50}s of the two FSH preparations were statistically the same, being (with 95% limits) 11.4 (10.1-13.0) or 13.4 (11.8-15.4) for FSH-NIH-S1 and 12.1 (10.6-13.9) for FSH-NIH-S2. The luteinizing potency of FSH-NIH-S2, relative to FSH-NIH-S1, was 1.2 (95% limits, 0.93-1.36). Relative potencies in terms of LH-NIH-B1 were also statistically the same for both preparations, being 0.13 (0.11-0.16) or 0.11 (0.09-0.13) for FSH-NIH-S1 and 0.12 (0.10-0.15) for FSH-NIH-S2.

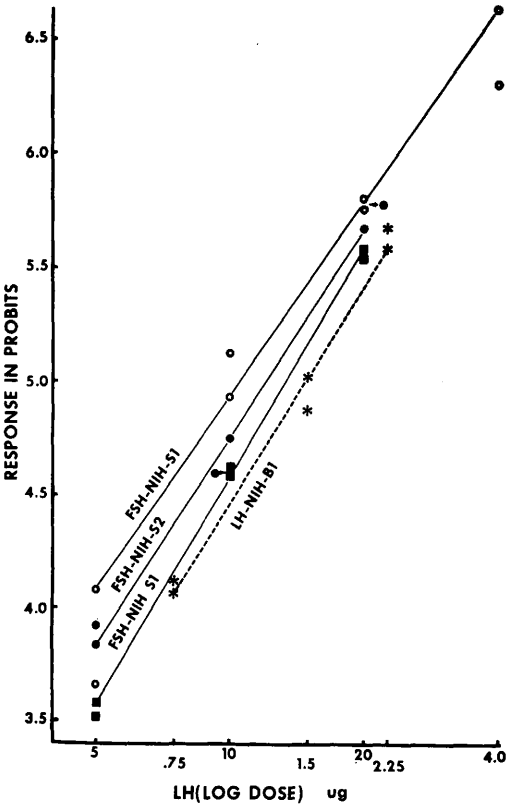

FIG. 1. Calculated regression lines for probit-response to log-dose (symbols *on* line), with empirical probit-responses (symbols *off* line), for FSH-NIH-S1 and S2 and LH-NIH-B1.

Anesthetized males; FSH-NIH-S1. The luteal response to 25 μg of FSH-NIH-S1, in 2 groups of mice, each bearing 22 to 25 grafts and tested simultaneously, was 86% with, and 84% without, subsequent pentobarbital anesthesia.

Atropinized males; FSH-NIH-S2 and LH-NIH-B1. The luteal response, in 4 groups of mice, each bearing 13 to 17 grafts and tested simultaneously, to 25 μg of FSH and to 2 μg of LH were 86% and 82%, respectively without, and 73% and 69% with, subsequent administration of atropine.

Androgenized males and females; FSH-NIH-S1 and S2 and LH-NIH-B1. Ovarian grafts in such males showed no differences from those in untreated males. The luteal response to 20 μg of FSH-NIH-S1, or to 2 μg of LH-NIH-B1, was similar to that in untreated males, being 74% and 68%, respectively, in 2 groups of mice each bearing 26 to 28 grafts, and tested simultaneously.

Ovarian grafts in androgenized females which had been ovariectomized after transplantation resembled those in untreated males, developing mature follicles only. The luteal response to 20 μg of FSH-NIH-S1 and of FSH-NIH-S2 was 80% and 73%, respectively, in 2 groups of mice, each bearing 15 grafts and tested simultaneously.

Timing of luteal response. The development of fully-formed corpora lutea occurred in 27% of 128 responding grafts on the 2nd, 66% on the 3rd, and 7% on the 4th day after administration of FSH-NIH-S1. The corresponding figures for 88 grafts, after FSH-NIH-S2, were 25%, 68%, and 7%; and, for 74 grafts after LH-NIH-B1, 18%, 74%, and 8%. The mean time for this development, after gonadotropin administration, did not differ significantly, being 2.828 ± 0.051 days after FSH-NIH-S1, 2.818 ± 0.057 days after FSH-NIH-S2, and 2.905 ± 0.058 days after LH-NIH-B1. Twenty-four per cent of newly formed corpora showed an initial moderate hyperemia.

Discussion. The present results indicate that the direct luteinizing activities of FSH-NIH-S1 and S2 are equal and equivalent to 12% of that of LH-NIH-B1. The latter has a luteinizing potency of 1.09, relative to that of LH-NIH-S1.† This is at variance with the ventral prostate (HRP) and ovarian hyperemia (OH) responses which give equivalents of 4.8% and 3.1%, respectively, relative to LH-NIH-S1(5,6). Ovine FSH-NIH-S1 may augment the action of contaminating LH in these assays; the LH activity of specially-purified FSH-2(1) was 0.52% by the HRP assay(5) but only 0.01% by the OAAD method(1). The discrepancy between the OAAD assay and the present direct luteal response is even greater, for the former gave a relative LH activity of 0.6% for FSH-NIH-S1(1) and 0.028% for FSH-NIH-S2.† The OAAD assay is not responsive to FSH or to LTH or STH(2); no luteal response occurred after single doses of 100 μg of LTH (prolactin, NIH-P-S3) or of STH (growth hormone, NIH-GH-B6) with the present method(7).

The parameters of the different responses to LH are very unlike. The HRP test depends on a relatively chronic effect of LH (production of testicular androgens), the OH test on an acute effect on ovarian vascularity, the OAAD test on an "acute biochemical response"(5) in ovaries already heavily luteinized and probably under the influence of LTH(8), and the present test on an all-or-none response of morphological luteinization. It appears that either these various responses are not directly related, or, especially in the present method, FSH has inherent luteinizing activity or can act as a releasing factor for LH from the host pituitary. The latter possibility seems unlikely, for such a release should also occur in the OAAD test. Further, the luteal response to FSH here was not abolished by agents such as pentobarbital(9) or atropine(10) that can temporarily block endogenous LH release (although the timing of their administration, relative to that of the hormone, may not have been at the critical period). It has been implied that PMS, with predominantly FSH-like activity, does cause LH release in the rat, for the ovulatory response to it can be blocked by pharmacological agents at specific times after administration and does not occur after hypophysectomy(11). However, the luteal response to FSH-NIH-S2 in the present work also occurred in androgenized females which, at the dose of androgen employed(12), should have a permanent block of LH release. Finally, the facts that the slope of the regression lines for FSH-NIH-S1 and S2 and for LH-NIH-B1 did not differ, and that the timing of luteal response was similar after administration of each hormone, suggest an identity of action.

Summary. The luteal response to FSH-NIH-S1 and S2 in ovarian isografts in male mice indicates that they have equal LH activity and that this is equivalent to 12% of that

of LH-NIH-B1. The response was unchanged by pentobarbital or atropine administration and occurred in androgenized females.

1. Reichert, L. E., Jr.. Parlow, A. F., Endocrinology, 1963, v73, 224.
2. Parlow, A. F., in Albert, A., ed., Human Pituitary Gonadotropins, C. C Thomas, Springfield, Ill., 1961, p282.
3. Browning, H. C., Larke, G. A., Proc. Soc. Exp. Biol. and Med., in press.
4. Finney, D. J., Statistical Method in Biological Assay, Hafner, New York, 1952.
5. Parlow, A. F., Reichert, L. E., Jr., Endocrinology, 1963, v73, 377.
6. ———, ibid., 1963, v72, 955.
7. Browning, H. C., Am. Zoologist, 1963, v3, 537.
8. Baird, J. M., Wolf, R. O., Rennels, E. G., Proc. Soc. Exp. Biol. and Med., 1961, v106, 362.
9. Everett, J. W., Sawyer, C. H., Endocrinology, 1953, v52, 83.
10. Sawyer, C. H., Everett, J. W., Markee, J. E., ibid., 1949, v44, 218.
11. Zarrow, M. X., Quinn, D. L., J. Endocrinol., 1963, v26, 181.
12. Barraclough, C. A., Gorski, R. A., Endocrinology, 1961, v68, 68.

Received September 2, 1964. P.S.E.B.M., 1965, v118.

Relation of Chick Kidney Arginase to Growth Rate and Dietary Arginine.* (29768)

B. L. O'DELL, W. H. AMOS AND J. E. SAVAGE
Departments of Agricultural Chemistry and Poultry Husbandry, University of Missouri, Columbia

Chicks cannot synthesize arginine and when fed a semi-purified diet based on casein as source of protein have a high requirement for this amino acid(1,2,3). Furthermore, chicks fed a sub-optimal amount of arginine show an unusually wide variation in growth rate(4). A study of arginine metabolism showed a considerably greater excretion of urea by chicks fed free arginine, and the authors suggested that the high requirement was due in part to the cleavage of arginine by kidney arginase(5). If this postulate is correct, the high variability in growth rate could result from differences in kidney arginase activity and one would expect an inverse relationship since high arginase activity would be detrimental when arginine is limiting.

Arginase is found in moderate amounts in chick kidney but only in trace amounts in the liver of this species(6,7). Rat liver is a rich source of arginase and the activity can be increased or the enzyme induced in this tissue by high protein diets(8). There is no evidence that this enzyme, or any other, can be induced in non-hepatic tissues of postnatal animals(9). However, arginase has been induced in a variety of animal cells cultured in the presence of ribonucleic acid (10). If kidney arginase is induced by dietary arginine, this phenomenon would accentuate the requirement for dietary arginine when the diet is supplemented with the free amino acid. This paper is concerned with the relationship between growth rate and kidney arginase levels in chicks fed a sub-optimal level of arginine and with the induction of arginase in chick kidney by dietary arginine.

Materials and methods. Male chicks of a crossbred broiler strain were reared from hatching to 4 weeks of age in battery brooders. Feed and water were supplied *ad libitum* and weights were recorded weekly. Two types of diets were fed. One was a semi-purified diet (S) of the following percentage composition: Casein 35, DL-methionine 0.5, L-arginine HCl 0.72, glycine 1.5, glucose hydrate 43.3, salts(11) 6.0, K acetate 2.7, soybean oil 10.0, choline Cl 0.2 and the vitamin supplement previously described(3). This diet contained about 30% of protein and 1.8% of arginine. Supplements were added

* Contribution from Missouri Agri. Exp. Station. Approved by Director as Journal Series No. 2795. Supported in part by a grant-in-aid from Monsanto Chemical Co., St. Louis, Mo.