A Systematic Analysis of the hCG Augmentation Assay for Follicle Stimulating Hormone[1] (37536)

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The basis for this study arises from the early observation of Steelman and Pohley (1) that the dose–response curve has a narrow range and begins to plateau above 0.3 mg of standard. Other implications of a plateau in response to high doses of follicle stimulating hormone (FSH) have been cited (2–4). In the data presented here the existence and extent of this plateau effect is systematically demonstrated under several experimental conditions. Additionally, optimal conditions for control of much of the imprecision and insensitivity encountered in the Steelman–Pohley human chorionic gonadotropin (hCG) augmentation bioassay are reported. These results are significant in view of numerous earlier attempts to improve upon the Steelman–Pohley procedure as applied to rats (2, 3, 5–11) and mice (12–15).

Materials and Methods. Female, 21–22 or 24–25 day old Sprague–Dawley rats were injected at 9:00 AM and 4:00 PM daily for 3 days according to the schedule recommended by Parlow and Reichert (7). At the end of the 3 day period each rat received a total of 20, 40, 60 or 80 IU hCG (purchased from Parke, Davis as Antuitrin "S," lot No. MC 262) in addition to a total of 20 to 1000 μg ovine NIH-FSH-S9 (kindly provided by the National Institute of Arthritis and Metabolic Diseases, Bethesda, MD) as designated in the text. Each injection volume was 0.5 ml. Either the hCG and NIH-FSH-S9 were subcutaneously injected in the head and back for separate site injections at each time interval, or alternatively the hCG and NIH-FSH-S9 were combined in saline for a single subcutaneous injection at each time interval. Five to 7 rats/group were used in all experiments. Control groups received injections of hCG alone. One day prior to the first injection the hCG and NIH-FSH-S9 solutions were prepared by dissolving the compounds in saline (0.9% NaCl) containing 0.1% bovine albumin (purchased from Armour Pharmaceutical Co.) unless designated otherwise. All solutions were stored at 4° until injections. Seventy-two hours following the first injection the rats were sacrificed by carbon dioxide inhalation and their ovaries were removed. On saline moistened filter paper, adherent fat and connective tissue were gently teased away from the ovarian tissue. Immediately following cleaning the ovaries were weighed to the nearest 0.2 mg on a Roller-Smith balance.

Results. Effect of bovine albumin on dose–response curve. Dose–response curves are presented in Fig. 1 for single site injections of hCG combined with FSH standard in saline with and without addition of 0.1% BSA, as designated in the legend. The dose-response curves are linear in all 3 cases with the highest overall response in rats receiving hCG and FSH in 0.1% BSA. The lower response was by rats receiving the same levels of hCG and FSH prepared in saline without additional BSA but prepared such that all solutions came in contact only with siliconized glassware. The presence of BSA seems effective in

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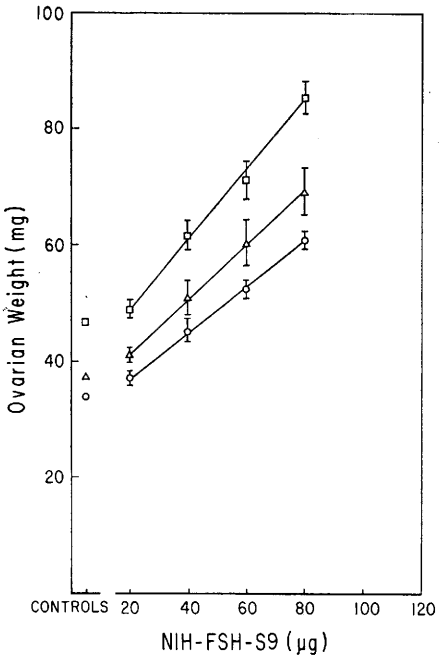

FIG. 1. Comparison of single site subcutaneous injections of 21–22 day old rats with increasing doses of NIH-FSH-S9 combined with 40 IU hCG in saline (□) containing 0.1% bovine albumin (BSA), (△) without BSA, and (○) without BSA but using siliconized glassware for preparation, storage and injection of gonadotropins. Each point represents the mean response for 5 rats/group and the standard error for each group is illustrated by vertical bars at each point.

preventing adherence of FSH to glass for assurance of injection of the designated amount of FSH for bioassay.

Effect of hCG on dose–response curve. Figure 2 illustrates the ovarian weight reponse to 20–100 μg NIH-FSH-S9 augmented by 20–80 IU hCG. As designated in the legend, in one set of experiments the FSH and hCG are prepared together in solution and injected at a single site in the rat. In a second set of experiments the hCG and FSH are prepared in separate solutions and injected into separate sites in the rat. Several observations can be made from the data in Fig. 2. Separate subcutaneous injections of hCG in the head and FSH in the back yields a considerably higher weight response than a single subcutaneous injection of FSH and hCG combined in solution. The slopes of the linear portion of all dose–response curves are equivalent (within standard error) with the exception of 20 IU hCG injected into the same site as FSH. In the latter case the weight response is too low for interpretation of the data to be of any great significance. Separate site injections of hCG and FSH yield linear dose–response curves between 90–180 μg NIH-FSH-S9, a plateau in response between 180–450 μg FSH standard and slight decrease in response to 1000 μg NIH-FSH-S9. This dose–response relationship for separate site injections is analogous for the 4 curves representing 20–80 IU hCG augmenting the FSH ovarian weight response. In no case can these curves be described as a log dose–response. Using either single or separate site injections 40 IU hCG yields data with the minimal standard error at each concentration of NIH-FSH-S9 tested. Hence for minimal variability and optimal response 40 IU hCG is the suggested aug-

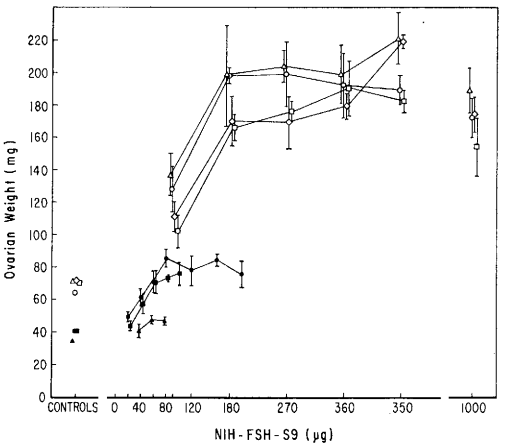

FIG. 2. Comparison of single site versus separate site injections of increasing doses of NIH-FSH-S9 and 20, 40, 60 or 80 IU hCG into 21–22 day old rats is made. Control values represent ovarian weight response to hCG alone. All gonadotropins are prepared in saline containing 0.1% BSA. Combination of 20–120 μg FSH standard with each of 3 augmenting doses of hCG for injection into a single site: (▲) 20 IU hCG, (●) 40 IU hCG, and (■) 80 IU hCG. Injection of 80–1000 μg FSH standard in the back with simultaneous injection in the head of either 20, 40, 60 or 80 IU hCG: (△) 20 IU hCG, (○) 40 IU hCG, (◇) 60 IU hCG and (□) 80 IU hCG. Mean values are displaced to allow illustration of standard error as indicated by the vertical bars.

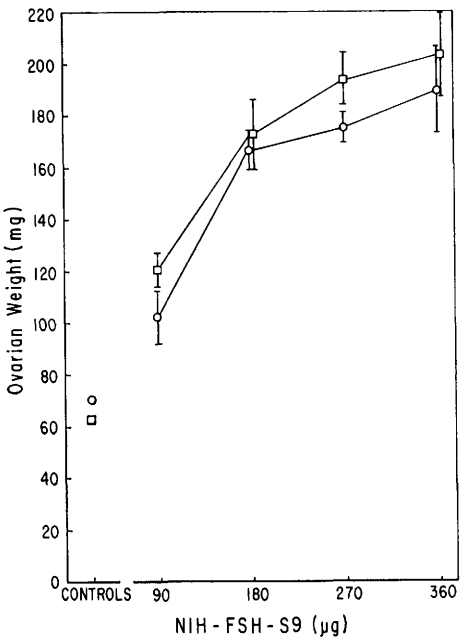

FIG. 3. Comparison of ovarian weight response to 90–360 μg NIH-FSH-S9 in 21–22 (—○—) and 24–25 (—□—) day old rats is made with standard error indicated by the vertical bars. The solutions of hCG and FSH are prepared separately in saline containing 0.1% BSA and are injected into separate subcutaneous sites on the rat. All assays are augmented by 80 IU hCG.

menting dose.

Effect of age on dose–response curve. Figure 3 illustrates the ovarian weight response of 24–25 and 21–22 day old rats injected with 90–360 μg NIH-FSH-S9 augmented with 80 IU hCG. Although there is some overlap in the standard error at 180 and 360 μg NIH-FSH-S9 the data indicates the ovarian weight response is slightly greater in 24–25 day old rats.

On the basis of the data presented in Figs. 1–3 optimal conditions for controlling the imprecision and insensitivity encountered in the Steelman and Pohley augmentation bioassay for FSH are indicated. Figure 4 is a composite of these conditions. Here, production of a dose–response curve of satisfactory slope for routine FSH bioassay is indicated if the following conditions are applied: (a) 90 to 180 μg NIH-FSH standard preparation comprise the standard curve dosages, (b) 40 IU hCG is used as the augmenting dosage, (c) the FSH and hCG are injected subcutaneously twice daily for 3 days in separate sites on the rat, (d) each gonadotropin is prepared in saline containing 0.1% BSA, (e) initial injections are administered 24–25 day old rats, and (f) the ovaries are cleaned and weighed immediately following autopsy. Again the plateau effect is indicated above 180 μg NIH-FSH-S9

In many of the foregoing experiments we are indebted to the suggestion of Dr. Leo Reichert (Emory University, Atlanta, GA) that we prepare the gonadotropins in separate solutions and inject them likewise. It had been his experience that some commercial preparations of hCG contained an apparent FSH-destroying activity. Whether or not this is a neuraminidase-like activity removing sialic acid which is reportedly necessary for complete expresson of FSH biological activity (16–18) is not clear. Figure 2 demonstrates, however, that separate site injections of the 2 gonadotropins markedly increases

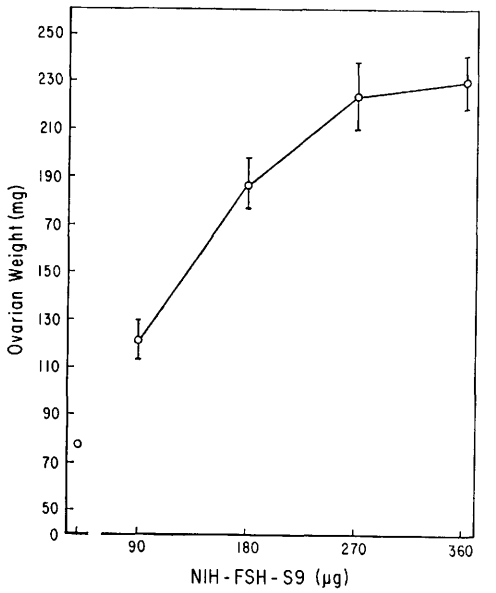

FIG. 4. Dose–response curve for 24–25 day old rats injected twice daily for 3 days with gonadotropins prepared in saline plus 0.1% BSA. Each rat received a total of 40 IU hCG injected subcutaneously in the head and either 90, 180, 270 or 360 μg NIH-FSH-S9 injected in the back. Control rats received only hCG. The vertical bars indicate standard error.

the ovarian weight response implying the presence of some FSH-destroying activity in the hCG preparation when FSH and hCG are prepared and injected together.

Summary. This study was prompted by the initial observation of Steelman and Pohley [Endocrinology **53**, 604 (1953)] that the dose–response curve was restricted to a narrow range of FSH standard and furthermore that at levels above 0.3 mg FSH (Armour standard 264-151-X of porcine origin) the curve begins to plateau. Later, indications of a similar plateau effect using NIH-FSH-(S1, S2, S3 and S8) all of ovine origin were noted (2–4). In this report optimal conditions for routine bioassay of FSH are presented. Separate site injections of NIH-FSH-S9 (90 and 180 μg) augmented with 40 IU hCG twice daily for 3 days yields a dose–response curve of satisfactory slope for bioassay. The validity and extent of the plateau effect in ovarian response to 180–1000 μg NIH-FSH-S9 augmented with 20, 40, 60 and 80 IU hCG are clearly illustrated. The possibility remains feasible that the plateau and apparent suppression of ovarian response to high FSH concentrations "may" be related to either the biological half-life of FSH in the plasma and/or negative feedback by gonadal steroid hormones.

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