Assuming that the exogenous hormone is relatively more active in suppressing the hypophysis than stimulating the ovaries, then the reduction in the resultant level of follicle-stimulating activity is bound to follow when the dose is within a certain range.

As the dose increases, the initial decline in ovarian response is the result of the rate of increase in hypophysis, suppression being greater than the rate of increase in the level of follicle-stimulating activity of the exogenous hormone.

However, as the suppression reaches its maximum, the situation becomes reversed, and the ovarian weight increases to increasing doses of exogenous hormone.

Therefore the evidence presented leads to the conclusion that the injection of low doses of FSH can reduce the level of activity of the endogenous follicle-stimulating system, and that this property is not specific to a single preparation.

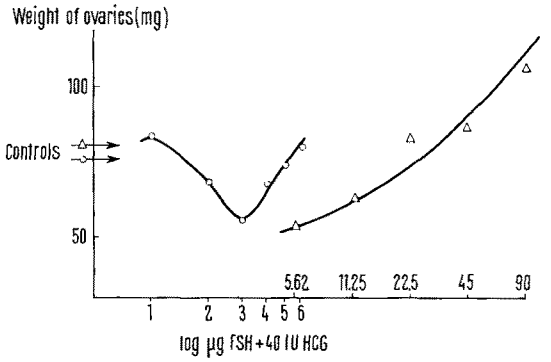

Fig. 2. FSH activity in the STEELMAN and POHLEY[2] test. Ovary weight after fixing in Bouin's liquid for 24 h. ○—○ Pig FSH (Mann Res. Lab.); △—△ NIH-FSH-S2 ovine.

Riassunto. Gli Autori riportano una interessante anomalia che si riscontra nel dosaggio di piccolissime quantità di FSH, sia nel test di STEELMAN e POHLEY quanto nel test di riduzione intravaginale del TTC. Tale anomalia si evidenzia in un netto e significativo abbassamento delle risposte rispetto ai controlli non trattati. Si fa l'ipotesi di una interazione tra l'ormone esogeno somministrato e la secrezione ipofisaria basale endogena dell'animale impubere, che si manifesterebbe in un feed-back a livello dell'ipofisi, diretto o indiretto tramite l'ipotalamo o centri ancora superiori. Tale effetto non è specifico di una singola preparazione.

G. LUGARO and M. M. CASELLATO

Institute of Organic Chemistry, University of Milano (Italy), 10 June 1968.

FSH-Releasing Effect of Clomiphene in the Female Rat

Clomiphene citrate (1-*p*-(β-diethylamino-ethoxy)-phenoxy-1, 2-diphenyl-2-chloroethylene), a derivate of the non-steroidal oestrogen chlorotrianisene, is a potent inducer of ovulation in man, but inhibits fertility in the rat. In the human its administration has been shown to increase the urinary excretion of total gonadotropines[1-3] and of LH[4]. In the rat a marked inhibition of pituitary gonadotropin secretion, probably due to blocking of hypothalamic receptor sites, was observed by HOLTKAMP[5]. To the contrary, IGARASHI et al.[6] found that clomiphene citrate (C.c.) caused a significant rise in plasma FSH- and LH-levels in the rat. As the implantation of C.c. crystals into the median eminence of the rat has been shown to decrease pituitary FSH content[6], we examined whether i.v. administered C.c. would affect the release of FSH in the ovariectomized, oestrogen-progesterone blocked rat.

Materials and methods. The FSH-releasing activity of C.c. was determined by a slight modification of the method of RAMIREZ et al.[7]. Wistar rats of approximately 180 g body weight which had been kept under standard condition were used. The experiments were performed 4 weeks after bilateral, dorsolateral ovariectomy. The certainty of anoestrous was assured by performing vaginal smears during the last week.

Pituitary blocking was achieved by injecting the animals s.c. with 50 µg oestradiol-17β and 25 mg progesterone on day 2 and 3 before administration of C.c.

C.c. was injected as a 0.03% aqueous solution into the tail vein. Each group contained 4 animals. In group I each animal received 300 µg and in group II 600 µg C.c. 15 min after the injection the animals were anaesthetized with ether and exsanguinated into heparinized test tubes. The blood of each group was pooled. Plasma was obtained by centrifugation at 3000 rpm for 15 min.

The plasma FSH-activity was assayed according to IGARASHI and MCCANN[8], utilizing NMRI mice. 7 animals were used per dose level. Each animal received 2 ml of a solution containing 1.5 ml pooled plasma and 0.5 ml HCG in normal saline. The HCG content of the final solution was 0.125 IU/ml. The injections were given in divided doses of 0.7 ml on the first and second day, and 0.6 ml on the third day. The mice were sacrificed on the day following the last injection. The uteri were removed immediately, and the wet weight was determined to the nearest 0.1 mg.

Since it was assumed that after 15 min circulation time plasma of C.c. treated animals still contained most of the substance, experiments were conducted to determine whether C.c. has a uterotropic effect in the mouse when administered together with HCG. 7 mice were used per dose level. Each animal received a total dose of 0.25 IU

[1] R. B. GREENBLATT, R. SOMNATH and V. B. MAHESH, Am. J. Obstet. Gynec. *84*, 900 (1962).
[2] G. BETTENDORF, M. BRECKWOLDT and P.-J. CZYGAN, Geburtsh. Frauenheilk. *25*, 637 (1965).
[3] J. ZANDER and G. BUNTRU, Geburtsh. Frauenheilk. *23*, 871 (1963).
[4] C. W. BARDIN, G. T. ROSS and M. B. LIPSETT, J. clin. Endocr. Metab. *27*, 1558 (1967).
[5] D. E. HOLTKAMP, J. G. GRESLIN, CH. A. ROOT and L. J. LERNER, Proc. Soc. exp. Biol. Med. *105*, 197 (1960).
[6] M. IGARASHI, Y. IBUKI, H. KUBO, J. KAMIOKA, N. YOKOTA, Y. EBARA and S. MATSUMOTO, Am. J. Obstet. Gynec. *97*, 120 (1967).
[7] V. D. RAMIREZ and S. M. MCCANN, Endocrinology *73*, 193 (1963).
[8] M. IGARASHI and S. M. MCCANN, Endocrinology *74*, 446 (1964).

HCG and 20, 100 or 200 µg of C.c., respectively, in 2 ml normal saline.

The mode of injection followed the scheme given above. The animals were sacrificed on the fourth day following the first injection, and the wet weight of the uteri was recorded to the nearest 0.1 mg.

Result and discussion. The results are summarized in Tables I and II. There was a significant difference ($p < 0.001$) in plasma FSH-activity between ovariectomized, oestrogen-progesterone blocked animals and those who had been ovariectomized but not been blocked. The injection of C.c. at the dose levels of 300 and 600 µg per animal caused a significant rise of plasma FSH-activity as compared to ovariectomized, oestrogen-progesterone blocked controls ($p < 0.001$). The difference between the 2 dose levels, however, was statistically not significant ($p > 0.05$). The plasma FSH-activity of C.c. treated animals was in the same order of magnitude as the one observed in ovariectomized, non-blocked animals. This seems to indicate that a maximal release of FSH was achieved at these dose levels.

Table I. Effect of clomiphene citrate upon FSH-release in the ovariectomized, oestrogen-progesterone blocked rat

Treatment (ovariectomized rats)	Dose of clomiphene citrate (µg)	Mouse uterine weight (mg)	*P v.* control
None	0	46.4 ± 1.3	< 0.001
50 µg oestradiol + 25 mg progesterone	0	29.3 ± 1.3	control
50 µg oestradiol + 25 mg progesterone	300	49.3 ± 2.9	< 0.001
50 µg oestradiol + 25 mg progesterone	600	47.0 ± 2.7	< 0.001

Determination of plasma-FSH according to IGARASHI and MCCANN[8].

Table II. Effect of clomiphene citrate and HCG upon uterine weight of infantile mice

Treatment	No. of mice	Uterine weight (mg)	*P v.* control
None	21	5.8 ± 0.2	–
2 ml normal saline	10	6.1 ± 0.5	–
0.25 IU HCG	11	19.1 ± 1.2	control
0.25 IU HCG + 20 µg clomiphene	7	20.9 ± 0.6	> 0.05
0.25 IU HCG + 100 µg clomiphene	7	20.2 ± 0.3	> 0.05
0.25 IU HCG + 200 µg clomiphene	7	19.8 ± 0.5	> 0.05

A direct uterotropic effect of C.c. still present in the plasma at the time of sacrifice could be excluded. As shown in Table II, there was a significant rise of uterine weight in immature mice after injection of a total dose of 0.25 IU HCG as compared to controls. No further increase of uterine weight was seen when 20, 100 or 200 µg of C.c./animal were injected together with HCG.

It is concluded, therefore, that the increased plasma FSH-activity observed in ovariectomized, oestrogen-progesterone blocked rats after i.v. injection of C.c. was due to an effect of this compound on the releasing mechanism for FSH. The results presented here are in agreement with the findings of IGARASHI et al. It appears likely that C.c. stimulates the release of FSH-RF in the rat, which leads to increased production and secretion of pituitary FSH.

Zusammenfassung. Der Effekt von Clomifendihydrogenzitrat auf den FSH-Release der Ratte wurde untersucht. Die i.v. Injektion von 300 bzw. 600 µg Clomifendihydrogenzitrat/Tier führte bei oophorektomierten, weiblichen Ratten, die mit Östradiol und Progesteron blokkiert worden waren, zu einer signifikanten Erhöhung der FSH-Aktivität im Plasma. Eine Beeinträchtigung des FSH-Nachweises durch noch im Plasma befindliches Clomifendihydrogencitrat konnte ausgeschlossen werden.

H. BAIER and H.-D. TAUBERT

Abteilung für Gynäkologische Endokrinologie der Universitäts-Frauenklinik, 6 Frankfurt a. M. Süd (Germany), 22 May 1968.

Liver Regeneration After Partial Hepatectomy in Rats Exposed Before the Operation to the Stress Stimulus

Some metabolic changes, for example the changes of proteosynthesis[1] and the changes of ATP metabolism[2] developing in the liver in the first 12 h after 65–70% hepatectomy, can be found even in the intact liver after the administration of different stress stimuli[1–4]. The conditions under which the development of these changes in the liver tissue is stimulated probably differ after partial hepatectomy (PH) from those after simple stress stimulus. Only the development of these changes after the application of stress stimulus can be prevented by adrenalectomy[1]. After PH the onset of the changes of proteosynthesis and of the changes of ATP metabolism precedes the development of the increased DNA synthesis[2,5]. To understand better the relationship between these changes, we decided to find out whether the changes of DNA synthesis are influenced if PH is carried out in time when the stress reaction is fully developed.

For our experiments, 75 female rats, aged 3–4 months, were used. At PH liver weight was reduced by 65–70%[6]. As the stress stimulus 8 h before PH the i.p. injection of the water suspension of hyflo-super-cell (5 mg/0.5 ml of saline/100 g of body weight), of compound similar to

[1] C. MAJUMDAR, K. TSUKADA and I. LIEBERMAN, J. biol. Chem. *242*, 700 (1967).
[2] P. OVE, S.-I. TAKAI and T. UMEDA, J. biol. Chem. *242*, 4963 (1967).
[3] O. W. NEUHAUS, H. F. BALEGNO and A. M. CHANDLER, Am. J. Physiol. *211*, 151 (1966).
[4] H. A. LEON, D. D. FELLER, E. D. NEVILLE and B. DALIGEON, Life Sci. *4*, 737 (1965).
[5] W. BUSANNY-CASPARI and M. DEIMEL, Z. ges. exp. Med. *136*, 456 (1963).
[6] G. M. HIGGINS and R. M. ANDERSON, Archs Path. *12*, 186 (1931).