Histamine and increased ovarian blood flow mediate LH-induced superovulation in the cyclic hamster*

A. Krishna, P. F. Terranova, R. L. Matteri† and H. Papkoff†

Department of Physiology, Ralph L. Smith Research Center, University of Kansas Medical Center, Kansas City, Kansas 66103, U.S.A. and †Hormone Research Laboratory, University of California, San Francisco, California 94143, U.S.A.

Summary. Constant infusion of LH (400 µg NIH-S24) through an osmotic minipump inserted on Day 1 (oestrus) of the cycle in the hamster resulted in spontaneous superovulation (≈ 29 ova) at the next expected oestrus, increased blood flow ($P < 0.001$) to the ovary on Day 3, and slight depletion ($0.1 > P > 0.05$) of histamine in the ovary. Treatment with antihistamine (alpha-fluoromethylhistidine, an irreversible inhibitor of histidine decarboxylase, or cimetidine, an H_2 blocker) by injections or by infusion using separate osmotic minipumps significantly ($P < 0.01$) reduced the number of ova shed in the LH-treated hamsters. Infusion of LH with alpha-fluoromethylhistidine in the same osmotic minipump reduced the bioactivity of the LH. Infusion of antihistamine alone did not alter the normal number of ova shed. The results suggest that the LH-induced superovulation involves stimulation of histamine release; the histamine then may increase ovarian blood flow thus allowing more gonadotrophins to reach the ovary.

Introduction

The study of Garza, Shaban & Terranova (1984) has shown that constant infusion of moderate amounts of LH during the cycle induced superovulation in the cyclic hamster. The superovulatory action of LH required the presence of the pituitary, suggesting that LH might synergize with FSH for spontaneous superovulation. The mechanism of LH-induced superovulation in the hamster remains unknown, but LH has been shown to increase ovarian blood flow (Niswender, Reimers, Diekman & Nett, 1976; Ellinwood, Nett & Niswender, 1978). Therefore, LH given during a superovulatory cycle could increase ovarian blood flow, and then the ovary would theoretically receive more FSH, a critical hormone necessary for superovulation.

Since LH has been shown to increase ovarian blood flow apparently by its ability to release histamine (Szego & Gitin, 1964; Lipner, 1971; Piacsek & Huth, 1971), we have examined whether, during an LH-induced superovulatory cycle, (1) ovarian blood flow increased, (2) ovarian histamine was altered and (3) antihistamines could reduce the number of ova shed.

Materials and Methods

Cyclic hamsters (80–100 g body wt) were maintained in a room at 22°C with a 14-h light:10-h dark daily lighting schedule and food and water were available *ad libitum*. Hamsters were used only after exhibiting three successive 4-day cycles.

* Reprint requests to Dr P. F. Terranova.

Effect of infusion of LH throughout the cycle on the number of ova shed. Osmotic minipumps (no. 2001, Alza Corp., Palo Alto, CA, U.S.A.) containing 400 µg ovine LH (S-24) in 0.01 M-phosphate-buffered normal saline (PBS) with 0.1% bovine serum albumin (BSA), pH 7.0, or PBS-BSA alone were inserted s.c. on Day 1 of the cycle at 09:00–10:00 h as previously described in detail by Garza *et al.* (1984). Each hamster received a single pump. On Day 5 (expected oestrus) after insertion of the pump, hamsters were autopsied, the oviduct irrigated and the number of oviducal ova was recorded.

Effect of infusion of LH on ovarian blood flow. LH-induced superovulation involved prevention of the normal reduction in the number of antral follicles that occurs between Days 3 and 4 of the cycle (Garza *et al.*, 1984). Day 3 may therefore be a critical period in the mechanism by which LH induces superovulation. In the present study, the blood flow to the ovary was measured on Day 3 in hamsters with and without LH-releasing pumps. The LH pumps (400 µg) were inserted on Day 1 of the cycle.

Radioactive microspheres (Wagner, Rhodes, Sasaki & Ryan, 1969; Niswender *et al.*, 1976) were used to estimate the flow of blood to the ovary. Blood flow studies were carried out under pentobarbitone sodium anaesthesia (60 mg/kg i.p.) at 12:00–15:00 h on Day 3. Physiological saline (9 g NaCl/l), 0.5 ml containing 300 000 c.p.m. of gadolinium-153 labelled microspheres with a diameter of 15 µm (sp. act. 10 mCi/g; New England Nuclear, Boston, MA, U.S.A.), was infused into the dorsal aorta through a catheter passed along the right common carotid artery. Blood was collected using a reverse-infusion pump (artificial organ) connected to the left femoral arterial catheter at a rate of 0.4 ml/min beginning 30 sec before the start of the infusion of microspheres; withdrawal continued for 1.5 min. Blood was withdrawn into a preweighed heparinized disposable 1 ml syringe. After weighing the syringe containing blood to determine blood volume, the blood was transferred to a scintillation vial. Immediately after the withdrawal of blood from the femoral artery, the hamster was killed by infusing an overdose of pentobarbitone sodium into the carotid cannula. The ovaries, uteri and kidneys were removed, weighed and dissolved overnight in 1.0 ml 30% (w/v) KOH. These organs and the blood withdrawn from the femoral artery were counted separately in a gamma counter for determination of radioactivity. Adequate mixing and streaming of the microspheres to each kidney was checked separately. When a disparity of > 30% existed between the counts of the right and left kidney, the experiment was discarded. Organ blood flow was calculated using simple proportions. For example, the ratio of the counts per minute in the artificial organ (A_{cpm}) and the blood flow of the artificial organ (A_{bf}) is directly proportional to the counts per minute in the ovary (O_{cpm}) and the blood flow of the ovary (O_{bf}):

$$A_{cpm}/A_{bf} = O_{cpm}/O_{bf}$$

A_{cpm} and O_{cpm} were measured experimentally; $A_{bf} = 0.4$ ml/min (withdrawal rate of artificial organ); and O_{bf} was calculated based on measurements of A_{cpm} , O_{cpm} and A_{bf} .

Effects of infusion of LH on the ovarian histamine level. The ovaries were removed from the body cavity on Day 3 at 09:00–10:00 h from animals treated with or without LH pumps inserted on Day 1 (methods described above). Ovaries from each animal were cleaned of surrounding tissues, weighed and then frozen immediately in 0.5 ml 0.05 M-sodium phosphate buffer (pH 7.9) containing 0.1% (w/v) Triton X-100 (PBST) until assayed for histamine by the method of Taylor, Krilis & Baldo (1980).

Tissues were prepared for histamine assay by the method of Taylor & Snyder (1972). Tissues were homogenized in ice-cold PBST. The homogenate was then heated in a boiling water bath for 10 min to precipitate proteins and to destroy endogenous *S*-adenosyl methionine. The mixture was then cooled to 4°C and centrifuged at 50 000 *g* for 10 min. A portion (20 µl) of the supernatant fluid was transferred to tubes for the assay of histamine. To 20 µl samples of tissue extract were added 10 µl histamine-*N*-methyl transferase-*S*-adenosyl methionine reaction solution. Histamine-

N-methyl transferase was prepared from Swiss male albino mice brains as described by Taylor *et al.* (1980). Mice were decapitated and the brain quickly removed and homogenized in 10 volumes of ice-cold phosphate buffer, pH 7.9. The supernatant fluid obtained after centrifugation at 50 000 *g* for 10 min was removed and kept on ice as the HMT-solution. Histamine-*N*-methyl transferase-*S*-adenosyl methionine solution was prepared by adding 0.5 ml HMT solution to the Eppendorf microfuge tubes containing 10 μ l *S*-adenosyl-L-[methyl- 3 H]methionine (1 mCi/ml; sp. act. 55.2 mCi/mmol; New England Nuclear Corp., Boston MA) immediately before use in the histamine assay. The enzymic reaction was allowed to proceed for 60 min at 37°C and then was stopped by the addition of 10 μ l 1 N-NaOH saturated with solid NaCl. The [3 H]-methylhistamine formed was extracted into 250 μ l chloroform. The chloroform (containing histamine) was dried in a scintillation vial. Then 9 ml scintillation fluid (3.81 litres toluene + 15 g 2a70 (Research Products International Corp., IL)) were added. The vials were then counted in an automatic liquid scintillation counter. The standard line ranged from 0.635 to 40 ng. The percentages for the coefficients of variation for the intra- and inter-assay variances were 5.1 and 5.7, respectively, for 6 assays in duplicate. Parallelism was evident amongst various concentrations of tissue histamine and the standard line.

Effect of antihistamine of superovulation. The effects of the antihistamine, alpha-fluoromethylhistidine mono-HCl hemihydrate (Merck, Sharp & Dohme Research Laboratories, Rahway, NJ) on LH-induced superovulation was determined by inserting on Day 1 (at 09:00–10:00 h), osmotic minipumps containing 400 μ g ovine LH with 50 mg alpha-fluoromethylhistidine on PBS-BSA together in the same pump, 400 μ g ovine LH alone in PBS-BSA or 50 mg alpha-fluoromethylhistidine alone in PBS-BSA. On Day 5 (expected oestrus) after insertion of the pumps, hamsters were autopsied, the oviducts irrigated and the number of ova was recorded.

Since alpha-fluoromethylhistidine reduced the biological activity of LH within the minipump (see 'Methods' below), LH and alpha-fluoromethylhistidine in 200 μ l PBS-BSA were infused in two separate osmotic minipumps at different locations s.c. on the back: 400 μ g ovine LH and 50 mg alpha-fluoromethylhistidine in 200 μ l PBS-BSA in separate pumps were inserted s.c. on Day 1 (at 09:00–10:00 h). On Day 5 (expected oestrus) after insertion of the pumps, hamsters were autopsied, the oviducts irrigated and the number of ova was recorded. Injections of alpha-fluoromethylhistidine and cimetidine (an H_2 receptor blocker) were also tested for the effects on LH induced superovulation. Hamsters with a 400 μ g ovine LH pump inserted on Day 1 were given 5 injections of alpha-fluoromethylhistidine (3.5 mg/0.1 ml saline per injection) or cimetidine (30 mg/0.1 ml saline per injection: Smith Kline & French, PA, U.S.A.) at 08:00, 12:00, 16:00 and 20:00 h on Day 3 and at 08:00 h on Day 4. Control animals without LH pumps were given the same doses of alpha-fluoromethylhistidine or cimetidine. Hamsters were autopsied on Day 5, the oviducts irrigated and the number of ova was recorded.

The stability of LH bioactivity was determined by measuring testosterone production in dispersed rat Leydig cells (Ramachandran & Sairam, 1975; Roser *et al.*, 1984), after the LH had been incubated with alpha-fluoromethylhistidine (H_2O) or acetate buffer (pH 4.0) for 16 h at 37°C. The reason for choosing pH 4.0 was that alpha-fluoromethylhistidine at a concentration of 50 mg/200 μ l PBS (250 mg/ml) resulted in a pH of 4.0. In the experiments, (1) purified ovine LH standard (G3-316B) was tested directly in the rat Leydig cell assay without prior incubation; (2) 4 mg ovine LH (G3-316B) and 250 mg alpha-fluoromethylhistidine were incubated in 0.5 ml distilled water at 37°C for 16 h. Then the mixture was desalted on Sephadex G25, lyophilized and tested in the bioassay for LH (G3-329B); (3) 4 mg ovine LH (G3-316A) was incubated in 0.5 ml sodium acetate buffer, pH 4.0 for 16 h and then placed in the bioassay (G3-329A); (4) the above materials (G3-329A and G3-329B) were incubated at pH 7.0 (phosphate buffer, 1 mg/1 ml) for 36 h at room temperature ($20 \pm 2^\circ C$) and then reassayed in the LH bioassay.

Statistics. Data were analysed by Student's *t* tests.

Results

Effects of infusion of LH throughout the cycle on the number of ova shed

In the hamster, infusion of 400 µg ovine LH-S-24 by an osmotic minipump resulted in spontaneous superovulation of 29.4 ± 0.9 ova ($N = 6$) which was significantly ($P < 0.001$) greater than in controls (12.9 ± 0.7 ova; $N = 6$).

Effect of infusion of LH on ovarian blood flow

Constant infusion of LH (400 µg LH pump) for at least 48 h (Days 1–3) resulted in a significant ($P < 0.001$) increase in the ovarian blood flow (2.9 ± 0.22 ml/min per g of ovary; $N = 6$) when compared with the control animals (0.76 ± 0.09 ml/min per g ovary; $N = 6$). In contrast to the ovary, blood flow (ml/min per g) to the kidneys (control = 2.22 ± 0.67 ; LH pump = 2.29 ± 0.67) and uterus (control = 0.27 ± 0.07 ; LH pump = 0.32 ± 0.08) did not increase significantly.

Effect of infusion of LH on the ovarian histamine concentration

There was a slight depletion ($0.1 > P > 0.05$) of the ovarian histamine concentration (1.05 ± 0.12 ng/mg ovarian wt; $N = 6$) in the animals with LH pumps when compared with that of control animals (1.74 ± 0.35 ng/mg ovarian wt; $N = 6$).

Effect of antihistamine on superovulation

Infusion of 50 mg alpha-fluoromethylhistidine with the superovulatory dose of 400 µg LH in the same minipump completely prevented the superovulatory action of LH. The 12.8 ± 0.59 ova ($N = 7$) ovulated was significantly ($P < 0.01$) lower than the number of ova shed after infusion of the LH only (29.4 ± 0.9 ; $N = 6$). Infusion of 50 mg alpha-fluoromethylhistidine alone did not affect the normal number of ova shed; the animals ovulated 12.5 ± 0.34 ova ($N = 6$) which is not significantly different from the number shed (12.9 ± 0.7 ova; $N = 6$) in the control animals without pumps. However, alpha-fluoromethylhistidine altered the bioactivity of LH when infused in the same pump (see below).

Table 1. Effects of alpha-fluoromethylhistidine (αFMH) and cimetidine on the number of ova shed in LH-treated hamsters

Treatment group	No. of ova shed
LH*	29.6 ± 1.6 (6)
LH + αFMH*	21.3 ± 0.6 (6)
LH + αFMH†	20.5 ± 0.8 (6)
LH + cimetidine†	21.7 ± 3.3 (5)
αFMH*	12.4 ± 0.3 (6)
Cimetidine†	16.3 ± 0.9 (3)

Values are mean $\pm$ s.e.m. for the no. of hamsters indicated.

*Given in separate osmotic minipumps at a dose of 50 mg αFMH/200 µl and 400 µg LH/200 µl, on Days 1–5 of the cycle; hamsters were autopsied on Day 5 (oestrus).

†Injections (3.5 mg αFMH/0.1 ml saline; 30 mg cimetidine/0.1 ml saline) were given at 08:00, 12:00, 16:00 and 20:00 h on Day 3 and 08:00 h on Day 4; hamsters were killed on Day 5 (oestrus).

Fig. 1. Effects of alpha-fluoromethylhistidine (α FMH) and acetate buffer (pH 4.0) on the bioactivity of LH in the rat Leydig cell assay. For details of incubation see 'Methods'.

Infusion of alpha-fluoromethylhistidine and the superovulatory dose of 400 μ g LH in separate pumps reduced, although it did not completely prevent the superovulatory action of LH (Table 1). Injection of alpha-fluoromethylhistidine or cimetidine also reduced significantly ($P < 0.01$) the superovulatory action of LH (Table 1).

Effects of alpha-fluoromethylhistidine on LH bioactivity

Incubation of 4 mg ovine LH with 250 mg alpha-fluoromethylhistidine in 0.5 ml distilled water at 37°C for ≈ 16 h reduced the bioactivity of the LH by $\approx 65\%$ in the rat Leydig cell assay (Fig. 1). Under similar conditions 4 mg ovine LH incubated in acetate buffer at pH 4.0 reduced the bioactivity of the LH by $\approx 85\%$; reincubation of the above treated LH preparations at a pH of 7.0 increased the bioactivity of each to near standard values (data not shown).

Discussion

A most interesting finding in the present study was the increased blood flow to the ovary on Day 3 (dioestrus II) after constant infusion of LH using the osmotic minipump inserted on Day 1 (oestrus) of the cycle. Thus the total quantity of circulating FSH, a critical hormone apparently necessary for superovulation (Garza *et al.*, 1984), reaching the ovary would be expected to be increased due to the enhanced flow of blood to the ovary. The ability of LH and hCG to induce ovarian hyperaemia has long been known in rodents (for review see Ellinwood *et al.*, 1978). As early as 1961, Ellis had quantified the hyperaemic response of the rat ovary to LH. Later, Wurtman (1964) reported that LH caused a rapid increase in the blood flow to the ovaries in rat but FSH and prolactin failed to do so. Piacsek & Huth (1971) also noted that LH increased ovarian blood flow in rats, and Janson (1975) demonstrated a significant increase in blood flow to the ovary of the rabbit within 2 min after injection of LH. All of these studies show acute effects of LH on increasing ovarian blood flow. The present study indicates that continuous infusion of LH can apparently

maintain elevated ovarian blood flow. Blasco, Wu, Flickinger, Pearlmuter & Mikhail (1975) observed ovarian hyperaemia for 9 h after a single injection of hCG in the rabbit.

Szego & Sloan (1961) postulated that the molecular basis of the hyperaemia induced by LH appears to be, in part, histamine mobilized from within the ovary. Later, Szego & Gitin (1964) demonstrated a significant decrease in the ovarian content of histamine 2 h after rats were injected with LH; they suggested that a primary effect of LH on the ovary was to induce release of histamine, resulting in increased ovarian blood flow. This hypothesis was further supported by the study of Wurtman (1964) who observed that histamine increased ovarian blood flow in rats and that it was similar to the increase obtained with LH. Piacsek & Huth (1971) found that anti-histamine blocked an acute LH-induced increase in ovarian blood flow. The present study indicates that LH causes a depletion of ovarian histamine as described by Szego & Gitin (1964).

Mast cells have been located in the ovarian hilum and surrounding the blood vessels that enter and leave the ovary (Krishna & Terranova, 1985). If LH induces the mast cells to release histamine, then very soon after release of the histamine it would be expected that ovarian histamine would increase; however, with prolonged LH stimulation and coincident depletion of mast cell histamine, it would be expected that the amount of histamine in the ovary and delivered to the ovary would be reduced. Mast cells undergo extensive degranulation at the peak of the gonadotrophin surge on the day of pro-oestrus (15:00 h) and the latter coincides with elevated concentrations of ovarian histamine (Krishna & Terranova, 1985) and increased utero-ovarian blood flow (Varga & Greenwald, 1979). LH-induced superovulation may therefore involve stimulation of mast cell release of histamine; this histamine may increase ovarian blood flow, thus allowing more gonadotrophins to reach the ovary. However, a non-mast cell source of histamine may play some role in these processes.

Infusion of alpha-fluoromethylhistidine + LH in the same pump reduced the bioactivity of the LH (Fig. 1). This apparently occurred due to the low pH (4.0) caused by the high concentration of alpha-fluoromethylhistidine monohydrochloride. The low pH probably caused dissociation of the alpha and beta subunits of LH (Papkoff, 1973). Therefore the bioactivity of the LH was reduced and only normal numbers of follicles ovulated. The infusion of the alpha-fluoromethylhistidine and LH in separate pumps at different locations on the back reduced the number of ova shed by about 8 ova/animal. In addition, i.p. injections of alpha-fluoromethylhistidine or cimetidine similarly reduced the number of ova shed. Although these treatments were not totally effective in reducing the number of ova shed to control values (≈ 12 ova) there is a possible explanation. The number of ova shed by the hamster during an LH-induced superovulation is the result of 2 components. First, at the follicular level, LH synergizes with FSH in promoting the growth, development and recruitment of follicles (Richards, 1980). Secondly, at the blood flow level, LH, acting alone or in synergy with other hormones, enhances the rate of blood flow to the ovaries; this results in more effective delivery of the hormones to the ovary, thus stimulating further follicular development. Blockage of the synthesis and/or action of histamine should reduce the ovarian blood flow component to control values. Previous studies have shown that antihistamines block LH-induced increases in ovarian blood flow (Piacsek & Huth, 1971). If ovarian blood flow was decreased 3-fold after treatment with alpha-fluoromethylhistidine and cimetidine, the circulating levels of LH still remain elevated and are capable of stimulating follicular development through its synergism with FSH. Until the ovarian blood flow measurements are recorded in antihistamine-treated hamsters and until no significant alteration in serum concentrations of FSH are shown in those animals, we can only speculate on the mechanisms involved. The ovaries of hamsters treated with LH + alpha-fluoromethylhistidine exhibited fresh corpora lutea (and no unruptured Graafian follicles), indicating that the reduction in ovulations was not due to a blockade of follicular rupture. This further indicates that histamine may be involved in the recruitment of the 'extra' follicles by the LH treatment. These results indicate that histamine may not be involved in the 'normal' recruitment of follicles during the cycle, although this must be interpreted with caution since only 1 dose of the antihistamine was used; higher doses might have reduced the number of ova shed. The reports of

Wallach, Wright & Hamada (1978), Knox, Lowry & Beck (1979) and Kobayashi, Wright, Santulli, Kitai & Wallach (1983) have shown that antihistamines can reduce the number of ova shed in the rabbit, probably by prevention of the rupture of the follicles. In a study by Lipner (1971) the factors reducing the number of ova shed in antihistamine- and PMS-hCG-treated rats are unknown; the fewer ova shed might have been due to retardation of follicular development since ovarian weights were reduced.

This work was supported in part by grants from NICHD (15526) to P.F.T. who was a Research Career Development Awardee (K00478). A.K. was supported in part by the Council of International Exchange of Scholars (Fulbright Foundation). We thank the National Pituitary Agency for supplies of gonadotrophins; Merck, Sharp and Dohme Research Laboratories for the generous supply of alpha-fluoromethylhistidine; and Dr R. T. Dowell and Mr Curtis Kauer, Department of Physiology at University of Kansas Medical Center, for their assistance with the blood flow measurements.

References

- Blasco, L., Wu, C.H., Flickinger, G.L., Pearlmutter, D. & Mikhail, G. (1975) Cardiac output and genital blood flow distribution during the preovulatory period in rabbits. *Biol. Reprod.* **13**, 581–586.
- Ellinwood, W.E., Nett, T.M. & Niswender, G.D. (1978) Ovarian vasculature: structure and function. In *The Vertebrate Ovary: Comparative Biology and Evolution*, pp. 583–614. Ed. R. E. Jones. Plenum Press, New York.
- Ellis, S. (1961) Bioassay of luteinizing hormone. *Endocrinology* **68**, 334–340.
- Garza, F., Shaban, M.A. & Terranova, P.F. (1984) Luteinizing hormone increases the number of ova shed in the cyclic hamster and guinea pig. *J. Endocr.* **101**, 289–298.
- Janson, P. O. (1975) Effects of luteinizing hormone on blood flow in the follicular rabbit ovary as measured by radioactive microspheres. *Acta endocr., Copenh.* **79**, 122–133.
- Knox, E., Lowry, S. & Beck, L. (1979) Prevention of ovulation in rabbits by antihistamine. In *Ovarian Follicular Development and Function*, pp. 159–163. Eds A. R. Midgley & W. A. Sadler. Raven Press, New York.
- Kobayashi, Y., Wright, K.H., Santulli, R., Kitai, H. & Wallach, E.E. (1983) Effect of histamine and histamine blockers on the ovulatory process in the in vitro perfused rabbit ovary. *Biol. Reprod.* **28**, 385–392.
- Krishna, A. & Terranova, P.F. (1985) Alterations in mast cell degranulation and ovarian histamine in the pre-ovulatory hamster. *Biol. Reprod.* **32**, 1211–1217.
- Lipner, H. (1971) Ovulation from histamine depleted ovaries. *Proc. Soc. exp. Biol. Med.* **136**, 111–114.
- Niswender, G.D., Reimers, T.J., Diekman, M.A. & Nett, T.M. (1976) Blood flow: a mediator of ovarian function. *Biol. Reprod.* **14**, 64–81.
- Papkoff, H. (1973) The chemistry of interstitial cell-stimulating hormone of ovine pituitary origin. In *Hormonal Proteins and Peptides*, Vol. 1, pp. 59–98. Ed. C. H. Li. Academic Press, New York.
- Piacsek, B.E. & Huth, J.F. (1971) Changes in ovarian venous blood flow following cannulation: effect of luteinizing hormone (LH) and antihistamine. *Proc. Soc. exp. Biol. Med.* **138**, 1022–1024.
- Ramachandran, J. & Sairam, M.R. (1975) The effect of interstitial cell-stimulating hormone, its subunits and recombinations on isolated rat Leydig cells. *Arch. Biochem. Biophys.* **67**, 294–300.
- Richards, J.S. (1980) Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular differentiation. *Physiol. Rev.* **60**, 51–89.
- Roser, J.F., Papkoff, H., Murthy, H.M.S., Chang, Y.S., Chloupek, R.C. & Potes, J.A.C. (1984) Chemical, biological and immunological properties of pituitary gonadotropins from the donkey (*Equis asinus*): comparison with the horse (*Equis caballus*). *Biol. Reprod.* **30**, 1253–1262.
- Szego, C.M. & Gitin, E.S. (1964) Ovarian histamine depletion during acute hyperaemic response to luteinizing hormone. *Nature, Lond.* **201**, 682–684.
- Szego, C.M. & Sloan, S.H. (1961) The influence of histamine and serotonin in promoting uterine growth in the rat. *Gen. comp. Endocr.* **1**, 295–305.
- Taylor, K.M. & Snyder, S.H. (1972) Isotopic microassay of histamine, histidine, histidine decarboxylase and histamine methyltransferase in brain tissue. *J. Neurochem.* **19**, 1343–1358.
- Taylor, K.M., Krilis, S. & Baldo, B.A. (1980) An enzymatic-isotopic microassay for measuring allergic release of histamine from blood and mast cells in vitro. *Int. Archs. Allergy appl. Immun.* **61**, 19–27.
- Varga, B. & Greenwald, G.S. (1979) Cyclic changes in utero-ovarian blood flow and ovarian hormone secretion in the hamster: effects of adrenocorticotropin, luteinizing hormone, and follicle stimulating hormone. *Endocrinology* **104**, 1525–1531.
- Wagner, H.N., Rhodes, B.A., Sasaki, Y. & Ryan, J.P. (1969) Studies on the circulation with radioactive microspheres. *Invest. Radiol.* **4**, 374–386.
- Wallach, E.E., Wright, K.H. & Hamada, Y. (1978) Investigation of mammalian ovulation with an in vitro perfused rabbit ovary preparation. *Am. J. Obstet. Gynecol.* **132**, 728–738.
- Wurtman, R.J. (1964) An effect of luteinizing hormone on the fractional perfusion of the rat ovary. *Endocrinology* **75**, 927–933.

Received 11 January 1985