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THE EFFECTS OF TREATMENT WITH GONADOTROPHINS OR WITH OESTROGEN ON THE THYROID GLAND OF THE IMMATURE RAT

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SUMMARY

The uptake of ^{131}I by the thyroid gland increased 72 hr. after the injection of pregnant mare serum gonadotrophin (PMS) into immature female rats whether ovulation occurred or not. PMS failed to produce this effect in male rats but oestrogen administration increased ^{131}I uptake in both male and female immature rats, suggesting that oestrogen was responsible for the effect of PMS in females. Both PMS and oestrogen may increase the uptake of radioactive phosphate by the thyroid of female but not male rats; oestrogen may stimulate thyroid-stimulating hormone (TSH) secretion in immature female rats. These effects of oestrogen in the female made it impossible to determine whether the 'ovulatory surge' in luteinizing hormone secretion in PMS-treated rats was associated with an increased secretion of TSH or not.

INTRODUCTION

The functional activity of the gonads of the immature rat can be stimulated before the normal time of puberty by the administration of exogenous gonadotrophin. One response that has been extensively studied is ovulation after the administration of a single injection of pregnant mare serum gonadotrophin (PMS), and it has been established that this response involves the release of luteinizing hormone (LH) from the animal's own pituitary (McCormack & Meyer, 1962; Zarrow & Quinn, 1963). This 'ovulatory surge' of LH secretion in the immature rat appears to be very similar to the period of LH release that occurs at pro-oestrus in the adult rat; it can be blocked by barbiturates and other pharmacological agents (Quinn & Zarrow, 1964; Zarrow & Brown-Grant, 1964), is absent in rats injected with testosterone during the neo-natal period (Brown-Grant, Quinn & Zarrow, 1964), is facilitated by progesterone administration and this facilitation is blocked by barbiturates (McCormack & Meyer, 1963) and the time of LH discharge is related to the environmental lighting conditions (Strauss & Meyer, 1962; Wagner & Brown-Grant, 1965). It has been suggested that the increase in thyroid-gland activity at the oestrous stage of the ovarian cycle in adult rats is related to a period of increased thyrotrophin secretion which is associated in some way with the 'ovulatory surge' of LH secretion (Brown-Grant, 1962,

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1963, 1965) and the possibility of a similar association in the immature rat has been investigated. In the event, it has not been possible to settle this question because of the complex effects of oestrogen produced by the stimulated ovary on the thyroid gland of these animals.

MATERIAL AND METHODS

The animals were immature rats from the closed Wistar colony maintained here; conditions of housing, diet, lighting and methods were the same as in earlier studies (Zarrow & Brown-Grant, 1964; Wagner & Brown-Grant, 1965). Animals raised in litters of eight or nine rats (large litters) or six to seven rats (small litters) were used but in any one experiment only rats from small or large litters were included. Despite attempts to ensure uniformity, considerable litter-to-litter variation was encountered which may account for the differences in the control values for ^{131}I uptake from experiment to experiment (Tables 1–4). Accordingly experiments were designed, as in an earlier study (Wagner & Brown-Grant, 1965), so that animals from different litters were equally distributed between different treatment groups.

Follicular growth was produced by a single s.c. injection of PMS (serum gonadotrophin, B.P., Gestyl, Organon Laboratories Ltd.) in 0·1 ml. water. The ages given are ages in days at the time of the initial injection. The optimal dose and age for animals from large litters was 45 i.u. at 30 days (Zarrow & Brown-Grant, 1964) and 30 i.u. at 27 days for animals from small litters. Usually most of the animals treated in this way ovulated spontaneously; fresh tubal ova were found when they were killed 72–76 hr. later (all times are given in hr. after the initial injection at 09.00–10.00 hr.). Younger animals do not ovulate spontaneously but ovulation can be induced by the injection of human chorionic gonadotrophin (HCG) (chorionic gonadotrophin, B.P., Pregnyl, Organon Laboratories Ltd.) 56 hr. after the injection of PMS. The dose used was 20 i.u. in 0·1 ml. water injected s.c. Animals from large litters received 60 i.u. PMS at 26 days of age followed by HCG, and animals from small litters 45 i.u. PMS at 23 days of age followed by HCG. The oestrogen preparation used was the cyclopentylpropionate ester of oestradiol-17β in cottonseed oil (Depo-estradiol, Upjohn Ltd., Kalamazoo, Michigan). This was diluted with sterile ethyl oleate so that 25 μg. were contained in 0·05 ml. of oil and the required dose was injected i.m. Androgen-treated rats received 1·25 mg. testosterone propionate in 0·05 ml. of oil s.c. on day 4 of post-natal life. Details of the procedure and of the effects on the thyroid have been published previously (Brown-Grant, 1965).

Animals were killed with chloroform 72–76 hr. after the first injection of PMS or oestrogen unless otherwise stated. Under the conditions of these experiments the 'ovulatory surge' of LH secretion occurs about 54 hr. after PMS injection (Wagner & Brown-Grant, 1965). The oviducts were removed and examined for eggs which were counted if present; the ovaries and uterus or testes and seminal vesicles plus coagulating gland were weighed. Thyroid-gland activity was assessed by measuring the uptake of ^{131}I by the thyroid gland 2·5 hr. after the i.m. injection of 0·5 μc of carrier-free $Na^{131}I$. Counts were made in a well-type scintillation counter to a statistical accuracy of $\pm 1\%$ or better and expressed as a percentage of the injected dose. The uptake of ^{32}P by the thyroid 4 hr. after the i.m. injection of 8 or 20 μc of ^{32}P as sodium orthophosphate was determined in some experiments. The gland was removed, dis-

solved in 2N-NaOH at 60° and counted in an M6H Geiger–Müller counter to a statistical accuracy of ±5% or better; the results were expressed as counts/200 sec./gland. Further details of these methods are given by Brown-Grant (1962).

When *t*-tests were carried out, $P < 0{\cdot}05$ was taken as the limit of significance. Values given in the text and tables are group means ± S.E.

RESULTS

Effects of treatment with gonadotrophins on ^{131}I uptake

A group of 20 female rats received 45 i.u. PMS at 30 days of age, a treatment expected to produce spontaneous ovulation in the majority of these animals. Uptake of ^{131}I by the thyroid was greater than in a group of eight control animals measured 72 hr. later: 10·45 ± 0·66% as compared with 6·36 ± 0·54% ($P < 0{\cdot}01$). A group of seven 26-day-old rats received 60 i.u. PMS; none of these animals had ovulated when killed 72 hr. later; seven other rats received 60 i.u. PMS plus 20 i.u. HCG and all had ovulated (mean egg count 37·9 eggs/rat). Uptake of ^{131}I was slightly but not significantly increased in both groups compared with eight control animals (Table 2).

Table 1. *The effect of PMS and oestrogen (OCP) on ^{131}I uptake by the thyroid gland and on organ weights (mg./100 g. body weight) in female rats.* (*Means* ± S.E.)

Age and treatment	No. of rats	Uptake (% dose)	Thyroid	Ovary	Uterus
30 days					
Controls	8	6·36 ± 0·54	10·2 ± 0·2	23·3 ± 0·9	67 ± 7
45 i.u. PMS, ovulated	8	10·26 ± 0·97**	11·7 ± 0·3	58·1 ± 4·5**	171 ± 13**
45 i.u. PMS, not ovulated	12	10·54 ± 0·91**	11·7 ± 0·3	62·2 ± 5·9**	192 ± 14**
27 days					
Controls	10	5·69 ± 0·36	9·7 ± 0·6	24·6 ± 0·6	55 ± 5
30 i.u. PMS, ovulated	12	7·84 ± 0·52**	10·8 ± 0·6	155·2 ± 14·9**	152 ± 5**
30 i.u. PMS, not ovulated	8	7·22 ± 0·46*	11·2 ± 0·4	129·8 ± 6·6**	213 ± 15**
15 μg. OCP	10	8·06 ± 0·59**	10·8 ± 0·5	30·4 ± 1·6**	196 ± 9**
Androgen-treated controls	10	4·38 ± 0·36	9·4 ± 0·3	18·0 ± 0·9	75 ± 19
Androgen-treated + 15 μg. OCP	11	6·73 ± 0·70*	10·0 ± 0·3	16·1 ± 0·8	157 ± 6**

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

These results suggested that a raised level of thyroid activity 72 hr. after the injection of PMS might be related to an 'ovulatory surge' of LH secretion the evening before. However, more detailed analysis of the results and additional experiments showed that this was improbable. First, analysis of the results obtained with 45 i.u. PMS in 30-day-old females showed that the increase in ^{131}I uptake was no higher in the eight injected rats that ovulated (mean egg count 21·2 eggs/rat) than in the 12 rats that failed to ovulate (Table 1). The experiment was repeated in 27-day-old females injected with 30 i.u. PMS. Again uptake was higher than in the controls in 12 rats that ovulated (mean egg count 23·7) but not significantly different from that of eight rats that failed to ovulate (Table 1). Secondly, the experiment on younger animals that consistently fail to ovulate spontaneously after PMS alone was repeated

on 23-day-old rats injected with 45 i.u. PMS. This time the increase in uptake was significant (Table 2) both in rats treated with PMS alone (none ovulated) and in rats treated with PMS plus HCG, which all ovulated (egg count 38·2). Finally, six androgen-treated females were injected with 45 i.u. PMS at 30 days of age; as expected from earlier studies (Brown-Grant *et al.* 1964) all these rats failed to ovulate. When killed 72 hr. after PMS there was evidence of ovarian growth (54·5 ± 5·2 mg./100 g., as compared with 14·1 ± 2·7 in six androgen-treated controls) and of oestrogen secretion (uterus weight 157 ± 9 mg./100 g. as compared with 42 ± 5) and, despite the absence of an 'ovulatory surge' of LH, the ^{131}I uptake of the thyroid was 8·49 ± 0·45% compared with 5·74 ± 0·29% in the controls ($P < 0·01$).

Table 2. *The effect of PMS or PMS plus* 20 *i.u. HCG* 56 *hr. later or oestrogen* (*OCP*) *on* ^{131}I *uptake by the thyroid gland and on organ weights* (*mg./*100 *g. body weight*) *in female rats.* (*Means* ± S.E.)

Age and treatment	No. of rats	Uptake (% dose)	Thyroid	Ovary	Uterus
26 days					
Controls	8	6·98 ± 1·04	11·7 ± 0·3	32·3 ± 2·4	56 ± 14
60 i.u. PMS	7	7·82 ± 2·10	12·4 ± 0·9	187·7 ± 23·1**	235 ± 31**
60 i.u. PMS + HCG	7	7·83 + 0·78	11·8 ± 1·0	240·9 ± 19·5**	213 ± 6**
23 days					
Controls	12	5·95 ± 0·38	11·4 ± 0·2	32·8 ± 1·2	50 ± 3
45 i.u. PMS	11	7·99 ± 0·82*	12·8 ± 0·4**	226·3 ± 14·0**	219 ± 8
45 i.u. PMS + HCG	11	7·73 ± 0·58*	13·1 ± 0·5**	259·3 ± 16·3**	190 ± 6
10 μg. OCP	11	7·25 ± 0·43*	12·2 ± 0·4	37·5 ± 2·1	222 ± 8

* $P < 0·05$. **$P < 0·01$.

Table 3. *The effect of PMS or PMS plus* 20 *i.u. HCG* 56 *hr. later or oestrogen* (*OCP*) *on the uptake of* ^{131}I *by the thyroid gland of male rats and on organ weights* (*mg./*100 *g. body weight*). (*Means* ± S.E.)

Age and treatment	No. of rats	Uptake (% dose)	Thyroid	Testis	Seminal vesicles
30 days					
Controls	7	5·71 ± 0·46	10·3 ± 0·2	911 ± 49	17·9 ± 1·3
45 i.u. PMS	7	6·16 ± 0·46	9·5 ± 0·4	1079 ± 39*	49·4 ± 2·8**
24 days					
Controls	8	5·12 ± 0·85	12·7 ± 0·5	545 ± 26	14·1 ± 0·8
60 i.u. PMS	12	5·89 ± 1·01	12·4 ± 0·6	860 ± 24**	44·5 ± 3·0**
60 i.u. PMS + HCG	7	6·08 ± 0·50	11·0 ± 0·3	948 ± 30**	46·4 ± 2·9**
24 days					
Controls	14	5·10 ± 0·32	10·3 ± 0·5	751 ± 40	17·7 ± 1·2
45 i.u. PMS + HCG	14	5·32 ± 0·39	9·7 ± 0·3	991 ± 41**	51·9 ± 2·4**
12·5 μg. OCP	15	7·32 ± 0·53**	11·1 ± 0·3	553 ± 23**	29·3 ± 1·3**

* $P < 0·05$. ** $P < 0·01$.

An increase in ^{131}I uptake was observed in female rats injected with PMS whether or not any evidence of endogenous LH release was obtained. The possibility that this rise was the direct consequence of PMS administration was tested by injecting immature male rats with similar amounts of PMS or PMS plus HCG. No effects on ^{131}I uptake were detected although stimulation by the gonadotrophins was shown in

the increase in the weight of the testis and accessory organs (Table 3). In all experiments on female rats the secretion of oestrogen was stimulated as shown by the increase in the weight of the uterus (Tables 1 and 2). The absence of this factor in the experiments on male rats could account for the differences observed. The effects of oestrogen administration on ^{131}I uptake were therefore determined.

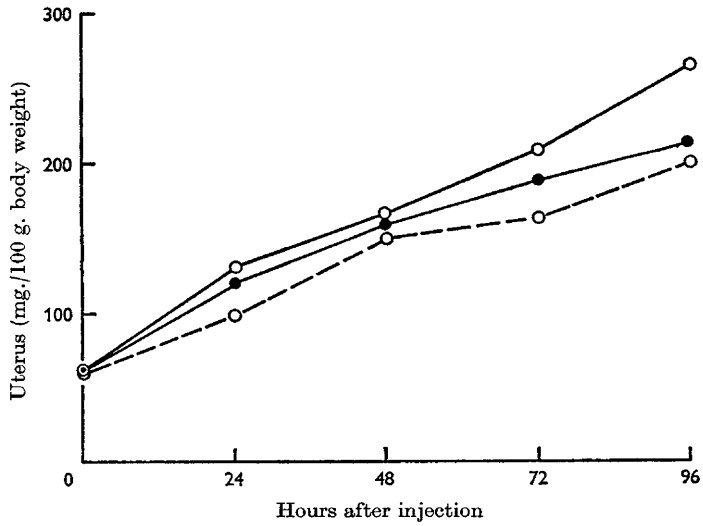

Fig. 1. Growth of the uterus in immature rats 30 days of age at the time of injection of 12·5 μg. (O- - -O) or 25 μg. (O—O) of oestradiol cyclopentylpropionate or 45 i.u. of PMS (●—●). Each point is the mean of values obtained from four to eight rats.

Effects of oestrogen administration on ^{131}I uptake

The first problem was to determine the dose of oestrogen that would produce uterine growth comparable to that seen after PMS injection; it was thought that a slowly absorbed preparation was most likely to be suitable and oestradiol cyclopentylpropionate (OCP) was tested. Female rats were injected with 45 i.u. PMS or 12·5 or 25 μg. OCP when 30 days of age and killed 24, 48, 72 or 96 hr. later and the uterus was weighed (mg./100 g. body weight). Values for animals 72 and 96 hr. after PMS injection were obtained only from animals that had not ovulated. The results are shown in Fig. 1; 25 μg. produced a somewhat greater and 12·5 μg. a rather smaller increase in uterine weight than PMS; the time-course of uterine growth was similar in all three cases. A dose of 15 μg. OCP was tested in five 30-day-old rats; the mean uterus weight was 183 ± 7 when they were killed 72 hr. later as compared with a value of 185 ± 10 in seven rats treated with 45 i.u. PMS that had failed to ovulate. The ^{131}I uptake of the oestrogen-treated rats was $6{\cdot}50 \pm 0{\cdot}84$, significantly higher than the value for five uninjected controls ($3{\cdot}65 \pm 0{\cdot}37$, $P < 0{\cdot}05$). The results of other experiments in which the effects of OCP on ^{131}I uptake 72 hr. later were determined are given in Tables 1, 2 and 3. A dose of 15 μg. in 27-day-old females, 10 μg. in 23-day-old females and 12·5 μg. in 24-day-old males (the body weights of the male rats were between those of the two groups of females) produced significant increases in ^{131}I uptake. Androgen-treated females also showed an increase in uptake after the injection of oestrogen (Table 1). The uterine weights in the females were

close to those of PMS-treated animals that failed to ovulate. The increase in ovarian weight after the injection of oestrogen in normal but not in androgen-treated females is in contrast to the marked decrease in the weight of the testes in oestrogen-treated males (Tables 3 and 4). The increase in seminal vesicle weight probably represents an effect on the smooth muscle rather than a stimulation of epithelial growth (Freud, 1933) but this was not investigated histologically.

Table 4. *The effect of treatment with PMS or PMS plus* 20 *i.u. HCG or with oestrogen (OCP) on the uptake of* ^{32}P *by the thyroid gland and on organ weights (mg./*100 *g. weight) in male and female rats. (Means* ± S.E.)

Age, sex and treatment	No. of rats	Counts/ 200 sec./ thyroid	Thyroid	Ovary or testis	Uterus or seminal vesicles
27 days, female					
Controls	12	311 ± 25	10·2 ± 0·5	26·7 ± 0·9	61 ± 4
30 i.u. PMS, ovulated	10	411 ± 22**	10·9 ± 0·4	141·0 ± 11·4**	166 ± 8**
30 i.u. PMS, not ovulated	9	392 ± 30	10·9 ± 0·2	114·3 ± 7·3**	181 ± 5**
15 μg. OCP	12	369 ± 20	11·2 ± 0·3	32·1 ± 2·1*	188 ± 5**
23 days, female					
Controls	11	287 ± 13	11·1 ± 0·4	36·1 ± 1·9	55 ± 4
45 i.u. PMS	11	358 ± 12**	13·2 ± 0·5**	234·3 ± 22·2**	211 ± 5*
45 i.u. PMS + HCG	11	389 ± 19**	13·2 ± 0·5**	243·5 ± 13·1**	189 ± 6*
10 μg. OCP	12	362 ± 14**	13·2 ± 0·5**	35·1 ± 1·5	240 ± 6*
24 days, male					
Controls	10	782 ± 44	10·5 ± 0·4	678 ± 31	16·4 ± 0·9
45 i.u. PMS + HCG	8	785 ± 61	10·6 ± 0·6	798 ± 31*	40·0 ± 3·4**
12·5 μg. OCP	8	852 ± 55	11·4 ± 0·3	432 ± 20**	30·1 ± 1·3**

* $P < 0·05$. ** $P < 0·01$.

These results show that oestrogen in amounts similar to those produced in PMS-treated female rats can increase the uptake of ^{131}I by the thyroid. Further evidence that the changes in PMS-treated rats may be due to oestrogen is provided by the finding that ^{131}I uptake was increased 48 hr. after the injection of 45 i.u. of PMS in 30-day-old rats; 14 controls gave a value of 3·43 ± 0·28, 14 PMS-treated rats 4·86 ± 0·32 ($P < 0·01$). At this time no surge of LH release has occurred but oestrogen secretion has begun, as shown by the increase in uterine weight (Fig. 1).

Effects of gonadotrophin and oestrogen on ^{32}P *uptake*

The reasoning behind these experiments was that thyroid-stimulating hormone (TSH) is known to increase the uptake of ^{32}P by the thyroid as well as ^{131}I uptake (see Discussion). If either PMS or oestrogen increased ^{32}P uptake this would be indirect evidence that a release of TSH was involved. The results obtained are shown in Table 4. In 27-day-old female rats only those that ovulated after PMS injection showed a significant increase; oestrogen or PMS or PMS plus HCG produced an increase in 23-day-old females but had no effects on ^{32}P uptake in 24-day-old males.

DISCUSSION

It has not proved possible to relate the increase in ^{131}I uptake by the thyroid gland in PMS-treated prepubertal female rats to the occurrence of an 'ovulatory surge' of LH secretion. In older rats the increase was similar whether the animals ovulated or not and comparable increases were found in younger rats and in androgen-treated rats which do not appear to release LH spontaneously. Similar doses of PMS or PMS plus HCG had no effect in immature male rats, suggesting that the effects in the female might be due to the secretion of oestrogen. Injection of oestrogen at a dose level that produced growth of the uterus similar to that seen in PMS-treated females resulted in an increase in ^{131}I uptake in both male and female rats.

Uptake of ^{131}I by the thyroid gland is a convenient index of the level of thyroid-gland activity but there are other available methods. The thyroid:serum concentration ratio for radioactive iodide could be determined but would be of little use since there is good evidence that oestrogen produces a rise in this ratio in the immature female rat, probably by a direct action on the thyroid (Boccabella & Alger, 1964). The measurement of the rate of release of ^{131}I-labelled hormone from the gland *in vivo* has been found to be technically very difficult in these small animals and the results were not reliable (Brown-Grant, unpublished). The uptake of ^{32}P by the thyroid might provide a means of distinguishing between the effects of oestrogen and the effects of an increase in TSH secretion; it has been shown that TSH will increase the uptake of both ^{131}I and ^{32}P by the thyroid of the rat but in adult castrated males and spayed female rats oestrogen has no effect on ^{32}P uptake although it increases both ^{131}I uptake and the thyroid:serum concentration ratio for iodide (Brown-Grant, 1962; Boccabella & Alger, 1964). The results in 27-day-old females shown in Table 4 suggest that an increase in TSH secretion may occur in association with the 'ovulatory surge' of LH; ^{32}P uptake was significantly increased as compared with controls in PMS-treated rats that ovulated spontaneously but not in those that did not ovulate or that received oestrogen. However, some increase above control levels did occur in these groups also and the mean value for rats that ovulated was not significantly different from that for rats which did not. Furthermore, in younger female rats which do not show evidence of an 'ovulatory surge' of LH release, PMS or PMS plus HCG or oestrogen increased ^{32}P uptake significantly. Oestrogen may act directly on the thyroid gland of the immature rat, to increase both ^{131}I and ^{32}P uptake in contrast to the situation in the adult castrated rat. Against this view is the finding that oestrogen does not increase the uptake of ^{32}P by the thyroid of the immature male although it increases the uptake of ^{131}I. Alternatively, it could be argued that oestrogen has a direct effect on the thyroid gland to increase ^{131}I uptake in both immature males and females as it has in the adult castrated rat but that in addition it may cause an increased secretion of TSH in the prepubertal female rat but not in the male. There appears also to be some evidence for a difference in the effect of oestrogen on the pituitary of immature males and females with regard to gonadotrophin secretion in that testis weight was uniformly decreased in oestrogen-treated males but ovarian weight was often increased by oestrogen treatment in normal though not in androgen-treated females.

It has not been possible to determine whether an increase in TSH secretion occurs

in association with the release of LH that leads to ovulation in PMS-treated immature female rats. It has been shown that sufficient oestrogen is produced to increase the uptake of ^{131}I by the thyroid and it is possible that in the female, but not in the male, rat this effect of oestrogen may involve an increase in the secretion of TSH.

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REFERENCES

Boccabella, A. V. & Alger, E. A. (1964). Influence of estradiol on thyroid-serum radio-iodide concentration ratios of gonadectomized and hypophysectomized rats. *Endocrinology*, **74**, 680–688.

Brown-Grant, K. (1962). Changes in thyroid gland activity during the oestrus cycle in rats. *J. Physiol., Lond.*, **161**, 557–574.

Brown-Grant, K. (1963). Inhibition of ovulation and thyroid gland activation in the rat by Nembutal. *J. Endocrin.* **26**, 299–300.

Brown-Grant, K. (1965). The effect of testosterone during the neonatal period on the thyroid gland of male and female rats. *J. Physiol., Lond.*, **176**, 91–104.

Brown-Grant, K., Quinn, D. L. & Zarrow, M. X. (1964). Superovulation in the androgen-treated immature rat. *Endocrinology*, **74**, 811–813.

Freud, J. (1933). Conditions of hypertrophy of the seminal vesicles of rats. *Biochem. J.* **27**, 1438–1450.

McCormack, C. E. & Meyer, R. K. (1962). Ovulating hormone release in gonadotrophin treated immature rats. *Proc. Soc. exp. Biol. Med.* **110**, 343–346.

McCormack, C. E. & Meyer, R. K. (1963). Ovulation induced by progesterone in immature rats pretreated with pregnant mare serum gonadotrophin. *Gen. comp. Endocr.* **3**, 300–307.

Quinn, D. L. & Zarrow, M. X. (1964). Inhibition of pregnant mare's serum-induced ovulation in the immature rat. *Endocrinology*, **74**, 309–313.

Strauss, W. F. & Meyer, R. K. (1962). Neural timing of ovulation in immature rats treated with gonadotrophin. *Science, N.Y.*, **137**, 860–861.

Wagner, J. W. & Brown-Grant, K. (1965). Studies on the time of luteinizing hormone release in gonadotrophin-treated immature rats. *Endocrinology*, **76**, 958–965.

Zarrow, M. X. & Brown-Grant, K. (1964). Inhibition of ovulation in the gonadotrophin-treated immature rat by chlorpromazine. *J. Endocrin.* **30**, 87–95.

Zarrow, M. X. & Quinn, D. L. (1963). Superovulation in the immature rat following treatment with PMS alone and inhibition of PMS-induced ovulation. *J. Endocrin.* **26**, 181–188.