PHOSPHOMONOESTERASES AND HISTAMINE IN THE UTERUS OF THE EWE DURING EARLY PREGNANCY

By R. N. MURDOCH*

[Manuscript received 12 July 1972]

Abstract

An attempt was made to assess whether histamine is involved in the regulation of sheep uterine endometrial acid and alkaline phosphatases during early pregnancy. Interacting effects of progesterone and oestradiol on the endometrial enzymes and the behaviour of the phosphatases in the caruncles during early pregnancy were also examined.

Histamine occurred in greater concentration in the intercotyledonary endometrium than in the caruncles during early pregnancy and increased in both tissues between days 14 and 22. The concentration of the monoamine fell on day 31 of pregnancy and only low levels in the tissues were recorded on day 44. The subcutaneous administration of the potent histamine-releasing agent, compound 48/80, or the injection of histamine directly into the uterine lumen of ovariectomized ewes treated with combinations of progesterone and oestradiol benzoate, did not significantly alter the phosphatase activity of the endometrial tissue.

Progesterone stimulated the acid and alkaline phosphatases in the intercotyledonary endometrium and maintained their activities at high levels for 21 days when administered at a dose rate of 10 mg/day. Oestradiol benzoate slightly decreased endometrial phosphatase activities when injected into ovariectomized ewes and limited the response of the enzymes to progesterone.

Alkaline phosphatase activity in the caruncles increased between days 0 and 8 of pregnancy and fell on day 14. Only low levels of activity were found in the caruncles on days 22, 31, and 44 of pregnancy. Acid phosphatase maintained a constant level of activity in the caruncles throughout early pregnancy and was not influenced by the increase in histamine concentration between days 14 and 22.

The results suggest the participation of factors other than histamine in the regulation of endometrial phosphatases during early pregnancy in the ewe. Progesterone and oestradiol may be involved to some extent but are not solely responsible for the control of the enzymes.

I. INTRODUCTION

Acid and alkaline phosphatases (orthophosphoric monoester phosphohydrolase, E.C.3.1.3.1 and E.C.3.1.3.2) in the intercaruncular areas of the uterine endometrium of the ewe are sensitive to changes in the production of progesterone by the corpus luteum and increase in activity between days 0 and 8 of the oestrous cycle and pregnancy (Murdoch and White 1968*a*; Murdoch 1970*a*). Although the corpus luteum remains fully functional and continues its production of progesterone following day 8 of pregnancy (Basset *et al.* 1969; Obst and Seamark 1970; Bindon 1971), acid and alkaline phosphatase activities rapidly decrease after this stage and on day 14 post mating reach similar levels to those recorded at oestrus. Alkaline phosphatase activity in the intercotyledonary endometrium continues to fall during the remaining

* Department of Veterinary Physiology, University of Sydney, Sydney, N.S.W. 2006.

period in which implantation of the conceptus is being established but acid phosphatase again increases its activity and remains high between days 22 and 31 of pregnancy (Murdoch 1970*a*). Since no significant change in plasma progesterone occurs in ewes between days 20 and 34 of pregnancy from the levels observed during the luteal phase of the oestrous cycle (Bassett *et al.* 1969; Obst and Seamark 1970), factors other than this steroid must also be involved in the regulation of the endometrial phosphomonoesterases during implantation.

Boshier (1969) has suggested a role for the increased acid phosphatase activity during implantation in the destruction or modification of the uterine surface epithelium. Numerous studies have implicated acid phosphatase activity with lysosomal activity (see Gahan 1967) and it is now a widely held belief that the release of lysosomal enzymes results in either the death or modification of the cell (De Duve 1963; Gahan 1967). Since histamine is generally liberated in localized areas of inflammation or at sites of tissue damage, such as may be the case in the uterine epithelium during implantation, the possibility that this monoamine may be at least one factor involved in the regulation of the endometrial phosphatases has been considered in the present study.

The studies described by Murdoch (1970*a*) did not include observations on the phosphatase activity of the maternal caruncles. Since changes in the histamine concentration of this tissue during early pregnancy are reported in the present work, the behaviour patterns of the caruncular enzymes have been investigated. Interacting effects of progesterone and oestradiol on the endometrial enzymes have also been examined.

II. Materials and Methods

(*a*) *Experimental Animals*

Adult Merino ewes were mated with fertile rams and slaughtered by cutting the throat and dislocating the cervical vertebrae on the day of mating (day 0) and on days 8, 14, 22, 31, and 44 of pregnancy (see Murdoch 1970*a*).

Some of the ewes were ovariectomized and, after 30 days, randomized into eight equal groups and treated according to the following schedule. All groups were injected with 30 μg of oestradiol benzoate on day 1 and killed on day 6.

Group 1: Controls; no further treatment received.

Group 2: 50 mg of histamine-liberating agent, compound 48/80 (*N*-methy-*p*-methoxyphenethylamine) (Sigma Chemical Company, St. Louis, Missouri), on days 3, 4, and 5.

Group 3: 10 mg of progesterone on days 3, 4, and 5.

Group 4: 10 mg of progesterone and 50 mg of compound 48/80 on days 3, 4, and 5.

Group 5: 30 μg of oestradiol benzoate on days 3, 4, and 5.

Group 6: 30 μg of oestradiol benzoate and 50 mg of compound 48/80 on days 3, 4, and 5.

Group 7: 10 mg of progesterone and 30 μg of oestradiol benzoate on days 3, 4, and 5.

Group 8: 10 mg of progesterone, 30μg of oestradiol benzoate, and 50 mg of compound 48/80 on days 3, 4, and 5.

The steroids were administered by intramuscular injection in 1·0 ml of peanut oil. Compound 48/80 was administered by subcutaneous injection in 0·154M NaCl. The histamine releaser at the dose rate employed did not cause the animals any apparent stress and, following injection, the ewes continued to feed normally, were alert, and attempted to avoid capture when subsequent

injections were to be administered. Dosages of compound 48/80 above 50 mg, however, proved to be toxic.

Another group of 16 ovariectomized ewes were injected with 30 μg of oestradiol benzoate on day 1 and then treated with 10 mg of progesterone and 30 μg of oestradiol benzoate on days 3, 4, and 5. The ewes were laparotomized on day 4 and histamine (free base, Sigma Chemical Company, St. Louis, Missouri) was injected in doses of 0·0, 0·1, 0·3, and 0·9 mg through a 26-gauge needle into the lumen of the uterus near the uterotubal junction. Individual dosages of histamine were dissolved in 0·6 ml of sterile 0·154M NaCl and 0·3-ml aliquots were administered into each uterine horn. The ewes were killed on day 6.

(*b*) *Preparation of Tissues*

Following slaughter the uteri were removed, placed in crushed ice, and quickly taken to the laboratory where the uterine horns were immediately dissected free of fatty and connective tissue and of the attached oviducts and cervix. All subsequent processing of the tissue was performed at 4°C. The uteri from ovariectomized ewes and from ewes on days 0, 8, and 14 of pregnancy were washed through with 10 ml of 0·154M NaCl to remove any contaminating endometrial secretion and to recover blastocysts. The uteri from ewes at more advanced stages of pregnancy were dissected and, after removal of the foetal fluids, the foetus and its supporting membranes were carefully separated from the maternal tissues. The endometrium was then gently rinsed with 0·154M NaCl to remove any contaminating cells or fluid. Each uterine horn was placed on a piece of filter paper and opened down the mesometrial side. The exposed endometrium was blotted with filter paper, and caruncular and endometrial tissue from the intercotyledonary areas were carefully dissected using fine scissors and forceps. Samples of tissue were homogenized in either 5 parts of 0·6N perchloric acid for histamine analyses or 10 parts of distilled water for phosphatase and protein analyses using a Potter–Elvehjem homogenizer. The homogenates were centrifuged for 10 min at 1000 *g* and the supernatants retained for analysis.

(*c*) *Analytical Methods*

The histamine concentration in the caruncular and intercotyledonary endometrial tissue was determined by the fluorimetric method of Shore, Burkhalter, and Cohn (1959) as modified by Burkhalter (1962).

Acid and alkaline phosphatase activities were determined by using *p*-nitrophenylphosphate as substrate (Bessey, Lowry, and Brock 1946; Andersch and Szcypinski 1947). One phosphatase unit is defined as being the amount of enzyme which liberates 1 mmole of *p*-nitrophenol at 37°C when contained in 1000 ml of sample.

The protein concentration in the samples was determined by the biuret method (Wales, Scott, and White 1961).

(*d*) *Statistical Analyses*

The significance of the results has been assessed by analysis of variance. All main effects and their first-order interactions were isolated and tested for significance using the within-group error mean square to calculate variance ratios.

III. Results

(*a*) *Histamine in the Uterus during Early Pregnancy*

Figure 1 shows the concentration of histamine in the maternal caruncles and intercotyledonary endometrium of ewes at 0, 8, 14, 22, 31, and 44 days of pregnancy.

Histamine occurred in significantly greater ($P < 0·01$) concentration in the intercotyledonary endometrium than in the caruncles during early pregnancy and increased in both tissues between days 14 and 22. The concentration of the monoamine fell on day 31 of pregnancy and only low levels in the tissues were recorded on day 44.

No statistically significant interactions between stages of pregnancy and uterine tissue were detected when the data were subjected to analysis of variance.

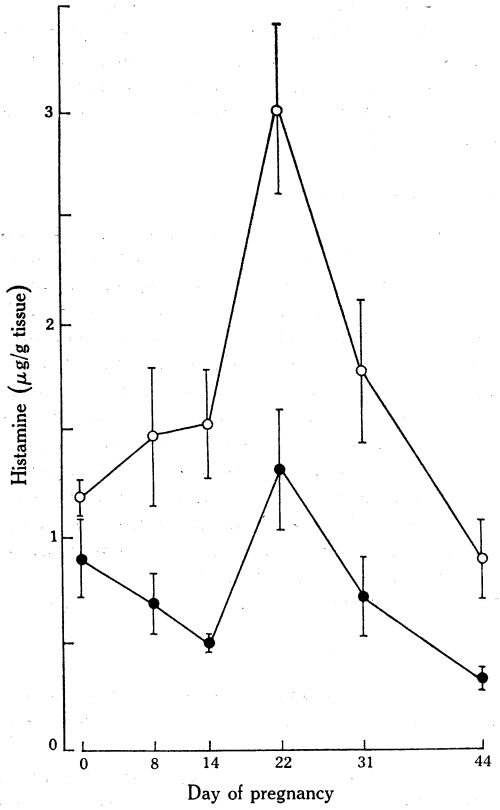

Fig. 1.—Concentration of histamine in the maternal caruncles and intercotyledonary endometrium of ewes during early pregnancy. ● Caruncles. ○ Intercotyledonary endometrium. Values represent the means ± S.E. for four ewes.

(*b*) *Effect of Compound 48/80, Progesterone, and Oestradiol on Endometrial Phosphatases*

The results of an experiment designed to examine the effects of injecting the histamine-releasing agent compound 48/80 on intercotyledonary endometrial acid and alkaline phosphatase activities in ovariectomized ewes treated with combinations of progesterone and oestradiol are shown in Figure 2.

Although the concentration of histamine in the endometrium was reduced by about 73% within 3 hr after the last injection of compound 48/80, no significant effect of the treatment on the activities of the endometrial enzymes was detected. Progesterone significantly ($P < 0{\cdot}01$) stimulated acid and alkaline phosphatase activities in the endometrial tissue with the latter enzyme being more responsive to the steroid than the former. Oestrogen significantly ($P < 0{\cdot}01$) decreased the phosphatase activities when injected into untreated ovariectomized ewes and limited the response of the enzymes to progesterone. The extent of the depressing effect of oestrogen on alkaline phosphatase activity was significantly greater ($P < 0{\cdot}01$) when the enzyme had been simultaneously stimulated by progesterone than when progesterone treatment had not been administered. No other significant interactions were apparent when the data were subjected to analysis of variance.

(c) Effect of an Intra-uterine Injection of Histamine on Endometrial Phosphatases

Intra-uterine injections of 0·1, 0·3, and 0·9 mg of histamine into ovariectomized ewes treated with progesterone and oestradiol benzoate had no significant effect on acid and alkaline phosphatase activities in the caruncles or intercotyledonary endometrium (Table 1). Phosphatase activities were significantly greater ($P < 0·01$) in the intercotyledonary endometrium than in the caruncles and differences between the tissues were most apparent with alkaline phosphatase. Alkaline phosphatase activity exceeded acid phosphatase activity in both tissues.

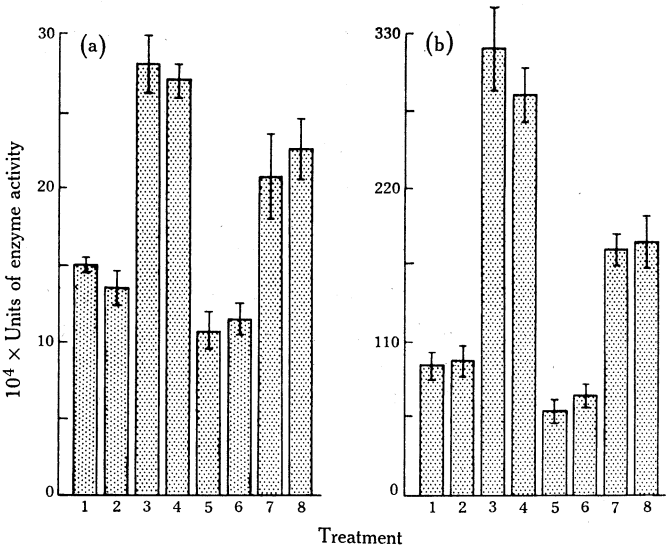

Fig. 2.—Effect of injections of compound 48/80, progesterone, and oestradiol on acid (*a*) and alkaline (*b*) phosphatase activities per milligram of tissue protein in the intercotyledonary endometrium of oviariectomized ewes. Values represent the means ± S.E. for five ewes. Treatments: 1, controls; 2, compound 48/80; 3, progesterone; 4, progesterone + compound 48/80; 5, oestradiol; 6, oestradiol + compound 48/80; 7, progesterone + oestradiol; 8, progesterone + oestradiol + compound 48/80.

(d) Effect of Prolonged Treatment with Progesterone on Endometrial Phosphatases

The rapid fall in acid and alkaline phosphatase activities in the endometrium of the ewe between days 8 and 14 of pregnancy, as described by Murdoch (1970*a*), occurs despite the continuous production of progesterone by the corpus luteum during this time. In order to assess whether the enzymes in the endometrium become refractory to the effects of progesterone, three groups of three ovariectomized ewes were treated with 10 mg of progesterone per day for 0, 3, or 21 days and killed 24 hr after the last injection. Acid and alkaline phosphatase activities were then assayed in the intercotyledonary endometrium.

Progesterone stimulated the endometrial phosphatases and maintained their activities at high levels for 21 days. Enzyme activities after 21 days of progesterone

treatment did not differ significantly from those after 3 days treatment with the steroid.

TABLE 1

EFFECT OF INTRA-UTERINE INJECTIONS OF HISTAMINE ON ACID AND ALKALINE PHOSPHATASE ACTIVITIES IN THE CARUNCLES AND INTERCOTYLEDONARY ENDOMETRIUM OF OVARIECTOMIZED EWES TREATED WITH PROGESTERONE AND OESTRADIOL BENZOATE

Values represent the means ± S.E. for four ewes and are expressed as $10^4 \times$ units of enzyme activity per milligram of tissue protein

Histamine (mg per uterus)	Acid phosphatase		Alkaline phosphatase	
	Caruncles	Intercotyledonary endometrium	Caruncles	Intercotyledonary endometrium
0·0	13·4±1·2	18·2±1·4	38·8±2·1	109± 8
0·1	14·2±1·0	16·3±0·9	45·5±5·4	119± 5
0·3	13·3±0·6	16·3±0·8	46·6±5·9	114± 8
0·9	12·9±1·1	15·0±1·2	38·1±5·0	113±10

(e) *Phosphatases in the Caruncles during Early Pregnancy*

This experiment was conducted to assess whether changes in the concentration of histamine in the caruncles during early pregnancy (see Fig. 1) are paralleled by changes in the tissue activity of acid phosphatase. Figure 3 shows the activity of

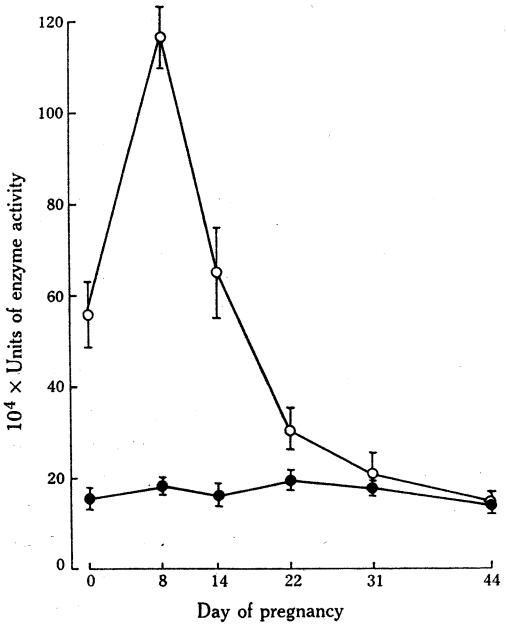

Fig. 3.—Acid and alkaline phosphatase activities per milligram of tissue protein in the maternal caruncles of the ewe during early pregnancy.
● Acid phosphatase.
○ Alkaline phosphatase.
Values represent the means ± S.E. for four ewes.

acid and alkaline phosphatase per milligram of tissue protein in the caruncular tissue of ewes on days 0, 8, 14, 22, 31, and 44 of pregnancy.

Alkaline phosphatase activity significantly increased ($P < 0{\cdot}01$) between days 0 and 8 of pregnancy but fell to levels similar to those recorded at oestrus on day 14. Activity continued to fall following day 14 of pregnancy and only low levels were found in the caruncles on days 22, 31, and 44. Acid phosphatase activity in the caruncles did not change significantly between the stages of pregnancy studied and occurred in the tissue in lower concentration than alkaline phosphatase, particularly between days 0 and 14.

IV. Discussion

The results of the present investigation demonstrate significant changes in the concentration of histamine in the uterine tissues of the ewe during early pregnancy. The increased concentration of the monoamine in the intercotyledonary endometrium on day 22 of pregnancy does not appear to be involved in processes leading to the stimulation of acid phosphatase activity during this time (see Murdoch 1970*a*), since the administration of the potent histamine-releasing agent, compound 48/80, or the injection of histamine directly into the uterine lumen of hormone-treated, ovariectomized ewes did not significantly alter the phosphatase activity of the endometrial tissue.

The physiological role of histamine formed in the body is largely unknown and controversy exists over its significance in reproductive processes. In the rat it has been proposed that histamine is the mediator of oestrogen action (Spaziani and Szego 1958) and is involved in decidualization and in oestrogen-induced metabolic reactions in the uterus (Shelesnyak 1957, 1960; Marcus, Shelesnyak, and Kraicer 1964; Shelesnyak and Kraicer 1964; Szego and Lawson 1964). This concept has been questioned by Finn and Keen (1962), Cecil, Bitman, and Wrenn (1964), Wrenn *et al.* (1964), Marcus and Shelesnyak (1967), Humphrey and Martin (1968), and Garg and Chaudhury (1971). Histamine, however, does appear to increase oestradiol uptake by the uterus *in vivo* (De Carli *et al.* 1971) while certain antihistamine drugs enhance the antifertility effects of oestrogens administered immediately after coitus (McColl, Robinson, and Sagritalo 1971).

The reason for the increase in the concentration of histamine in the uterine tissues of sheep during implantation is not apparent. In the rat uterus, the concentration of the monoamine decreases rather than increases around the time of implantation (Wrenn, Wood, and Bitman 1969). Cooper, Hawk, and Scommegna (1970) showed that the histamine concentration of the stroma and caruncles of the sheep uterus increases after ovariectomy but does not change appreciably throughout the oestrous cycle. The values recorded between days 0 and 14 of pregnancy in the present study are in good agreement with those reported by Cooper, Hawk, and Scommegna (1970) and confirm their observations that higher concentrations of histamine exist in the intercotyledonary endometrium than in the caruncles.

The increased phosphatase activities in the intercotyledonary endometrium of ovariectomized ewes treated with progesterone support the results of previous studies (Murdoch and White 1968*b*; Murdoch 1970*b*). The ability of the steroid to maintain high enzyme activities in the endometrium for a period of at least 21 days when administered daily suggests that the phosphatases do not become refractory to the

effects of progesterone during this time. However, between days 8 and 14 of pregnancy the endometrial phosphatases rapidly decrease in activity (Murdoch 1970*a*) despite the continuous production of progesterone by the corpus luteum (Bassett *et al.* 1969; Obst and Seamark 1970; Bindon 1971) indicating the possibility that factors which either inhibit the enzymes or prevent the stimulating effect of progesterone are brought into play in the early stages of gestation. Since the results presented in Figure 2 show that oestradiol limits the stimulating effect of progesterone on the enzymes, it is tempting to suggest that this steroid may have a role in regulating endometrial phosphatase activities. However, this possibility must remain speculative until further information on the production of oestrogens during early pregnancy in the ewe becomes available.

The constant level of acid phosphatase activity in the maternal caruncles during early pregnancy is in contrast to the previously reported activity pattern for the enzyme in the intercotyledonary endometrium (Murdoch 1970*a*). Murdoch and White (1968*b*) showed that the caruncular enzyme is much less responsive to ovarian hormones than the enzyme in the intercotyledonary area of the uterus and it is possible that acid phosphatase exists in different molecular forms in the tissues or is activated by a different chain of biochemical events within the two sites. The failure of acid phosphatase activity to parallel changes in the concentration of histamine in the caruncular tissue provides further evidence to indicate that the monoamine is not involved in the regulation of uterine phosphatases.

In conclusion, the results suggest the participation of factors other than histamine in the regulation of endometrial phosphatases during early pregnancy in the ewe. Progesterone and oestradiol may be involved to some extent but are not solely responsible for the control of the enzymes.

V. Acknowledgments

The author is indebted to Professor C. W. Emmens for interest and criticism and to Associate Professor R. G. Wales for his advice and assistance in the fluorimetric determination of histamine. The work was aided by a grant from the Ford Foundation.

VI. References

Andersch, M. A., and Szcypinski, A. J. (1947).—*Am. J. clin. Path.* **17**, 571.

Bassett, J. M., Oxborrow, T. J., Smith, I. D., and Thorburn, G. D. (1969).—*J. Endocr.* **45**, 449.

Bessey, O. A., Lowry, O. H., and Brock, M. J. (1946).—*J. biol. Chem.* **164**, 321.

Bindon, B. M. (1971).—*Aust. J. biol. Sci.* **24**, 149.

Boshier, D. P. (1969).—*J. Reprod. Fert.* **19**, 51.

Burkhalter, A. (1962).—*Biochem. Pharmac.* **11**, 315.

Cecil, H. C., Bitman, J., and Wrenn, T. R. (1964).—*Endocrinology* **74**, 701.

Cooper, B. S., Hawk, H. W., and Scommegna, A. (1970).—*J. Anim. Sci.* **31**, 1191.

De Carli, D. N., Castro-Vazquez, A., Macome, J. C., and Rosner, J. M. (1971).—*J. Endocr.* **50**, 541.

De Duve, C. (1963).—In "Lysosomes". CIBA Foundation Symposium. (Eds. A. V. S. de Reuck and M. P. Cameron.) (J. & A. Churchill: London.)

Finn, C. A., and Keen, P. M. (1962).—*Nature, Lond.* **194**, 960.

Gahan, P. B. (1967).—*Int. Rev. Cytol.* **21**, 1.

Garg, G. P., and Chaudhury, R. R. (1971).—*J. Reprod. Fert.* **27**, 287.

HUMPHREY, K. W., and MARTIN, L. (1968).—*J. Endocr.* **42**, 129.
MARCUS, G. J., and SHELESNYAK, M. C. (1967).—*Endocrinology* **80**, 1028.
MARCUS, G. J., SHELESNYAK, M. C., and KRAICER, P. F. (1964).—*Acta endocr., Copnh.* **47**, 255.
MCCOLL, J. D., ROBINSON, S., and SAGRITALO, G. (1971).—*Nature, Lond.* **230**, 245.
MURDOCH, R. N. (1970*a*).—*Aust. J. biol. Sci.* **23**, 1089.
MURDOCH, R. N. (1970*b*).—*Aust. J. biol. Sci.* **23**, 1289.
MURDOCH, R. N., and WHITE, I. G. (1968*a*).—*Aust. J. biol. Sci.* **21**, 123.
MURDOCH, R. N., and WHITE, I. G. (1968*b*).—*J. Endocr.* **42**, 187.
OBST, J. M., and SEAMARK, R. F. (1970).—*J. Reprod. Fert.* **21**, 545.
SHELESNYAK, M. C. (1957).—*Recent Prog. Horm. Res.* **13**, 269.
SHELESNYAK, M. C. (1960).—*Endeavour* **19**, 81.
SHELESNYAK, M. C., and KRAICER, P. F. (1964).—*J. Reprod. Fert.* **8**, 287.
SHORE, P. A., BURKHALTER, A., and COHN, V. H. (1959).—*J. Pharmac. exp. Ther.* **127**, 182.
SPAZIANI, E., and SZEGO, C. M. (1958).—*Endocrinology* **63**, 669.
SZEGO, C. M., and LAWSON, D. A. (1964).—*Endocrinology* **74**, 372.
WALES, R. G., SCOTT, T. W., and WHITE, I. G. (1961).—*Aust. J. exp. Biol. med. Sci.* **39**, 455.
WRENN, T. R., BITMAN, J., CECIL, H. C., and GILLIAM, D. R. (1964).—*J. Endocr.* **28**, 149.
WRENN, T. R., WOOD, J. R., and BITMAN, J. (1969).—*J. Reprod. Fert.* **19**, 511.