THE FALLOPIAN TUBE OF THE SHEEP

II. THE INFLUENCE OF PROGESTERONE AND OESTROGEN ON THE SECRETORY ACTIVITIES OF THE FALLOPIAN TUBE

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Summary

Secretion of fluid by the fallopian tubes was examined in normal ewes during their sexually active period and in ovariectomized ewes which were treated with progesterone and oestradiol benzoate. The normal ewes showed a cyclical variation in fluid secretion with a peak output of 0·98 ml/day in oestrus dropping to 0·37 ml/day in dioestrus. In ovariectomized animals oestradiol benzoate produced a cyclical variation similar to that seen in the normal ewe. The effect of progesterone was to shorten the response to oestrogen from 6 to 4 days. Progesterone given alone did not raise fluid secretion above that observed in untreated ovariectomized ewes.

There was a significant difference in height of the tubal epithelium between the ampulla (maximum height 26·6 μ) and the isthmus (maximum height 19·9 μ). Progesterone prevented the complete regression of the epithelium. When it was given in combination with oestradiol benzoate, the height of the epithelium was comparable to the tubal epithelium of normal ewes. Oestradiol benzoate given alone produced different effects on the ampulla and isthmus. The ampulla responded and the epithelium reached a height of 26·2 μ. The isthmus did not respond and the height of the tubal epithelium (12·8 μ) was similar to that observed in untreated ovariectomized ewes. These findings are discussed.

I. INTRODUCTION

Fertilization occurs in the oviduct (Austin 1959) and spermatozoa require a period in the genital tract before they can fertilize ova (Austin 1951; Chang 1951; Mattner 1963). However, little is known of the fluids providing the environment for these important phenomena. In recent years there has been an increase in studies of these fluids and species investigated include the rabbit (e.g. Clewe and Mastroianni 1960), the cow (Olds and Van Demark 1957), the monkey (Mastroianni, Shah, and Abdul-Karim 1961), and the sheep (Black, Duby, and Riesen 1963; Restall 1964*a*). The cyclic variation of the epithelium of the fallopian tube has been demonstrated in a number of species (Novak and Everett 1928; Casida and McKenzie 1932; Hadek 1955; Borell *et al.* 1956) and the influence of exogenous hormones on the secretion of the fallopian tube has been studied in rabbits (Bishop 1956; Greenwald 1958; Mastroianni *et al.* 1961). However, no information is yet available on the hormonal control of secretion in the fallopian tube of the sheep.

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II. Materials and Methods

(a) *Animals*

Twenty mature Merino ewes were used in this study.

(b) *Treatments*

The fallopian tubes of the 20 ewes were cannulated and fitted with a fluid-collecting device as described by Restall (1966). Sixteen ewes were ovariectomized and four left entire. They were housed singly in pens and fed lucerne pellets *ad libitum*. The observations were carried out during June and July, 1964.

The 20 ewes were divided into five groups of four ewes and in a 17-day cycle period were treated as follows:

(1) Four entire ewes—no treatment.

(2) Four ovariectomized ewes—no hormone treatment.

(3) Four ovariectomized ewes were given 10 mg/day of progesterone for 12 days, commencing on day 2 of each cycle, followed by 5 days without any hormone treatment.

(4) Four ovariectomized ewes were given 30 μg of oestradiol benzoate on day 15 of each cycle.

(5) Four ovariectomized ewes were given 10 mg/day of progesterone for 12 days, commencing on day 2 of each cycle, followed 48 hr later by 30 μg of oestradiol benzoate, then no hormone treatment for 3 days.

The 17-day cycle was arbitrarily divided into three periods henceforth referred to as stage 1 (days 1 and 2 of cycle), stage 2 (days 3–7), and stage 3 (days 8–16). In the case of the entire ewes day 1 was the day oestrus was first observed and stages 1 and 2 were of the periods stated above, but where the oestrous cycle in these ewes exceeded 17 days, then stage 3 was longer than indicated.

The hormone regimes used as treatments for the ovariectomized ewes were selected because they have been shown to induce behavioural oestrus and normal vaginal cycles in these ewes. They closely approximate the levels and duration of action of the ovarian hormones in the entire ewe (Robinson 1955; Robinson and Moore 1956; Robinson, Moore, and Binet 1956; Moore and Robinson 1957). The division of the treatment cycle into three arbitrary stages was made after considering the time lengths of oestrus, metoestrus, and dioestrus in the sheep. Robinson and Moore (1956) have shown that behavioural oestrus occurs 48 hr after oestrogen administration, and oestrus generally lasts for 1–2 days (Restall 1961). Casida and McKenzie (1932) and Hadek (1955) have considered a 5-day metoestrus in their observations in sheep. The remainder of the cycle has been termed dioestrus. Thus the arbitrary stages used here approximate the generally accepted time lengths of the physiological states of oestrus, metoestrus, and dioestrus. These physiological states will only be apparent in the entire ewes and those ewes receiving ovarian hormones in dose levels and duration of treatment approximating those in the entire animal (i.e. progesterone+oestradiol benzoate group and the oestradiol benzoate alone group). For the other two groups these physiological states will not be apparent and their division into three stages is purely for comparison.

All hormones were given intramuscularly in 1 ml of peanut oil between 8.30 a.m. and 9.30 a.m. All sheep were given one cycle of treatment before observations commenced and the observations were continued for a further two cycles before the animals were slaughtered. Each morning the amount of accumulated fluid was recorded and then aspirated from the collection apparatus. Oestrus was detected by examining the vagina for copious mucus (Restall 1961).

(*c*) *Histological Examination*

The entire reproductive tract was removed at slaughter and placed in buffered formol–saline fixative. The tracts from two ewes in each group were selected for examination, and tissue sections were taken from points midway between the infundibulum and the ampulla–isthmic junction and midway between the transected end of the fallopian tube and the ampulla–isthmic junction. The tissues were dehydrated, embedded in paraffin, sectioned at a thickness of 8 μ, and stained with haemotoxylin and eosin. The height of the tubal epithelia was measured with an eyepiece micrometer at three points in two sections taken at different depths in each tissue. These sections were compared with those taken from similar positions in two non-cannulated entire ewes killed at a similar stage of the cycle (stage 2).

(*d*) *Statistical Analysis*

For the purposes of analysis the mean daily fluid output in each stage of the cycle for each oviduct was calculated. Each mean could then be classified as to type of treatment, oviduct (left or right), cycle of treatment (first or second), and stages of cycle (1, 2, or 3). The ewes were treated as replications and the data subjected to an analysis of variance. Main effects and the significant first-order interactions were partitioned using orthogonal coefficients and the main-effect components were tested against the components of the significant first-order interactions.

In the two groups receiving oestradiol benzoate, length of secretory response was examined. The length of response was regarded as the number of days from maximum fluid output following oestradiol benzoate administration to the return to the pre-maximum level.

The six epithelial height measurements were treated as replications. Each set of six measurements could be classified according to treatment, oviduct (left and right), and position (ampulla or isthmus). The results were subjected to an analysis of variance. A between-animal, within-treatment source of variation was used as the error variance, and main effects and first-order interactions partitioned by using orthogonal coefficients. The main-effect components were tested against the components of the significant first-order interactions.

In three ewes, one from each of groups receiving oestradiol benzoate alone, progesterone alone, and the untreated ovariectomized ewes, a cannula failed before observations were complete. Subsequently one ewe selected at random was dropped from the other two treatment groups, and the analyses performed on the observations of the remaining three ewes in each treatment.

III. Results

(a) *Secretion Rate*

In the normal ewes peak fluid output coincided with oestrus and gradually fell away levelling off approximately 5–6 days after oestrus and remaining at this low level until the following oestrus. The mean daily fluid output of three entire ewes for two complete cycles is shown in Figure 1. The maximum fluid output observed in oestrus was 1·2 ml/day and the lowest output in the luteal phase of the cycle was 0·1 ml/day.

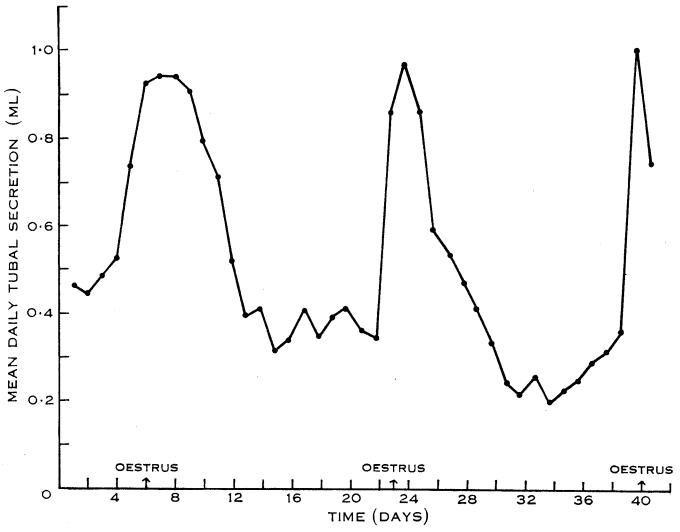

Fig. 1.—Mean daily fluid secretion in the fallopian tubes of three normal ewes.

The results of the treatments are given in Table 1 and a summary of the analysis of variance is given in Table 2. There were two highly significant interactions, the treatment × cycle stage and the treatment × cycle interaction. The treatment × cycle stage interaction is shown in Figure 2, the entire ewes and the groups receiving oestradiol benzoate having a distinct cyclic variation, the others showing little variation. The analysis shows that the entire group is different from the rest ($P < 0·01$), mean daily fluid output falling from 0·95 ml/day in stage 1 to 0·33 ml/day in stage 3. Within the ovariectomized ewes there is a highly significant effect due to oestradiol benzoate, those groups receiving it plus progesterone falling from a maximum fluid output of 0·81 ml/day and 0·73 ml/day (oestradiol benzoate alone) in stage 1 to 0·24 ml/day (both hormones) and 0·22 ml/day (oestradiol benzoate alone) in stage 3. There is little change between stages in the untreated ovariectomized group and the group receiving progesterone alone, the levels being generally below 0·2 ml/day, although a significant quadratic effect is shown in the analysis. This quadratic effect is small in comparison to the other components of the treatment × cycle stage interaction.

The treatment × cycle interaction is shown in Figure 3. The analysis shows a highly significant difference between the entire ewes and the rest, the mean fluid out-

put of entire ewes dropping from 0·72 ml/day in cycle 1 to 0·60 ml/day in cycle 2. Within the ovariectomized ewes there is a highly significant effect due to oestradiol benzoate, the two groups receiving it showing no change between cycles (mean output 0·5 ml/day) whilst the group receiving progesterone alone and the untreated ovariectomized group showing a decline in fluid output from cycle 1 to cycle 2.

TABLE 1

MEAN DAILY SECRETION OF FLUID FROM THE FALLOPIAN TUBES OF EWES RECEIVING VARIOUS HORMONAL TREATMENTS

Treatment	Stage of Cycle	Mean Daily Secretion (ml) in Cycle 1		Mean Daily Secretion (ml) in Cycle 2	
		Right Tube	Left Tube	Right Tube	Left Tube
Entire ewes, no treatment	1	1·02	0·95	0·85	1·00
	2	0·89	0·74	0·57	0·59
	3	0·36	0·38	0·27	0·30
Ovariectomized ewes treated with progesterone plus oestradiol benzoate	1	0·85	0·78	0·83	0·78
	2	0·43	0·44	0·45	0·45
	3	0·27	0·24	0·23	0·22
Ovariectomized ewes treated with oestradiol benzoate	1	0·67	0·77	0·75	0·75
	2	0·48	0·55	0·55	0·52
	3	0·23	0·28	0·19	0·19
Ovariectomized ewes treated with progesterone	1	0·18	0·15	0·15	0·18
	2	0·27	0·26	0·17	0·14
	3	0·23	0·20	0·13	0·16
Ovariectomized ewes, no hormone treatment	1	0·25	0·20	0·12	0·12
	2	0·25	0·26	0·13	0·14
	3	0·17	0·17	0·13	0·13

The main effect, cycle stage, had a significant linear component, mean fluid production falling from 0·57 ml/day in stage 1 through 0·42 ml/day in stage 2 to 0·23 ml/day in stage 3.

The effect of progesterone on the length of secretory response in ovariectomized ewes receiving oestradiol benzoate is a follows:

Cycle	Mean Length of Response (days) ± S.E.	
	Oestradiol Benzoate Alone	Progesterone plus Oestradiol Benzoate
1	6·00 ± 0·57	4·33 ± 0·34
2	6·67 ± 0·34	4·33 ± 0·34

There was a highly significant difference between treatments ($P < 0·01$), the ewes receiving progesterone prior to oestradiol benzoate adminstration having their response shortened by 2 days. There was no significant difference between successive cycles.

(*b*) *Histology*

All ewes were slaughtered during stage 2 of the oestrous cycle and it was seen that tracts from the untreated ovariectomized ewes and the group receiving progesterone alone were much reduced in size. The size of the tracts from the two groups receiving oestradiol benzoate were similar to those from the entire ewes.

TABLE 2

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 1

Source of Variation	Degrees of Freedom	Variance Ratio
Treatment	4	
Entire ewes *v.* rest	(1)	5·04
Within ovariectomized ewes:		
Effect of oestradiol benzoate	(1)	2·09
Effect of progesterone	(1)	0·12
Oestradiol benzoate–progesterone interaction	(1)	0·03
Stage of cycle	2	
Linear	(1)	11·27*
Quadratic	(1)	0·05
Cycle	1	4·70
Fallopian tube	1	0·005
Treatment × cycle stage interaction:	8	
Linear		
Entire ewes *v.* rest	(1)	97·54**
Within ovariectomized ewes:		
Effect of oestradiol benzoate	(1)	248·10**
Effect of progesterone	(1)	0·15
Oestradiol benzoate–progesterone interaction	(1)	2·38
Quadratic		
Entire ewes *v.* rest	(1)	2·34
Within ovariectomized ewes:		
Effect of oestradiol benzoate	(1)	3·85
Effect of progesterone	(1)	5·41*
Oestradiol benzoate–progesterone interaction	(1)	5·98*
Treatment × cycle interaction	4	
Entire ewes *v.* rest	(1)	8·56**
Within ovariectomized ewes:		
Effect of oestradiol benzoate	(1)	7·18**
Effect of progesterone	(1)	0·20
Oestradiol benzoate–progesterone interaction	(1)	0·43
Other first-order interactions	9	0·82
Higher-order interactions	30	1·00
Replications (error)	120	0·0061 (mean square)

* $P < 0·05$. ** $P < 0·01$.

The fallopian tubes were lined with a pseudostratified columnar epithelium, much folded in the ampulla region and very much less folded in the isthmic region. Relatively few cells in the ampulla region were ciliated and most showed cytoplasmic projections extending into the lumen. These projections were observed in the ampulla

region of the tubes from all the treatment groups. Extruding nuclei were also observed in the ampulla region. The isthmic region contained mainly ciliated cells, cytoplasmic projections and extruding nuclei being absent.

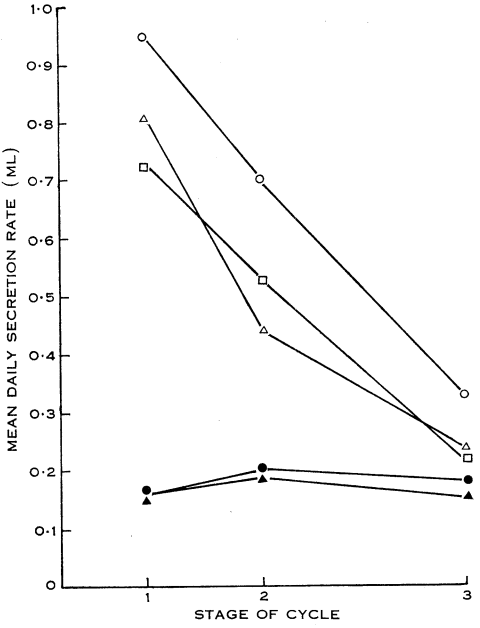

Fig. 2.—Mean daily tubal secretion rate in ewes receiving various hormone treatments and showing treatment × stage of cycle interaction.
○ Entire ewes.
△ Ovariectomized ewes receiving progesterone plus oestradiol benzoate.
□ Ovariectomized ewes receiving oestradiol benzoate alone.
● Ovariectomized ewes receiving progesterone alone.
▲ Ovariectomized ewes, no hormone treatment.

The results of the measurements of epithelial height are shown in Table 3 and a summary of the analysis of variance appears in Table 4. There was significant between-animal variation and the epithelium of the ampulla portion was significantly

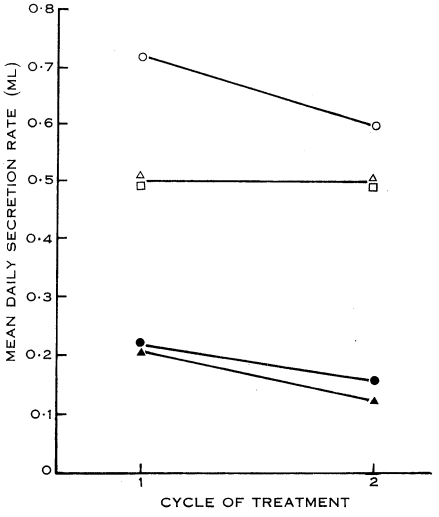

Fig. 3.—Mean daily tubal secretion rate in ewes receiving various hormone treatments and showing treatment × cycle of treatment interaction.
○ Entire ewes.
△ Ovariectomized ewes receiving progesterone plus oestradiol benzoate.
□ Ovariectomized ewes receiving oestradiol benzoate alone.
● Ovariectomized ewes receiving progesterone alone.
▲ Ovariectomized ewes, no hormone treatment.

taller than in the isthmus. There were significant treatment differences with the entire ewes having a taller epithelium than the rest, and there was significant effect due to oestradiol benzoate and progesterone. The significant treatment by position

interaction is shown in Figure 4. The treatment groups fall into three broad height levels with the exception of the group receiving oestradiol benzoate alone. This group shows a height in the ampulla (26·2 μ) comparable to the entire group and the group receiving both hormones. However, the height of the epithelia in the isthmus in the group receiving oestradiol benzoate alone (12·8 μ) is below that of the group receiving progesterone alone and just above that of the untreated castrate group.

There was no significant difference in the height of the epithelium from cannulated entire ewes and those not cannulated.

TABLE 3

MEAN HEIGHT OF THE TUBAL EPITHELIUM OF EWES RECEIVING VARIOUS HORMONE TREATMENTS

Hormone Treatment	Ewe No.	Mean Epithelial Height (μ)			
		Right Tube		Left Tube	
		Ampulla	Isthmus	Ampulla	Isthmus
Entire ewes, not cannulated, no treatment	601	24·2	19·0	24·7	18·5
	619	23·9	19·6	24·2	19·3
Entire ewes, cannulated, no treatment	303	26·8	17·7	26·5	19·3
	315	26·0	20·9	27·1	20·9
Ovariectomized ewes, treated with progesterone plus oestradiol benzoate	308	25·5	18·5	28·7	18·5
	316	22·0	16·9	23·6	19·6
Ovariectomized ewes, treated with oestradiol benzoate alone	671	28·2	12·9	24·7	12·9
	318	26·3	12·6	26·6	12·9
Ovariectomized ewes, treated with progesterone alone	309	25·3	14·0	20·1	14·0
	306	19·0	14·5	20·6	14·8
Ovariectomized ewes, untreated	312	17·7	10·8	18·2	11·6
	317	18·0	11·0	13·4	10·2

IV. DISCUSSION

The results indicate that in entire ewes there is a well-defined cyclic pattern of fluid secretion in the fallopian tubes. High levels of fluid output are observed during oestrus and metoestrus when the ewe is under the influence of endogenous oestrogen. Low levels of fluid output are observed during dioestrus when the ewe is under the influence of endogenous progesterone. This agrees with the observations of Black, Duby, and Riesen (1963) although the levels of fluid reported by them are higher than those given here.

In ovariectomized ewes the results indicate that oestrogen (oestradiol benzoate) produces a distinct cyclic variation in secretion rate with a peak fluid output at the expected time of oestrus. When progesterone is given before oestrogen its effect is to shorten the response to oestrogen by approximately 2 days. Given alone progesterone has no effect on secretion rate and it remains the same as in the untreated ovariecto-

mized ewes. The fact that the entire ewes have a higher secretion rate than the group receiving progesterone and oestrogen (Fig. 2) indicates that either

(1) other hormones are concerned in the control of secretion, or

(2) the dose levels and duration of treatments used here are not optimum.

In the entire ewes and in the untreated ovariectomized ewes and the group receiving progesterone alone a decline in fluid output occurred between cycles. In the entire ewes this may be due to the fact that the studies were carried out when the ewes were past the peak of the breeding season (Dun, Ahmed, and Morrant 1960)

TABLE 4

SUMMARY OF ANALYSIS OF VARIANCE OF DATA IN TABLE 3

Source of Variation	Degrees of Freedom	Variance Ratio
Treatments	5	
Entire ewes *v.* cannulated entire ewes	(1)	3·30
All entire ewes *v.* rest	(1)	69·00**
Within ovariectomized ewes:		
Effect of oestradiol benzoate	(1)	69·45**
Effect of progesterone	(1)	26·14**
Oestradiol benzoate–progesterone interaction	(1)	2·66
Position (ampulla *v.* isthmus)	1	252·67**
Fallopian tube (left *v.* right)	1	0·001
Treatment × position interaction	5	
Entire ewes *v.* cannulated entire ewes	(1)	3·30
All entire ewes *v.* rest	(1)	5·02*
Within ovariectomized ewes:		
Effect of oestradiol benzoate	(1)	10·01**
Effect of progesterone	(1)	6·68*
Oestradiol benzoate–progesterone interaction	(1)	12·03*
Other interactions	11	1·85
Between animals within treatments	23	6·34*
Between readings within treatments (error)	241	2·55 (mean square)

*$P < 0·05$. **$P < 0·01$.

and it probably reflects a decline in endogenous oestrogen levels. In the other two groups the decline was presumably due to gradual atrophy of the tubes. The two groups receiving constant levels of oestrogen showed no such decline between cycles.

Reports on the endocrine control of fluid secretion in the fallopian tube are sparse. Greenwald (1958) has shown that mucin production in the rabbit oviduct is modified by exogenous oestrogen. Fredricsson (1958) has shown that oestrogen causes an increase in the number of secretory cells in the rabbit oviduct, while Bishop (1956) and Mastroianni *et al.* (1961) have shown that oestrogen increases secretion in the ovariectomized rabbit. The latter authors have further shown that progesterone in the presence of oestrogen decreases secretion rate, but does not affect the level of secretion when given to ovariectomized rabbits. These results are in general agreement with those reported here for the sheep.

It is clear that progesterone and oestrogen have definite effects on the height of the epithelium in the fallopian tube. Progesterone prevents the epithelial height from regressing to that observed in the untreated ovariectomized ewes. When progesterone is given in combination with oestradiol benzoate, the epithelium is higher and is comparable with the entire animal in both the ampulla and isthmic regions of the tube. When oestradiol benzoate is given alone the height of the epithelium in the ampulla region is comparable to that of the entire animals while the height in the isthmic

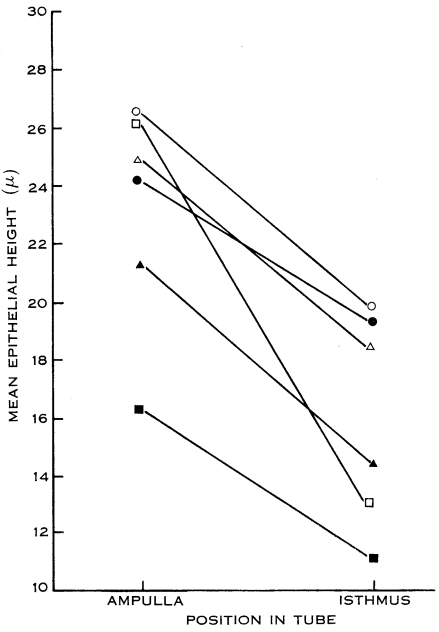

Fig. 4.—Mean height of the tubal epithelium in ewes receiving various hormone treatments and showing treatment × position interaction.
○ Entire ewes with cannulae.
● Entire ewes without cannulae.
△ Ovariectomized ewes receiving progesterone plus oestradiol benzoate.
□ Ovariectomized ewes receiving oestradiol benzoate alone.
▲ Ovariectomized ewes receiving progesterone alone.
■ Ovariectomized ewes, no hormone treatment.

region is comparable to that of the untreated ovariectomized ewes. There are two possible explanations for these phenomena:

(1) the isthmic epithelium requires treatment with progesterone to sensitize it to oestrogen whereas the ampulla does not, or

(2) the isthmic region develops a refractory condition due to oestrogen administration, and this refractory condition is prevented from developing by pretreatment with progesterone.

In regard to the second possibility, Moore and Robinson (1957) have shown that progesterone does not increase vaginal sensitivity to oestrogen, but its effect is to prevent the development of a refractory state induced by oestrogen stimulation. It emerges that both progesterone and oestrogen are required to produce a normal response in the fallopian tube, and that the response of the ampulla is different from that of the isthmus.

It is interesting to consider the secretory contribution of the isthmus. Hadek (1955) has shown that there are few secretory cells in the isthmus in sheep and the observations here agree. The cells observed in the isthmus were mainly ciliated and no cells with cytoplasmic extrusions were noted. Considered in relation to the observation that oestrogen given alone does not increase epithelial height in the

isthmus but does cause an almost normal production of tubal fluid (Fig. 2), these results indicate that the contribution of the isthmus to fluid output is small. The bulk of the fluid produced would appear to be secreted by the cells of the ampulla.

Borell *et al.* (1956) observed that secretory cells were absent in the oviduct of the ovariectomized rabbit. However, Bishop (1956) and Mastroianni *et al.* (1961) reported a fluid output from ovariectomized rabbits. In this study cytoplasmic extrusions were observed in the ampulla of the oviducts of all groups including the ovariectomized ewes, and although this may indicate cell activity, the possibility remains that in the untreated ovariectomized ewes a portion of the fluid output may be a transudate.

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VI. References

Austin, C. R. (1951).—*Aust. J. Sci. Res.* B **4**: 581.

Austin, C. R. (1959).—"Reproduction in Domestic Animals." (Eds. H. H. Cole and P. T. Cupps.) Ch. 12. p. 405.

Bishop, D. W. (1956).—*Am. J. Physiol.* **187**: 347.

Black, D. L., Duby, R. T. and Riesen, J. (1963).—*J. Reprod. Fert.* **6**: 257.

Borell, V., Nilsson, O., Wersall, J., and Westman, A. (1956).—*Acta Obstet. Gynec. Scand.* **35**: 35.

Casida, L. E., and McKenzie, F. F. (1932).—Res. Bull. Mo. Agric. Exp. Stn. No. 170.

Chang, M. C. (1951).—*Nature, Lond.* **168**: 697.

Clewe, T. H., and Mastroianni, L. (1960).—*J. Reprod. Fert.* **1**: 146.

Dun, R. B., Ahmed, W., and Morrant, A. J. (1960).—*Aust. J. Agric. Res.* 11: 805.

Fredricsson, B. (1958).—*Acta Morph. Neerl. Scand.* **11**: 193.

Fredricsson, B. (1959).—*Acta Obstet. Gynec. Scand.* **38**: 109.

Greenwald, G. S. (1958).—*Anat. Rec.* **130**: 477.

Hadek, R. (1955).—*Anat. Rec.* **121**: 187.

Mastroianni, L., Beer, F., Shah, U., and Clewe, T. H. (1961).—*Endocrinology* **68**: 92.

Mastroianni, L., Shah, V., and Abdul-Karim, R. (1961).—*Fert. Steril.* **12**: 417.

Mattner, P. (1963).—*Nature, Lond.* **199**: 772.

Moore, N. W., and Robinson, T. J. (1957).—*J. Endocr.* **14**: 297.

Novak, E., and Everett, H. S. (1928).—*Am. J. Obstet. Gynec.* **16**: 499.

Olds, D., and Van Demark, N. L. (1957).—*Fert. Steril.* **8**: **345**.

Restall, B. J. (1961).—In "Artificial Breeding of Sheep in Australia". (Ed. E. M. Roberts.) (Proceeding of a conference held at University of New South Wales, August 1961.) p.67.

Restall, B. J. (1964).—*Proc. Aust. Soc. Anim. Prod.* **5**: 29.

Restall, B. J. (1966).—*Aust. J. Biol. Sci.* **19**: 181.

Robinson, T. J. (1955).—*J. Endocr.* **12**: 163.

Robinson, T. J., Moore, N. W., and Binet, F. E. (1956).—*J. Endocr.* **14**: 1.

Robinson, T. J., and Moore, N. W. (1956).—*J. Endocr.* **14**: 97.