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RESPONSE OF PINEAL β-ADRENOCEPTORS IN DIFFERENT BREEDS OF SHEEP TO IMMUNIZATION AGAINST SELECTED STEROIDS

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Abstract—Active immunization of Merino and Wiltshire Horn × Merino cross-bred sheep against a range of gonadal steroids has revealed that pineal β-adrenoceptors in both breeds are sensitive to hormonal modification by androgens, but only in the less seasonal, non-shedding Merino do these receptors appear to be sensitive to regulation by estrogens. Neither breed showed sensitivity of its pineal β-adrenoceptors to active immunization against the pineal hormone melatonin under either normal or reversed photoperiod treatment. These results (a) suggest that pineal related seasonal differences between the breeds (i.e. wool shedding and/or reproductive function) may reside in differential sensitivity of the pineal gland to regulation by specific circulating steroid hormones, and (b) indicate an absence of end-product regulation of β-adrenoceptor-mediated pineal melatonin biosynthesis in both seasonally shedding and non-shedding sheep.

Changing photoperiod has been suggested [1] to be the trigger for seasonal shedding of wool in primitive breeds of sheep, such as the Wiltshire Horn. Improved breeds, such as the Merino, do not respond in the same way to this stimulus [2], suggesting that, as well as having different regulatory mechanisms operating at the level of the skin and/or wool follicle [3, 4], the breeds may differ in their central photosensitive mechanisms [5].

The pineal gland has been implicated in mediating responses to changing photoperiod. These responses include seasonal physiological changes in a number of species [6, 7] and have been shown recently to include the timing of the annual moult in two different primitive breeds of sheep [8, 9].

Synthesis of melatonin in the pineal gland is regulated by photoperiod, the regulation being exerted via the superior cervical ganglion [10] and pinealocyte β-adrenoceptors [11, 12]. In Merino pineal glands, these receptors, which also regulate diverse other metabolic pathways in the glands, were shown to be modified by specific gonadal steroids, using both active immunization and steroid implantation techniques [13].

When initial studies indicated no significant differences in basal β-adrenoceptor parameters between pineals of Merino and Wiltshire Horn rams (A. Foldes, unpublished observation), it was suggested that the breeds may differ in the sensitivity of their pineal β-adrenoceptors to modification by endogenous hormones. We now report results of our experiments on pineal β-adrenoceptor function in seasonally shedding Wiltshire Horn × Merino sheep actively immunized against a range of gonadal hormones and against melatonin.

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MATERIALS AND METHODS

Materials. [^{3}H]Dihydroalprenolol (47.4 Ci/mmole), [^{14}C]5-hydroxytryptamine (58.5 mCi/mmole), [^{3}H]-3′,5′-cyclic adenosine monophosphate (39.1 Ci/mmole) and [8-^{14}C]adenosine-5-triphosphate (54.2 mCi/mmole) were purchased from Searle Nucleonics, Sydney, Australia, and their radiochemical purity was monitored by thin-layer chromatography. Radiolabeled steroids for antibody titre determination were purchased from the Radiochemical Centre, Amersham, U.K. Other reagents were of commercially available analytical grade.

Animals. Merino (M) ewes, 30–45 kg in weight, maintained under field conditions were used in experiments at Prospect, NSW (latitude 33°49′S). F_2 Wiltshire Horn × Merino (WH × M) ewes and rams (9 months of age) were used in experiments at Yeerongpilly, Qld (latitude 27°28′S). These sheep were also maintained under field conditions except in the case of the melatonin-immunized (and matched sham-immunized) ewes which were housed indoors in two groups. One group of six immunized and six sham-immunized WH × M ewes was maintained for 13 weeks in individual pens under a natural light regimen (daylight period of 11 hr 16 min at the winter solstice on 21 June) and a similar group of 12 WH × M ewes was maintained for 13 weeks under a reversed lighting regimen exactly 12 hr out of phase with natural light. Light was provided by cool white fluorescent tubes. All ewes housed indoors were offered a diet of 800 g lucerne pellets per day and water *ad lib.*

Sheep were killed by exsanguination and cervical dislocation. Within 1 min of slaughter, pineal glands were removed and placed into 25 vol. (w/v) of ice-cold 80 mM Tris–5 mM Mg^{2+} buffer, pH 7.4, containing 0.6 mM ethyleneglycolbis (aminoethylether)tetra-acetate (EGTA). Slaughter of the

ewes maintained under reversed photoperiod and collection of their pineal glands were carried out under dim red light (40 W, $\lambda = 660$ nm, Philips, NSW, Australia). The ovarian status of all ewes was assessed at slaughter.

Immunization and antibody titre measurement. Antigens were prepared by conjugation of steroid or melatonin derivatives to human serum albumin (HSA) as previously described [13]. Groups of five to seven Merino ewes at Prospect were immunized (or sham-immunized) against the steroids estrone, 17β-estradiol, testosterone or androstenedione, or against melatonin, in May or June 1981 using the synthesized HSA conjugates [13]. In May 1982, groups of six or eight WH × M ewes at Yeerongpilly were immunized against estrone, 17β-estradiol, or melatonin, and similar groups of control ewes were sham-immunized. At the same time, groups of six WH × M rams, also at Yeerongpilly, were immunized against the androgens testosterone or androstenedione using an immunization protocol similar to that employed at Prospect. The ewes immunized against estrogens, the rams immunized against androstenedione, and their corresponding sham-immunized controls were slaughtered in early August, whereas the melatonin-immunized ewes, the testosterone-immunized rams, and their sham-immunized controls were slaughtered in late October.

Antibody titres were measured in jugular venous plasma prepared from blood samples taken 2 weeks after each booster immunization and again immediately prior to slaughter. Titres were measured as previously described [13]; the values reported here refer to the samples taken just prior to slaughter.

Assays of pineal variables. Pineal β-adrenoceptors were quantitated by Scatchard analysis of [^{3}H]dihydroalprenolol binding to washed pineal membranes at 25° as previously described [5]. Pineal serotonin *N*-acetyltransferase activity was determined by radioisotope assay involving two-dimensional thin-layer chromatography [14]. Adenyl cyclase activity in whole pineal homogenates was determined according to the method of Weiss and Costa [15], with minor modifications. Recovery of cyclic AMP averaged over 80%, determined by addition of [^{3}H]–cAMP prior to the Dowex chromatography step. Protein was determined by the method of Lowry *et al.* [16] using bovine serum albumin as standard.

Wool-related measurements. The percentage of brush end fibres in wool staples plucked at two-weekly or monthly intervals from the fibre-shedding region of the neck of WH × M sheep was determined as previously described [17].

Statistical methods. β-Adrenoceptor parameters, as well as serotonin *N*-acetyltransferase (NAT) and adenyl cyclase activities, were determined by analysis of the binding or enzyme activity data on an Apple II (Europlus) microcomputer using a program based on a "Visicalc" Program Diskette (Personal Software, U.S.A.). The statistical significance of results was determined using Student's paired or unpaired *t*-test, as appropriate, or by a modified Chi square test in the case of the ovarian data.

RESULTS

Immunization of Merino (M) and Wiltshire Horn × Merino (WH × M) sheep against estrogens. Results of immunization of Merino ewes at Prospect against estrone and 17β-estradiol have been reported [13] and are summarized for comparative purposes in Table 1, together with the results of similar immunization of WH × M ewes at Yeerongpilly. The M ewes were immunized in May/June, when they were cycling; immunization of the WH × M ewes also commenced in May, but their reproductive status at the time of immunization was not known (cf. Table 4). The data clearly show that, whereas

Table 1. Effects of active immunization against estrogens*

Immunization	(N)	Location	Reciprocal antibody titre × 10^{-3}	β_{max} (pmoles/mg)	K_D (nM)	NAT activity (μmoles/hr/g)
WH × M ewes		Yeerongpilly				
Sham	8			6.6 ± 0.7	6.4 ± 1.1	15.5 ± 2.3
Estrone	8		8.8 ± 3.4	5.4 ± 0.5	7.6 ± 1.0	17.9 ± 2.6
Sham	8			6.8 ± 0.8	6.5 ± 0.7	18.1 ± 1.5
17β-Estradiol	8		>50.0	6.7 ± 0.9	7.5 ± 0.7	20.2 ± 1.2
M ewes†		Prospect				
Sham‡	7			1.6 ± 0.3	12.9 ± 0.9	30.3 ± 2.8
Estrone	7		21.8 ± 5.5	0.9 ± 0.1§	6.5 ± 0.8‖	22.1 ± 2.0
Sham	6			9.2 ± 1.2	17.2 ± 3.0	ND
17β-Estradiol	6		>500	5.9 ± 0.9¶	10.0 ± 2.0¶	ND

* Means ± S.E. of (N) determinations are shown. Abbreviations: M, Merino (non-shedding); WH × M, Wiltshire Horn × Merino cross-bred (seasonally shedding) ewes; β_{max}, pineal β-receptor density; K_D, dissociation constant; NAT, serotonin *N*-acetyltransferase (NAT activity is expressed in terms of μmoles of serotonin acetylated per hr per g of pineal protein); and ND, not determined.

† Data from Ref. 13.

‡ Pineals of estrone-immunized and corresponding sham-immunized ewes were assayed for β-adrenoceptor parameters at 37°; other pineals were assayed at 25°.

§–¶ Values of §$P < 0.05$, ‖$P < 0.001$ and ¶$P < 0.01$ were determined using Student's paired *t*-test. (Pairing was based on assays, pineals from one immunized and one sham-immunized ewe being assayed simultaneously.)

Table 2. Effects of active immunization against androgens*

Immunization	N	Location	Reciprocal antibody titre × 10^{-3}	β_{max} (pmoles/mg)	K_D (nM)	NAT activity (μmoles/hr/g)
WH × M rams		Yeerongpilly				
Sham	6			12.3 ± 0.7	21.2 ± 3.7	23.6 ± 1.7
Testosterone	6		1.8 ± 0.5	5.6 ± 0.8†	9.5 ± 1.3‡	23.7 ± 4.1
Sham	6			5.7 ± 0.6	5.4 ± 1.5	20.4 ± 2.6
Androstenedione	6		5.4 ± 2.1	12.9 ± 2.1‡	17.3 ± 2.2§	20.0 ± 2.0
M ewes‖		Prospect				
Sham	6			3.0 ± 0.4	22.1 ± 2.9	36.6 ± 3.3
Testosterone¶	6		13.4 ± 8.6	2.0 ± 0.2‡	13.6 ± 1.0‡	36.4 ± 4.3
Sham	5			5.1 ± 0.7	4.4 ± 0.7	56.3 ± 4.0
Androstenedione	5		44.3 ± 3.4	7.5 ± 0.6‡	9.3 ± 0.6§	53.0 ± 3.8

* Means ± S.E. of (N) determinations are shown. Abbreviations are defined in Table 1.
†–§ Values of †$P < 0.001$, ‡$P < 0.05$ and §$P < 0.01$ were determined using Student's paired *t*-test.
‖ Data from Ref. 13.
¶ β-Receptor parameters in testosterone and matching sham-immunized ewes at Prospect were determined in whole pineal homogenates; all other parameters were determined in washed pineal membrane preparations.

pineal β-adrenoceptor density and dissociation constant are both decreased in M ewes by immunization against estrogens, in WH × M ewes these parameters remained unaltered following active immunization against either estrone or 17β-estradiol.

Immunization of M and WH × M sheep against androgens. Active immunization of M ewes against testosterone resulted in decreases in both pineal β-receptor density and dissociation constant, arguably due to indirect effects on estrogen biosynthesis [13]. The same treatment in WH × M rams at Yeerongpilly caused similar, statistically significant, decreases in these parameters (Table 2). This table also shows results obtained with testosterone-immunized Merino ewes at Prospect; the Merino data has been reported in part previously [13] and is included here for comparative purposes only.

Active immunization of M ewes against androstenedione, on the other hand, causes a significant enhancement of pineal β-adrenoceptor density and a decrement in binding affinity (Table 2, data derived from Ref. 13). This observation is supported by the results of androstenedione implantation in M ewes [13]. Similar active immunization of WH × M rams against androstenedione at Yeerongpilly resulted in changes in β-adrenoceptor parameters in the same direction and slightly greater in magnitude than those seen in the M ewes (Table 2).

Immunization against melatonin. Active immunization of WH × M ewes at Yeerongpilly against melatonin failed to affect pineal β-adrenoceptors or pineal serotonin-*N*-acetyltransferase activity, regardless of the photo-environment at the time of slaughter (Table 3). This is in keeping with the previously reported lack of effect of immunizing M ewes against melatonin under comparable photoperiod regimens at Prospect, and with the lack of effect on the receptors of incubating M pineals with melatonin *in vitro* [13].

Effects of the various immunization treatments on cycles of wool shedding in WH × M sheep. The WH × M sheep used in the experiments at Yeerongpilly were observed to cast wool from their neck, belly and breech regions on a seasonal basis. In both immunized and sham-immunized sheep, brush end fibre formation increased, commencing in June, in agreement with previous observations [17]. Immunization of WH × M rams against androstenedione, and to a lesser extent also against testosterone, retarded brush end formation compared to sham-immunized controls (Fig. 1). Subjective fleece casting scores ([18]; data not shown) support the suggestion that immunization against testosterone delayed fleece casting.

Immunization of WH × M ewes against estrogens failed to have significant effects on brush end for-

Table 3. Immunization of Wiltshire Horn × Merino ewes against melatonin*

Photoperiod	Treatment	N	Reciprocal antibody titre × 10^{-3}	β_{max} (pmoles/mg)	K_D (nM)	NAT activity (μmoles/hr/g)
Normal	Sham	6		8.8 ± 2.7	11.0 ± 1.2	22.5 ± 2.0
	Immunized	6	2.6 ± 1.8	7.3 ± 0.6	11.1 ± 1.6	22.4 ± 0.8
Reversed	Sham	6		8.0 ± 1.5	11.4 ± 1.8	80.0 ± 8.5
	Immunized	6	0.4 ± 0.1	7.9 ± 1.2	10.5 ± 1.1	88.9 ± 22.2

* Means ± S.E. of six determinations are shown. No significant effect of immunization was observed under either photoperiod, compared with sham-immunized controls. All abbreviations are defined in Table 1.

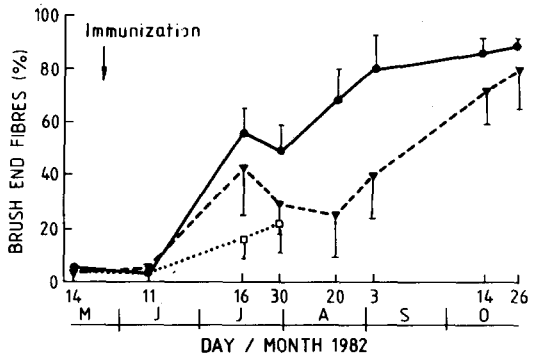

Fig. 1. Effects of active immunization of WH × M rams against androgens on wool fibre brush end formation. Percentage brush end formation is plotted against date of sampling for sham-immunized (●—●), testosterone immunized (▼—▼) and androstenedione-immunized (□—□) rams. The immunization treatment commenced on 25 May as indicated by the arrow. Brush end formation differed significantly between treated and sham-immunized rams during August and September in the case of testosterone-immune rams, and after 11 June 1982 in the case of androstenedione-immune ewes.

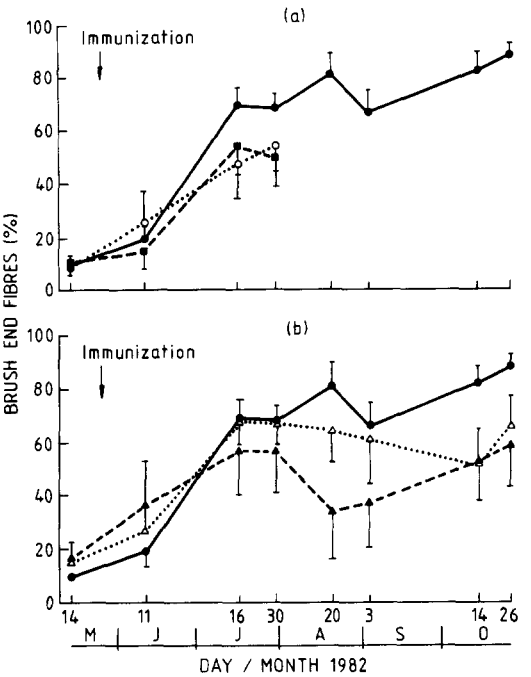

Fig. 2. Effects of active immunization of WH × M ewes against estrogens or melatonin on wool fibre brush end formation. Panel (a) shows the lack of significant effect of immunizing WH × M ewes against estrone (○—○) or 17β-estradiol (■—■) compared to sham-immunized controls (●—●) on wool fibre brush end formation up to 10 weeks post treatment. Panel (b) shows the effect of immunization against melatonin of WH × M ewes, maintained under natural (▲—▲) and reversed (△—△) photoperiods, on wool fibre brush end formation. Statistically significant effects were obtained with the former group from August through October compared to sham-immunized controls (●—●), but not with the latter group at any time during the experiment. For all groups immunization treatment commenced on 25 May and photoperiod treatment on 17 July.

mation during the period of observation (Fig. 2a). Immunization against melatonin under normal photoperiod significantly depressed brush end formation. The same treatment under reversed photoperiod had no significant effect on brush end formation in the follicles (Fig. 2b) during the period of study.

Effects of immunization treatments on ovarian cycles of WH × M ewes. The ovarian status of ewes at slaughter in August or October is shown in Table 4. All eight ewes immunized against 17β-estradiol were cycling in August, whereas only three of eight sham-immunized ewes were cycling at this time ($P < 0.005$, modified Chi square test). Of twelve ewes housed under reversed photoperiod, six were cycling in October, while only one of twelve ewes housed under normal photoperiod was cycling at that time ($P < 0.1$, NS, modified Chi square test). Melatonin immunization had no effect on the ovarian status of the ewes slaughtered in October under either photoperiod.

DISCUSSION

Gonadal steroid feedback regulation of central nervous function [19] is well documented and, in sheep, gonadal steroids have been shown to regulate seasonal changes in reproductive function [20]. Feedback regulation of pineal β-adrenoceptor function in Merino sheep by selected steroid hormones has also been suggested [13], and the differential effects of different gonadal steroids on these receptors, shown both by active immunization and implantation techniques, lend strong support to a physiological role for this mechanism. The current results provide further

Table 4. Effects of active immunization on ovarian cycling in WH × M ewes

Immunization	Photoperiod	Number of ewes treated	Cycling at slaughter August	October
Sham estrone	Normal	7	1	
Estrone	Normal	8	2	
Sham 17β-estradiol	Normal	8	3	
17β-Estradiol	Normal	8	8*	
Sham melatonin	Normal	6		0
Melatonin	Normal	6		1
Sham melatonin	Reversed	6		3
Melatonin	Reversed	6		3

* $P < 0.005$ (modified Chi square test).

evidence for an effect of gonadal steroids on pineal β-adrenoceptors and thus suggest that the gonads have the potential to regulate aspects of pineal function in Merino sheep. This, in turn, could have important biochemical and physiological effects on reproductive and central nervous function [21, 22] as well as on wool growth [8].

The results of active immunization of WH × M ewes and rams against steroids indicated that androgen effects on pineal β-adrenoceptors, similar to those seen in Marino ewes, were also observed in WH × M rams (Table 2). The differences seen in the table between pineal β-adrenoceptor parameters of the two groups of sham-immunized WH × M rams may reflect seasonal differences, since the rams immunized against androstenedione and the corresponding controls were slaughtered in early August; the testosterone-immunized rams and their controls were killed in late October. However, active immunization against estrogens had markedly different effects on pineal glands in M compared to WH × M ewes. Whereas in cycling Merinos, immunization against either estrone or 17β-estradiol significantly decreased pineal β-adrenoceptor density and concomitantly increased ligand binding affinity, immunization of WH × M ewes against the same antigens failed to have any effect at all on the receptors (Table 1). It is suggested that these differences in estrogen-sensitivity of the pineal gland may be breed related, in keeping with similar findings of differences in estrogen sensitivity of reproduction-related neuroendocrine function in different breeds of ewes [20, 23]. However, the possibility that the difference in steroid sensitivity arises from differences in nutrition, in the stage of the reproductive cycle of the ewes, or in the climatic or photoperiodic environment between the two sites where the experiments were carried out requires further investigation.

Immunization against melatonin failed to affect pineal parameters in WH × M ewes under two different light regimens in this study and, similarly, failed to affect the same parameters in Merino ewes maintained under the same light conditions in an earlier study [13]. These results indicate that plasma melatonin levels do not regulate pineal β-adrenoceptors and suggest that in neither breed of sheep does melatonin exert an end-product inhibition effect on its own biosynthesis, regardless of the time of day and hence the level of endogenous melatonin present. This conclusion is supported by the observations of Kennaway *et al.* [24] in Merino sheep *in vivo*.

Since differences in pineal function between shedding (WH) and non-shedding (M) rams were not reflected by differences in basal pineal β-adrenoceptor parameters (A. Foldes, unpublished observation), the suggestion was advanced that the two breeds may differ in the sensitivity of their pineal β-adrenoceptors to modification by endogenous hormones [25]. The current data provide some experimental support of this suggestion in that it shows that shedding and non-shedding breeds may differ in the sensitivity of their pineal β-adrenoceptors to modification by endogenous estrogens. The breed which is less seasonal in its reproductive cycle (viz. Merino) appears to be sensitive to feedback regulation by estrogens.

The pineal gland has been implicated in regulation of seasonal pelage changes in a variety of species (see, for example, Plotka *et al.* [26] and Rust and Meyer [27]) as well as of seasonal wool growth rates and shedding in two breeds of seasonally moulting sheep [8, 9]. To investigate whether or not differences in pineal sensitivity to steroid hormones may be related to wool follicle activity, we studied the effects of our active immunization treatments on brush end formation in wool staples plucked from the shedding region of the neck of WH × M sheep at regular intervals before and after immunization. A high percentage of brush ends indicates that the follicles at that site are in a resting phase (telogen) or are about to shed; conversely, a low percentage of brush ends suggests that the fibres are in a growing phase (anagen). The results (Figs. 1 and 2) indicate that immunization against androstenedione decreased brush end formation in the follicles, and immunization against testosterone had a similar effect. These results, supported by the effects of immunization against testosterone in decreasing subjective scores of fleece casting (not shown), suggest a possible accelerating effect of the androgens on seasonal wool fibre shedding. On the other hand, immunization against estrogens affected neither wool follicle activity (for up to 10 weeks post treatment) nor pineal function in WH × M ewes.

Fibre shedding in WH × M ewes immunized against melatonin appears to vary under normal and reversed lighting. Since both lighting regimens used in this study involved comparable durations of dark and light, the observation is unlikely to be related to photoperiod directly. The reason for the differences seen under the two lighting regimens is not known, but possible contributing factors could include (1) differences between natural daylight and cold fluorescent light in activating light-sensitive processes in sheep, and (2) the observed difference in the level of melatonin immunity achieved in the two groups of sheep. Results of the current study suggest that, in WH × M ewes maintained under normal lighting conditions, immunity against melatonin depressed brush end formation and, hence, increased wool follicle activity. Further studies are required to demonstrate that immunity against melatonin enhances, or does not enhance, wool growth in shedding and non-shedding breeds of sheep.

The apparent effect of 17β-estradiol immunization in promoting ovulation in WH × M ewes seems unexpected as well as previously unreported. Again, further research in this area is required to confirm or deny the observed effect.

Finally, steroid feedback on the pineal gland on the one hand and selected steroids affecting shedding in seasonally moulting sheep on the other suggest a possible association, at the level of the CNS, between regulation of seasonal breeding and of seasonal wool follicle activity in these sheep. This association may be reciprocal in nature, since wool growth appears to correlate with the anestrous, and cessation of wool growth with the reproductive, season. This suggested association also remains to be investigated further.

Thus, in summary, the current report provides

experimental confirmation of the suggestion [25] that pineal-related differences between seasonally shedding and non-shedding breeds of sheep may reside in differential sensitivity of the pineal, and specifically of the pinealocyte, β-adrenoceptors to circulating hormones.

Immunization of the crossbreeds against androgens but not estrogens resulted in an apparently altered wool follicle activity pattern in the shedding region of the neck. Thus, while differences in reproductive function may be associated with differences in pineal sensitivity to circulating estrogens, it is suggested, but remains to be clearly demonstrated, that differences in seasonal wool follicle activity patterns between the breeds may also be associated with steroidal regulation of the pineal gland.

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REFERENCES

1. J. Slee, in *Biology of the Skin and Hair Growth* (Eds. A. G. Lyne and B. F. Short), pp. 545–63. Angus & Robertson, Sydney (1965).
2. M. L. Ryder, *Aust. J. agric. Res.* **18**, 683 (1967).
3. M. L. Ryder and G. C. Priestley, *Res. vet. Sci.* **23**, 310 (1977).
4. B. A. Panaretto, in *Physiological and Environmental Limitations to Wool Growth* (Eds. J. L. Black and P. J. Reis), pp. 327–36. University of New England Publishing Unit, Armidale (1979).
5. A. Foldes, C. A. Maxwell, N. T. Hinks, R. M. Hoskinson and R. J. Scaramuzzi, *Biochem. Pharmac.* **31**, 1369 (1982).
6. S. Binkley, *Scient. Am.* **240**(4), 50 (1979).
7. G. C. Brainard, L. J. Petterborg, B. A. Richardson and R. J. Reiter, *Neuroendocrinology* **35**, 342 (1982).
8. G. A. Lincoln, M. Klandorf and N. Anderson, *Endocrinology* **107**, 1543 (1980).
9. A. H. Williams, in *Proceedings of the Second National Conference on Wool Harvesting Research and Development* (Ed. P. R. W. Hudson), pp. 37–40. Australian Wool Corp., Sydney (1981).
10. D. C. Klein, J. L. Weller and R. Y. Moore, *Proc. natn. Acad. Sci. U.S.A.* **68**, 3107 (1971).
11. T. Deguchi and J. Axelrod, *Proc. natn. Acad. Sci. U.S.A.* **69**, 2547 (1972).
12. J. A. Romero, M. Zatz, J. W. Kebabian and J. Axelrod, *Nature, Lond.* **258**, 435 (1975).
13. A. Foldes, R. M. Hoskinson, R. J. Scaramuzzi, N. T. Hinks and C. A. Maxwell, *Neuroendocrinology* **37**, 378 (1983).
14. D. C. Klein, G. R. Berg and J. L. Weller, *Science* **168**, 979 (1979).
15. B. Weiss and E. Costa, *J. Pharmac. exp. Ther.* **161**, 311 (1968).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
17. B. J. McDonald, A. Cotton and P. S. Hopkins, in *Proceedings of the Second National Conference on Wool Harvesting Research and Development* (Ed. P. R. W. Hudson), pp. 47–52. Australian Wool Corp., Sydney (1981).
18. M. L. Tierney, *Wool Technol. Sheep Breed.* **26**(3), 21 (1978).
19. B. S. McEwen and B. Parsons, *A. Rev. Pharmac. Toxic.* **22**, 555 (1982).
20. R. L. Goodman and F. J. Karsch, *J. Reprod. Fert.* Suppl. 30, 1 (1981).
21. R. F. Seamark, D. J. Kennaway, C. D. Matthews, A. J. Fellenberg, G. Phillipou, P. Kotaros, J. E. A. McIntosh, E. Dunstan and J. M. Obst, *J. Reprod. Fert.* Suppl. 30, 33 (1981).
22. D. P. Cardinali, *Endocr. Rev.* **2**, 327 (1981).
23. R. B. Land, *J. Reprod. Fert.* **48**, 217 (1976).
24. D. J. Kennaway, T. A. Gilmore and R. F. Seamark, *Endocrinology* **110**, 2186 (1982).
25. A. Foldes, C. A. Maxwell, R. M. Hoskinson and N. T. Hinks, in *Dynamics of Neurotransmitter Function* (Ed. I. Hanin), pp. 301–309. Raven Press, New York (1984).
26. E. D. Plotka, U. S. Seal, M. A. Letellier, L. J. Verme and J. J. Ozoga, in *Pineal Function* (Eds. C. D. Matthews and R. F. Seamark), pp. 45–56. Elsevier, North Holland (1981).
27. C. C. Rust and R. K. Meyer, *Science* **165**, 921 (1969).