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STRESS IN SHEEP RESULTING FROM MANAGEMENT PRACTICES

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SUMMARY

Preliminary studies on behaviour of sheep in the closed-field apparatus at Ruakura have indicated the development of severe stress in some animals. Plasma cortisol levels used as an indicator of this stress have also been measured with sheep under other management situations such as trucking, dipping, shearing and killing to assess the stressful nature of these conditions.

Individual sheep show significant differences in elevation of cortisol levels in a standard stress situation such as immobilization. The basal level of cortisol in sheep is of the order of 4 μg/100 ml plasma.

Sheep as a species have a uniform response-time of 30 to 40 minutes between onset of stressful stimuli and maximum blood cortisol level. The severity of the stress after peak output of cortisol can be determined by the slope of the decline to basal level. The sheep responds to small amounts of endogenous ACTH release by a return to baseline conditions before the peak response to stress occurs.

Betamethasone-treated sheep show depressed "basal" levels of cortisol and the peak levels of cortisol in response to adrenocorticotrophic hormones are lower than in control animals.

In the sheep, isolation from the mob and subjection to an unfamiliar environment are the major management variables which induce elevated blood cortisol levels.

ALMOST any change in the external environment of an animal such as physical injury or emotional upset is potentially capable of producing deleterious effects, but within limits the adaptive mechanisms of the animal allow it to accommodate to most changes or adverse situations. Brown (1967) has discussed stress stimuli and nominated such diverse things as sonic booms, surgical trauma, situations involving emotions such as fear, apprehension, anger and anticipation as able to elicit stress in animals; stress was defined as the generic term for immensely dissimilar events which have the common property of stimulating adrenocorticotrophic hormone (ACTH) secretion. Plasma corticoids provide a means for the routine assay of ACTH. Amoroso (1967) has suggested a definition and mnemonic of STRESS as Situations That Release Emergency Signals necessary for Survival.

Most psycho-endocrinal studies relating to the adrenal glands have been conducted in rats, dogs or man. There are few data on sheep. Reid and Mills (1962) measured plasma cortisol and blood glucose levels in ewes and showed changes associated with transport to unfamiliar environments, and Falconer and Hetzel (1964), after exteriorizing the thyroid gland, studied the influence of barking dogs, loud noises and restraint on changes in plasma protein bound ^{131}I. Holcombe (1957) has discussed urinary excretion of corticoids in both sheep and cattle.

STRESS IN THE CLOSED-FIELD TEST

Observations on twelve 3-year-old ewes in the closed-field apparatus at Ruakura have shown that handling techniques and general treatment of the animals were stressful to some of them. The sheep weighed 40 to 55 kg and were put on to a deprivation schedule and fed fresh grass (0.45 kg) and sheep nuts (0.35 kg) each day; one third of the animals gained weight. The sheep were required to move through a 22 m (72 ft) square enclosure to receive this food, and the inside barriers were altered to form a new detour problem each day for 12 days. The animals were run singly. All sheep at first showed a reluctance to move through the area. Several feigned lameness and others showed "displacement grazing" — *i.e.*, giving every appearance of grazing behaviour just above the level of the shavings on the floor. One animal became more and more difficult to drive around to the start, putting its head into the corners and refusing to move. A day or so later it died. A second animal also showed similar symptoms and, although immediately returned to pasture, it died. There was no post-mortem evidence of disease in both sheep. When the "bunt order" was analysed, it was found that these animals were lowest on the Dominance-Subordinance hierarchy and it was considered that they died from stress.

CORTISOL LEVELS AS A MEASURE OF STRESS

The correlation between stress and adrenal activity is well established (Ganong, 1963; Mason, 1968). In the sheep the ratio of cortisol to corticosterone is 20:1 (Hechter and Pincus, 1954). In the present studies, cortisol levels were determined fluorimetrically as described by Mattingly (1962) and modified by de Langen and Whittlestone (1970). The standard deviation of estimates of plasma

cortisol based on the mean of duplicate extracts of the sample and a regression line fitted to duplicates of blank, 10 and 20 μg of cortisol is ± 0.3 μg per 100 ml of plasma.

Blood samples were collected between 8 and 10 a.m. to allow for diurnal rhythm changes which may occur in sheep. In a small number of animals two samples (from left and right jugular veins) were taken to determine the repeatability of the cortisol levels in a particular animal. Similarly, in two sheep with jugular vein cannulae, repeated samples were obtained at intervals of approximately 30 seconds. These results are shown in Table 1. Only two pairs of samples of the 21 taken for comparison showed a difference greater than the expected error for the cortisol determination used — *i.e.*, ± 0.3μg per 100 ml of plasma.

TABLE 1: CORTISOL LEVELS IN PLASMA SAMPLES OBTAINED SIMULTANEOUSLY OR AT SHORT INTERVALS

Sheep	*Cortisol μg/100 ml* Right Vein	Left Vein	*Difference*
1	7.0	6.7	0.3
2	5.6	5.7	0.1
3	5.8	5.6	0.2
4	6.6	6.9	0.3
5	7 7	7.5	0.2
Average			0.2

Sheep	*Minutes*	*Cortisol μg/100 ml*	*Minutes*	*Cortisol μg 100/ml*	*Difference*
	4.5	3.5	5.5	3.5	0.0
	14.5	8.3	15	8.0	0.3
	24.5	7.3	25	7.0	0.3
	34.5	7.3	35	7.7	0.4
A	44.5	9.1	45.5	9.1	0.0
	55	9.4	55.5	10.4	1.0
	65	10.7	65.5	10.6	0.1
	75	12.4	76	12.4	0.0
	10	7.1	10.5	7.0	0.1
	20	8.5	20.5	8.8	0.3
	30	10.6	30.5	10.9	0.3
	39.5	10.5	40	10.3	0.2
B	50	12.5	51	12.5	0.0
	60	12.7	60.5	12.4	0.3
	70	12.4	70.5	12.4	0.0
	80	11.7	80.5	11.7	0.0

BASAL SECRETION RATES OF CORTISOL

Most workers consider that there is a basal secretion rate of cortisol to which is added the increase due to the ACTH produced by stressful stimuli. In studying blood cortisol in sheep an initial task was to determine the basal level. The mere handling of the animal may elevate cortisol level quite significantly. Very quiet sheep which had been handled over a period of months and which would approach and eat sheep nuts directly from the hand were sampled from the jugular vein while they were so eating. This was augmented by samples from regularly handled penned sheep and from pet lambs reared for behaviour studies. Table 2 gives these results. The average level of cortisol from these three groups of animals was 3.6 μg per 100 ml plasma; this value has been taken as the basal level.

During the next closed-field test of 6-tooth ewes, greater care was taken to avoid stress but one sheep showed the behavioural problems previously described and died. On the afternoon before death, 23 μg of cortisol per 100 ml of plasma was recorded, the highest level yet measured. From this preliminary work it was expected that the cortisol levels taken from animals during various farm management practices such as shearing and dipping might fall between baseline and this elevated level — *i.e.*, between 4 and 23 μg/100 ml plasma.

TABLE 2: BASAL PLASMA CORTISOL LEVELS (μg/100 ml) IN SHEEP

Hand-fed While Being Bled	*Regularly Handled Individually Penned Cannulated*				*Pet Lambs 10 Months Old*
3.5	3.5	4.3	2.2	2.5	5.0
3.2	3.3	4.5	3.3	2.3	2.9
2.8	4.6	4.3	3.8	3.8	2.7
3.7	2.5	3.0	2.9	2.5	3.9
3.7	3.3	3.5	2.3	1.9	4.1
5.8	3.5	4.0	3.7	4.0	2.7
3.8	3.1	5.1	2.9	2.2	7.4
6.5	3.1	4.8	4.0	2.5	3.7
5.6	3.6	4.6	2.7	1.9	
3.5	3.7	5.3	3.5	2.6	
	4.3	5.9	4.0	2.2	
	3.1	2.8	3.0	3.0	
—	—	—	—	—	—
Av. 4.2	3.5	4.3	3.2	2.6	4.1
—	—	—	—	—	—

KILLING METHODS AND STRESS

Considerable discussion has arisen regarding the humane slaughter of lambs by the present New Zealand killing method as compared with stunning prior to slaughter either by the captive bolt pistol or by the electric stunner. Thorpe (1967) has emphasized the necessity to distinguish between pain, a mechanism which safeguards the animal body, and "suffering", which is the prolonged anxiety and imaginative anticipation of further pain. As animal slaughter is a final process, it is meaningless to talk in terms of "suffering", but the stress of procedures immediately prior to death could be ascertained. Comparison of blood cortisol levels at death in animals killed at Horotiu freezing works or at Ruakura by either of the three above methods (10 sheep/group) were made. The results are shown in Table 3.

No significant differences in cortisol levels were found between lambs killed at the freezing works using either of the three methods but significant differences between methods existed for lambs killed at Ruakura. At the Ruakura abattoir the lambs were run into a small pen and left relatively undisturbed until seized and immediately slaughtered.

At the freezing works, the lambs are subject to a very strange environment, they are herded by dogs and shouting men, many after travelling some distance by road or rail. Finally, at the approach to the killing point they are

TABLE 3: PLASMA CORTISOL LEVELS (μg/100 ml) OF LAMBS RELATIVE TO METHOD OF SLAUGHTER

N.Z. Method	*Electrical Stunning*	*Captive Bolt Pistol*	*Ruakura*
12.5	9.6	7.6	7.9
13.8	11.0	7.7	5.1
10.0	10.2	5.8	2.3
10.9	11.1	13.3	5.0
9.3	8.3	8.8	4.9
10.2	12.3	10.9	5.4
10.7	9.5	11.9	4.4
15.0	10.1	12.5	3.8
7.8	12.0	8.7	5.2
11.1	7.7	10.8	8.1
Av. 11.1	10.2	9.8	5.2

forced into single file. It seems clear that the increased cortisol levels in these animals originated in prior events and it can be concluded that, provided the killing method is quick and accurate, which is not always the case with electrical stunning of woolly animals, the killing method contributes little or nothing to the stress of the animal involved.

FARM PRACTICES AS A SOURCE OF STRESS

Throughout the year, a small group of sheep which had been left undisturbed was sampled by jugular vein puncture at the termination of several management practices. These activities together with the intervals from commencement to the times of blood sampling were:

(1) Fast shearing — 5 minutes.

(2) Slow shearing — 10 to 15 minutes.

(3) Dipping (4 minutes immersion) — 5 minutes.

(4) Mobbed and held in pen for 3 minutes, using dog — 5 minutes.

(5) Trucked for 90 minutes — 90 minutes.

Table 4 shows the blood cortisol levels found after these treatments.

TABLE 4: PLASMA CORTISOL LEVELS (μg/100 ml) IN SHEEP FOLLOWING MANAGEMENT ACTIVITIES

Shearing 5 min	*Shearing 10-15 min*	*Dipping 5 min*	*Trucking 90 min*	*Dog-chasing 5 min*
8.3	15.8	5.7	4.6	5.7
7.0	11.6	7.6	6.7	4.3
5.6	7.7	9.1	4.4	8.6
5.8	13.7	7.0	8.3	6.3
6.6	12.4	6.4	8.1	9.6
7.7	10.3	5.3	7.0	16.0*
6.2	10.0	4.2	7.1	5.7
8.9	6.5		5.9	9.1
7.8	15.3		4.8	10.6*
7.8	10.0		7.0	8.7
			8.7	
			7.1	
—	—	—	—	—
Av. 7.2	11.3	6.5	6.7	8.5
—	—	—	—	—

*Bitten by dog.

The sheep sampled after fast shearing gave lower levels of cortisol than those sampled after the slow shearing routine. The variation in cortisol level between sheep was greatest in the group shorn slowly. The results from sheep after dipping in a circular dip where they were kept as a mob during the 4 minute soaking showed cortisol levels not significantly higher than baseline. The sheep chased in the mob by the dog, apart from two animals which were bitten, did not attain very high levels. Similarly, after trucking the mob of sheep, the cortisol levels were not high. In each of these latter situations, the animals were kept together as a group and this appears to be less stressful than if the sheep were isolated as for shearing. In view of the flocking behaviour of sheep as a species, these results were not unexpected. It is surprising, however, that during shearing cortisol may reach levels in excess of those obtained in sheep at slaughter at a freezing works.

RESULTS FROM SITUATIONS OF CONTINUOUS STRESS

Cortisol levels of sheep in the above trials were not as great as found in some animals subjected to a closed-field test. The possibility exists that continuous stress may lead to very high levels of blood cortisol. To examine this

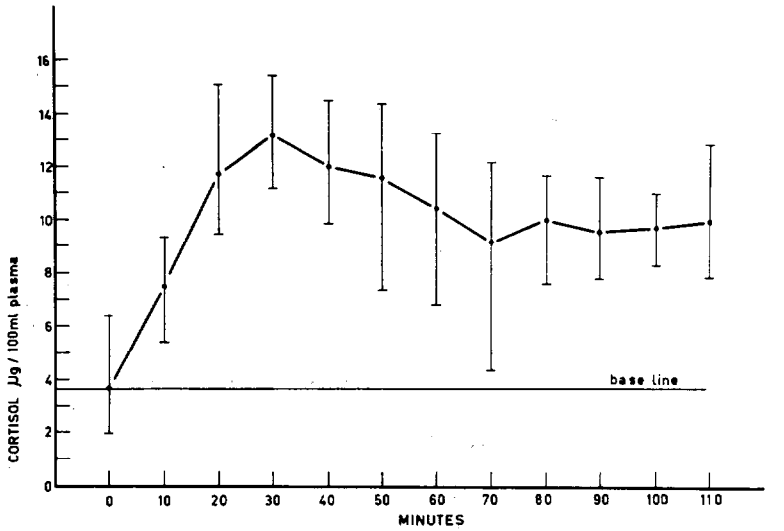

FIG. 1: *Average cortisol excretions during continuous stress by immobilization (5 sheep).*

further, several sheep with jugular vein cannulae were immobilized by tying the legs for several hours and blood collected every 10 minutes. The results from five animals are plotted in Fig. 1.

The cortisol levels reached a maximum about 30 minutes following the original stressful stimuli but then declined during the next one hour. At later observations, the graphs of cortisol levels in individual animals diverged, probably owing to additional release of ACTH, depletion of cortisol reserves, or other factors. It was obvious, therefore, that the timing of blood sampling is critical in comparative work in the sheep, especially as the response to stressful stimuli is slow. Responses for individual sheep also varied considerably.

CHANGES IN CORTISOL LEVELS AFTER INJECTION OF ACTH

The effect of ACTH in modifying the cortisol response to stress was studied using pairs of sheep with jugular vein cannulae and kept undisturbed in a room. Betamethazone to suppress the internal secretion of ACTH (Beaven *et al.*, 1964), and 0.1 to 50 i.u. doses of ACTH (Synacthen) were injected intravenously into one animal while the other acted as a control. Blood samples were taken at 10 minute intervals to follow changes in cortisol level and sheep were examined over several days. Figure 2 shows results from two sheep.

The blood samples taken prior to the injection of ACTH indicate levels of cortisol below baseline and show the result of betamethasone treatment. Cortisol levels increased slightly after injection of 0.1 i.u. ACTH and markedly after injection of 1 i.u. ACTH. There was very little difference between the responses to 10 and 50 i.u. and indicated an upper limit to ACTH treatment. The speed of the increase in circulating cortisol rises uniformly to the peak at 40 minutes after ACTH injection and either falls or is maintained dependent upon the amount of ACTH injected.

The overall levels of cortisol in the blood of betamethazone-treated animals was lower than in the control animals (Fig. 2) or in the immobilized sheep (referred to earlier), even though the peak levels occurred at similar times. The suppression of the basal secretory rate of cortisol by betamethazone could explain these observations. Nelson (1962) has reported that dogs responding to trauma maintained higher secretion rates of corticosteroids than could be obtained from increasing amounts of ACTH given intravenously to hypophysectomized dogs.

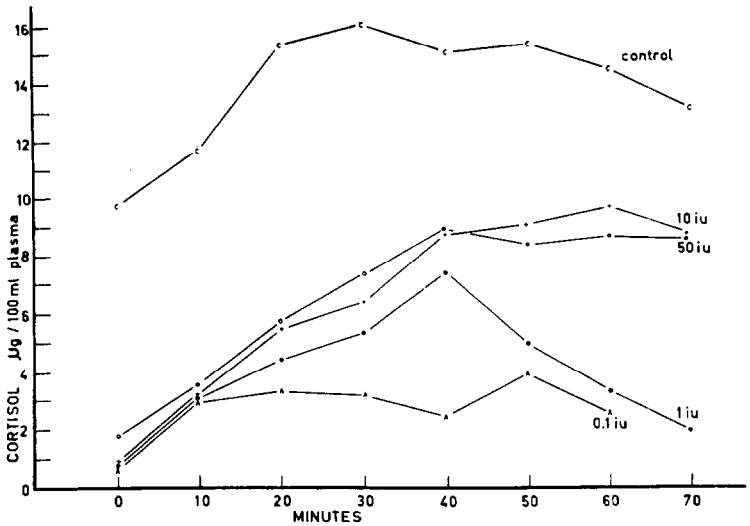

FIG. 2: *Cortisol response curves from sheep treated with betamethazone given different doses of ACTH.*

The maximum cortisol level attained by an animal on consecutive days varied. On the first day followed cannulation, levels of over 18 µg cortisol occurred 30 and 40 minutes after sampling began, but on the second and third days the maximum level of response was 12-13 µg cortisol and in spite of the increased ACTH dose. This observation suggests the possibility that effects of adrenal depletion may be shown in animals subjected to severe stress on consecutive days; alternatively, there may have occurred lowered reactions of the animal to handling after the first stressful experience.

COMPARISONS BETWEEN SPECIES

The cortisol responses to differing doses of ACTH in betamethazone-suppressed sheep can be compared with results from other species (either dexamethanzone-suppressed or hypophysectomized animals). Figure 3 shows diagrammatically results from rat, dog, man and sheep. In the dog, the first detectable rise in cortisol level occurs after 3 minutes and with increased dosage of ACTH up to 7 milliunits the levels rise to a maximum in 6 minutes. With increased ACTH, the peak response is

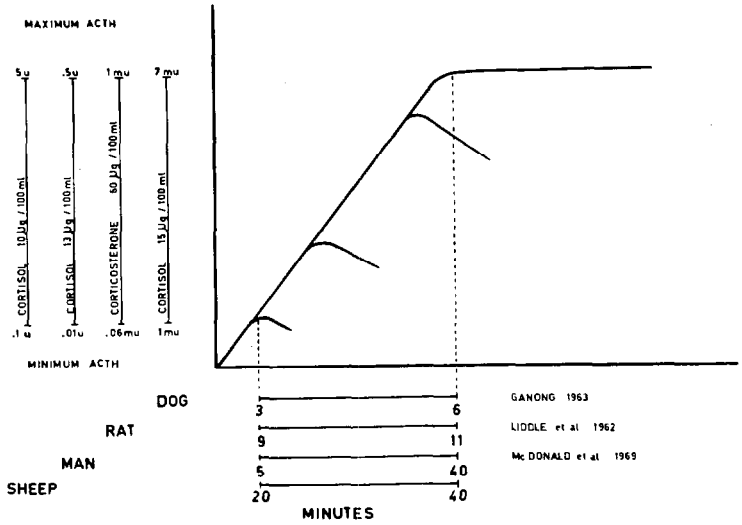

FIG. 3: *Cortisol response curve from several species.*

maintained over a longer period before levels return to baseline. In the rat, the response to ACTH is equally rapid; the time-interval between the first detectable changes in cortisol and the maximum response is 2 minutes. In man, the same interval is 35 minutes with the peak levels at approximately 40 minutes.

Figure 3 also records the amount of injected ACTH required for both minimum and maximum responses used by various workers and the peak response levels shown in each of the species described.

The intact animal was used in each of the above studies, with the blood samples being drawn from a similar point in the circulatory system. Beaven *et al.* (1964) took blood samples almost directly from the adrenal gland which had been transplanted into the neck of the sheep and obtained elevated levels of blood cortisol from 4 to 20 minutes after the injection of ACTH into dexamethazone-suppressed animals.

Time is an important factor in sampling the corticoid levels of different animal species if the data obtained are to be meaningful or useful for comparative purposes. Within one species, however, provided the point of sampling and the time after the stressful stimuli occurs are constant, valid comparisons can be made. With these restrictions, the effect of fast or slow shearing as stress-

ful stimuli may be considered. In the animals shorn fast, blood was sampled 5 minutes after initiation of stress. Cortisol levels were rising towards the peak which will result from the release of a given amount of ACTH. The average level was 7.2 μg cortisol per 100 ml plasma (range 5.6 to 8.9 μg). If the sampling were to be delayed until 10 minutes after stress, as is the case with sheep shorn slowly, then some animals with smaller amounts of ACTH will be past their peak while other animals would still show increasing levels of cortisol. Average level of 11.3 μg cortisol per 100 ml plasma (range 6.5 to 15.3 μg) were recorded in the sheep shorn slowly.

CONCLUSIONS

(1) Individual sheep show significant differences in elevation of blood cortisol levels in a standard stress situation such as immobilization. This is influenced by the present social status of the animal in the group and the nature of its previous experience of similar stressful stimuli.

(2) Sheep, in contrast to other species, give a uniform response-time of 30 to 40 minutes between the onset of stressful stimuli and the maximum cortisol output. The severity of the stress after peak output of cortisol can be determined by the slope of the decline to basal level. The sheep responds to small amounts of endogenous ACTH release by a return to baseline conditions before the peak response to stress occurs.

(3) The basal level of cortisol in sheep is of the order of 4 μg per 100 ml plasma.

(4) Betamethazone-treated animals show a depressed basal level of cortisol secretion and with known doses of ACTH injected intravenously the peak levels of cortisol in these sheep are lower than in control animals.

(5) In the sheep, isolation from the mob and subjection to an unfamiliar environment are the major management variables which induce elevated blood cortisol levels.

ACKNOWLEDGEMENT

The assistance of the staff of the Meat, Physiology and Hygiene, and Biometrics sections at Ruakura is gratefully acknowledged.

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