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CORTISOL IN PREGNANCY TOXAEMIA OF SHEEP

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SUMMARY

Of 31 pregnant ewes with clinical signs of pregnancy toxaemia, 24 had hypoglycaemia and hyperketonaemia at the time that a single blood sample was obtained. Twenty-five of these had a plasma cortisol concentration in excess of 10 ng/ml and six had a value below this. All the seven animals which did not show both hypoglycaemia and hyperketonaemia had a plasma cortisol concentration in excess of 10 ng/ml. Taking all the sheep together, 80% had a high plasma cortisol concentration. This could be the consequence of increased adrenal output or reduced excretion by the liver.

INTRODUCTION

Pregnancy toxaemia, which affects ewes carrying two or more fetuses in advanced pregnancy, has been a problem for flockmasters for many years. Although the incidence of the disease can be reduced by applying advances in knowledge of the nutritional requirements of pregnant sheep, it still occurs, especially when adverse weather interferes with the ewes' access to the food provided. Unlike acetonaemia of cattle, a disease which has some aspects in common with pregnancy toxaemia, the response to treatment is variable. One problem is the lack of a detailed knowledge of the metabolic disturbances which occur in pregnancy toxaemia. Some of these may be aspects of the disease itself and others may be a consequence of the inappetence which is a consistent clinical sign. Because many cases develop hypoglycaemia and hyperketonaemia and because acetonaemia of cattle responds to the injection of synthetic corticosteroids, some investigators have thought that cortical dysfunction may be an essential component of pregnancy toxaemia but others have suggested it may be a consequence of the disease.

Lindner (1959) measured cortisol, the main corticosteroid of sheep, by a method which detected concentrations above 15 ng/ml and found that values remained below this in fasted pregnant ewes which showed no clinical signs of the disease, but in 42 samples from 22 ewes which developed clinical signs, values rose to 43 ± 6 ng/ml. He could not say whether this result was due to increased adrenal output or to an impaired ability of the fatty liver, which was a consistent finding in the disease, to metabolize and excrete the hormone. Reid (1960) included Lindner's results in a paper which also reported that, in plasma samples from field cases of pregnancy toxaemia, 23 of 52 values were above 50 ng/ml. On the other hand, Saba *et al.* (1966) found that, although a combination of

transport to a new environment and fasting produced subacute signs of pregnancy toxaemia, plasma cortisol concentration of $13.7 \pm 0.29(8)$ ng/ml was less than that of $23.1 \pm 0.47(8)$ in ewes which although fasted, showed no signs of the disease. Furthermore, Saba & Cunningham (1971) reported the normal range of ovine plasma cortisol as 0–20 ng/ml with four of nine cases providing higher values. Furthermore, plasma cortisol concentration was also raised in clinical cases of hypocalcaemia. The authors concluded that both diseases caused sufficient 'stress' to produce an increase in the concentration of circulating cortisol.

In view of this equivocal situation we decided to carry out further investigations on the concentrations of cortisol in field cases of pregnancy toxaemia and to use a recently developed specific and sensitive method of measurement of cortisol based on an immunological technique.

MATERIALS AND METHODS

Several practitioners agreed to collaborate by completing a questionnaire when they encountered cases of pregnancy toxaemia and by providing blood samples before treatment had been given. It was agreed that, after the collection of a heparinized blood sample and a sample in oxalate-fluoride by vacutainer, the veterinary clinicians would apply whatever treatment they thought appropriate.

After centrifugation, plasma from the oxalate-fluoride sample was used to measure the concentration of glucose by the method of Morley *et al.* (1968). The concentration of 3-hydroxybutyrate was measured in plasma from the heparinized sample by the method described by Bergmeyer (1974). This plasma was also used to measure cortisol concentration by an enzyme linked immunoassay (Ford *et al.*, 1990). All clinical signs considered relevant were recorded on the report form, but rather than defining the disease as the presence of all or a particular number of specific signs, we accepted the diagnosis of pregnancy toxaemia made by the practitioner.

RESULTS

Table I gives the results of analysis of samples obtained before treatment from 31 sheep diagnosed as suffering from pregnancy toxaemia on clinical evidence. Their known ages ranged from 3 to 8 years although some may have been older as judged by their loss of incisor teeth. The plasma concentrations of glucose and of 3-hydroxybutyrate are given in columns 4 and 5 of the table and that of cortisol in column 3.

In previous work on sheep pregnant with twins (Ford & Evans, 1986), we had established a normal range of plasma glucose of 3.1–3.9 mmol/l and of 3-hydroxybutyrate of 0.8–2.2 mmol/l. If the concentration of glucose in plasma was below and that of 3-hydroxybutyrate was above its normal range, the word 'yes' in column 6 indicates that the original clinical diagnosis had been confirmed by plasma analysis. Twenty-four of the 31 sheep fell into this category, seven did not. Five of the seven, i.e. Nos. 5, 16, 20, 23 and 29, in spite of showing clinical signs of pregnancy toxaemia, had a plasma glucose concentration within or above the normal range and plasma 3-OH butyrate within or below the normal range. Two sheep were in neither category. No. 2 had low glucose and low 3-OH butyrate and No. 26 had low glucose and normal 3-OH butyrate.

TABLE I
Concentrations of glucose, 3-OH butyrate and cortisol in the plasma of 31 sheep with clinical signs of pregnancy toxaemia

Sheep number	*Age*	*Plasma cortisol (ng/ml)*	*Plasma glucose conc. (mmol/l)*	*Plasma 3-OH butyrate (mmol/l)*	*Pregnancy toxaemia confirmed by plasma analysis*	*Cortisol > 10 ng/ml*
1	8	4	1.3	2.4	Yes	No
2	4	7	1.7	1.0	No	No
3	7	8	0.4	2.4	Yes	No
4	6	8	1.0	4.3	Yes	No
5	4	9	4.1	0.5	No	No
6	3	9	0.8	6.0	Yes	No
7	4	11	1.25	3.4	Yes	Yes
8	4	11	2.6	3.2	Yes	Yes
9	5	12	2.7	4.4	Yes	Yes
10	4	12	1.1	6.0	Yes	Yes
11	5	14	1.1	6.1	Yes	Yes
12	5	16	0.7	5.7	Yes	Yes
13	5	20	1.22	3.6	Yes	Yes
14	4+	21	2.3	3.1	Yes	Yes
15	4	21	1.25	4.3	Yes	Yes
16	4	25	4.1	1.4	No	Yes
17	4+	25	1.9	2.4	Yes	Yes
18	4	26	1.1	5.0	Yes	Yes
19	5	30	2.8	4.0	Yes	Yes
20	6	64	5.1	0.6	No	Yes
21	4+	73	2.5	4.1	Yes	Yes
22	5	73	0.8	3.7	Yes	Yes
23	4	74	4.0	1.4	No	Yes
24	5	85	2.7	6.4	Yes	Yes
25	5	93	1.4	4.1	Yes	Yes
26	6	95	1.7	1.6	No	Yes
27	4+	120	2.7	1.9	Yes	Yes
28	5	125	1.0	6.6	Yes	Yes
29	4+	160	3.4	2.4	No	Yes
30	4+	160	1.4	6.1	Yes	Yes
31	3.5	240	2.3	4.4	Yes	Yes

DISCUSSION

Our results have confirmed that most cases of pregnancy toxaemia, i.e. 24 of 31, have a low concentration of glucose and a high concentration of 3-OH butyrate (the major ketone body of ruminants) in their plasma whereas seven animals did not fit into this category. A possible explanation is that all cases are not seen at the same stage of their development and that change of concentration from the normal range to either above or below such a value requires a period of time. Perhaps if the seven sheep of our series had been resampled some hours later, they might have shown the same pattern as the other 24.

The range of plasma cortisol concentrations from 4 to 240 ng/ml in these sheep is wide. Saba & Cunningham (1971), using a chemical extraction method, reported a normal range of 0–20 ng/ml.

We have found that plasma cortisol concentration measured by our enzyme immunoassay is influenced by the amount of disturbance and handling of the animals. Individually penned sheep used to handling, when bled through an indwelling catheter, have shown values of 3.01 ± 0.39 ng/ml whereas animals kept in groups in the open have mean values of $8.0 \pm 1.0(16)$ ng/ml. If we accept the normal range of samples collected by needle puncture to be up to 10 ng/ml then 25 of our 31 sheep had an increased plasma cortisol concentration. All the six with cortisol concentration within our suggested normal range showed clinical signs of pregnancy toxaemia and four of them also had low plasma glucose and high plasma 3-OH butyrate concentration.

However, our results cannot indicate whether the high concentrations were the result of increased adrenal production of cortisol or due to the inability of the fatty liver found in pregnancy toxaemia to break down and excrete the circulating cortisol. We hope to gain some information on this aspect of the problem by isotope infusion experiments.

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