Onderstepoort J. Vet. Res. (1966), 33(1), 161–168
Printed in the Republic of S. Afr. by the Government Printer, Pretoria

OVINE KETOSIS. V. KETONE BODY AND BLOOD GLUCOSE LEVELS OF MERINO EWES DURING THE PRECLINICAL, CLINICAL AND POSTCLINICAL STAGES OF PREGNANCY DISEASE

J. PROCOS* and F. M. C. GILCHRIST*, Veterinary Research Institute, Onderstepoort

Most of the workers who have studied pregnancy disease (Fraser, Godden, Snook & Thomson, 1938; Groenewald, Graf, Bekker, Malan & Clark, 1941, and Gill & Thomson, 1954) have drawn attention to the presence of variable degrees of hypoglycaemia while a number have also noted a degree of hyperketonaemia. There is, however, little or no information available concerning the fluctuations which occur in the blood levels of ketone bodies and glucose from day to day prior to, and throughout, the course of the disease. We felt that such information, and particularly that obtained during the preclinical stages, was more likely to contribute to our understanding of the basic causes of this metabolic disorder than information confined to the clinical stage. Accordingly an attempt was made to induce the disease in Merino ewes, and to follow the blood levels of these two metabolites through the preclinical, clinical and postclinical stages.

Three treatments, including two previously employed by other research workers, were used to induce the disease. These were sudden changes of diet (Groenewald *et al.*, 1941), short term starvation (Holmes, 1953, 1958; McClymont & Setchell, 1955) and oral dosing of Sulphadimidine. Daily determinations of blood glucose and ketone levels were performed throughout the experimental period.

Materials and Methods

Animals

Twenty three well-nourished, adult Merino sheep were used. Of these, four were wethers, three non-pregnant ewes, while 16 were pregnant ewes selected for being in approximately the same stage of pregnancy. Each animal was housed under cover in an individual pen.

* Employed by the Stock Diseases Research Fund of the Dairy, Meat and Wool Boards of the Republic of South Africa

Received for publication on 11 August 1965.—Editor

7219177–11

Treatments

Initially the animals were divided into two groups. Those belonging to the first group (12 animals) each received treatment A, while those of the second group (11 animals) each received treatment B. After a seven-day period in which the blood glucose and ketone body levels on these basic diets were established, the animals were subjected to the following treatments or combinations thereof:

A: maintained on good quality lucerne hay 1,250 gm, concentrates (yellow maize meal 65 per cent, wheat bran 10 per cent, lucerne meal 10 per cent, white fish meal 5 per cent, groundnut flour 5 per cent, 4:1 bone meal: salt 5 per cent) 300 gm daily;

B: maintained on freshly cut green lucerne 2,000 gm, concentrates 300 gm daily;

C: three days starvation;

D: changed to poor quality grass hay (3 per cent crude protein) 400 gm daily;

E: changed to fresh green lucerne 2,000 gm daily;

F: dosed intra-ruminally with Sulphadimidine, 0·2 gm/kg body weight on the first day, followed by 0·1 gm/kg body weight, daily for five days.

The hay or the green lucerne was given in two equal portions at 9 a.m. and 12 noon, and a record was kept of the food consumed by each animal. Water was freely available at all times. Samples of jugular blood (3 ml) were obtained from each sheep every morning at 8 a.m. before feeding.

Analytical

(*a*) Ketone bodies: Total blood ketone bodies were estimated by the method of Thin & Robertson (1952).

(*b*) *Blood glucose:* Blood glucose was determined using Lehmann & Silk's (1952) modification of the Folin & Wu (1920) method.

Results

Lamb production and gestation periods

Of the 16 pregnant ewes, 13 gave birth to single healthy lambs. The lamb of ewe 22 died two hours after birth. Ewe 15 carrying a single lamb died before giving birth, and when ewe 16 was killed *in extremis*, she was found, at *post mortem*, to be carrying twins. The average length of gestation was 149 days with a range of 143 to 156 days.

Effect of treatments on the incidence of clinical symptoms

The clinical symptoms of pregnancy disease observed ranged from loss of appetite, nervousness, lethargy, imbalance due to partial or total blindness, and nervous spasms. Of the 17 animals subjected to the various treatments, eight showed clinical symptoms which were either severe and irreversible (15, 16, 22) or mild and transient in nature (8, 10, 19, 20, 21).

TABLE 1.—*Relation between initial diets, treatments and clinical symptoms*

Initial Green Lucerne Diet

Changed to:	No change	Starvation then Poor Hay	Poor Hay and Sulphadimidine	Poor Hay
	13 (W) 14 (N.P.E.) 18 (P.E.)	17 (P.E.) 19* (W) 15* (P.E.)	21* (N.P.E.) 16* (P.E.) 22* (P.E.)	20* (P.E.) 23 (P.E.)

Initial Lucerne Hay Diet

Changed to:	No change	Starvation then Poor Hay	Poor Hay and Sulphadimidine	Poor Hay
	1 (W) 2 (P.E.) 6 (P.E.)	7 (W) 9 (N.P.E.) 10* (P.E.)	4 (P.E.) 5 (P.E.) 11 (P.E.)	3 (P.E.) 8* (P.E.) 12 (P.E.)

*Clinical Symptoms. W = wether. N.P.E. = non-pregnant ewe. P.E. = pregnant ewe.
Numbers indicate individual experimental animals.

The relationships between treatments and clinical symptoms are presented in Table 1. The treatment most conducive (three out of three sheep) to clinical symptoms was that in which the original green lucerne diet was substituted by poor hay with simultaneous dosing of Sulphadimidine. Two clinical cases were obtained in a group of three sheep whose initial green lucerne diet was replaced by one of poor hay after a three-day period of starvation The remaining treatments appeared to be either less effective or completely ineffective as in the case of the treatment in which Sulphadimidine dosing was instituted without any alteration of the original lucerne hay diet.

The relation between clinical symptoms, ketone body and blood glucose levels

The ketone blood levels (mean 4·9, range 0·6 to 12·9 mg per cent) of the animals in the initial seven day period before they were subjected to the various treatments were generally higher than those reported previously for well-nourished sheep (Procos, 1961). This was in all probability due to the large diurnal fluctuations in ambient temperature (mean difference between maximum and minimum temperature 17·5, range 15·0 to 21·5°C) encountered during this period, and which have been shown to affect ketone body levels of sheep (Procos, 1961).

TABLE 2.—*The relation between clinical symptoms, ketone body and blood glucose levels*

Sheep No.	Ketone bodies (mg %)		Blood glucose (mg %)	
	At appearance of symptoms	Maximum	At appearance of symptoms	Minimum
22	13·4	48·6	25·0	13·5
16	17·7	42·1	24·0	14·0
20	23·7	44·7	20·0	15·5
10	12·3	14·1	30·0	16·0
8	9·9	15·4	22·0	19·0
15	35·3	53·6	33·5	19·5
21 (N.P.E.)	12·3	12·6	44·5	23·0
19 (W)	13·2	17·4	34·5	34·5

At the time of the appearance of symptoms the ketone body levels of all the affected animals were greater than or equal to 10 mg per cent as shown in Table 2. By contrast, only four of the animals were hypoglycaemic with blood glucose levels equal to or less than 25 mg per cent at this stage. The highest ketone body levels obtained were found among pregnant sheep with clinical symptoms. However, the minimum blood glucose levels of these animals (13·5, 14·0, 15·5, 16·0, 19·0, 19·5, 23·0, 34·5 mg per cent) were of the same order as those encountered in non-pregnant sheep (16·0, 19·0, 20·0, 21·0 mg per cent) which had at no stage shown any clinical symptons. In no instance did the highest blood ketone and the lowest blood glucose levels coincide with the first appearance of symptoms (Table 3).

TABLE 3.—*Time of occurrence of clinical symptoms, maximum ketone body and minimum blood glucose levels*

Sheep No.	Days after commencement of treatment		
	Symptoms	Ketone body maximum	Blood glucose minimum
22	7	16	4
16	2	4	4
20	16	10	15
10	8	2	3
8	17	12, 16	18
15	9	10	6
21 (N.P.E.)	14	12	16
19 (W)	4	9	4

The relation between clinical symptoms and duration of ketosis and hypoglycaemia

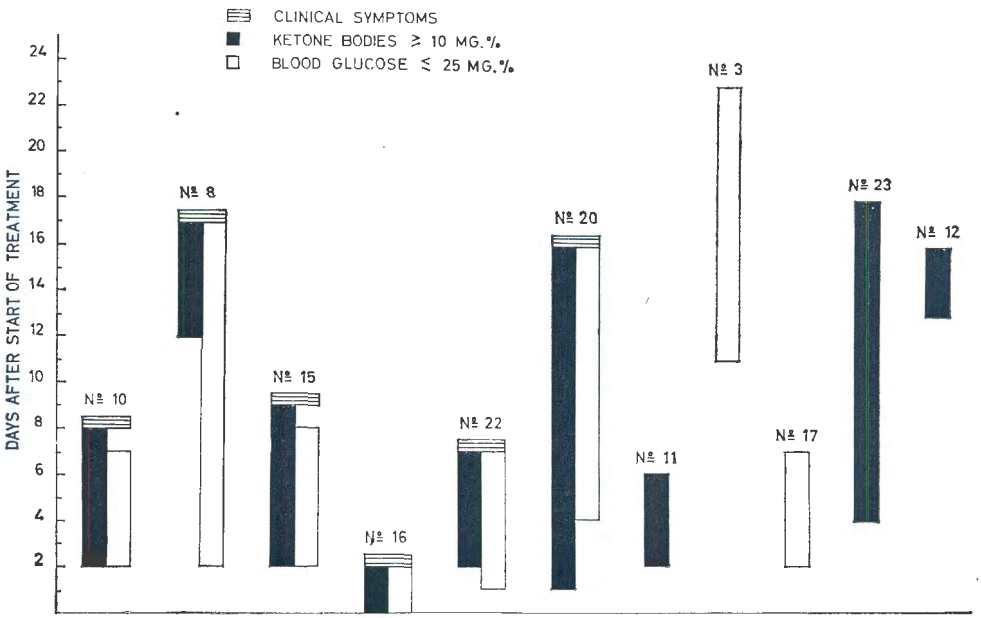

FIG. 1.—Duration of ketosis and hypoglycaemia before appearance of symptoms

The appearance of clinical symptoms amongst pregnant ewes was invariably preceded by both persistent (lasting at least three consecutive days) ketosis and hypoglycaemia (Fig. 1). By contrast, animals who had only displayed either persistent ketosis: 12 (3 days), 11 (4 days) and 23 (14 days) or persistent hypoglycaemia 17 (5 days) and 3 (12 days) had at no stage exhibited symptoms of pregnancy disease. These findings were less clearly defined amongst the non-pregnant ewes and wethers.

DISCUSSION

Despite the enormous amount of work that has been done on pregnancy disease, final agreement has not been reached on the following fundamental aspects:

(*a*) the underlying metabolic and hormonal disturbances leading to hypoglycaemia and ketosis; and the interrelationship between these two symptoms, and

(*b*) the exact cause of the nervous symptons of cerebral dysfunction.

It is felt that the present work throws some light on the latter.

Ruminant cerebral tissues appear capable of adapting to low levels of circulating glucose which would evoke symptoms of cerebral dysfunction in non-ruminant mammals. The change from lamb to adult status in sheep is accompanied by a fall in the normal blood glucose levels from 80 to 90 (Reid, 1953; Jarret, Jones & Potter, 1964) to 25 to 50 mg per cent (Reid, 1950). In the present work a sudden reduction in the nutrition (Treatments D; C + D) of ewes 3 and 17 in their last month of gestation, resulted in a fall in blood glucose to hypoglycaemic levels of less than 25

mg per cent, for twelve and five days, without the appearance of any symptoms of cerebral dysfunction. Similar observations have been reported by McClymont & Setchell (1955) and Reid & Hogan (1959). These findings would seem to indicate that the nervous symptoms of pregnancy disease in sheep are not due to low levels of circulating blood glucose *per se*.

On the other hand, it is possible that the adrenal cortical hyperactivity associated with the disease (Reid, 1960) impairs progressively the utilization of the glucose relative to the blood glucose level (Bassett, 1963). Thus the glucose consumption rate of the cerebral tissues which is ten times that of the body as a whole (Moss, 1964) would be diminished at hypoglycaemic levels. This could account for the nervous symptoms, since glucose constitutes an obligatory energy source for these tissues (Fazekas, 1958), and a diminution in the rate of glucose utilization invariably results in impairment of cerebral function (Moss, 1964). Evidence in favour of this hypothesis is the fact that, in the present work, nervous symptoms occurred only among those hypoglycaemic (blood glucose < 25 mg per cent) animals with a persistent (≥ 3 days) hyperketonaemia (blood ketones ≥ 10 mg per cent), which was very likely brought about by adrenal cortical hyperactivity elicited by the stress of suddenly reduced nutrition (treatments C + D; D + F; D), particularly in the heavily pregnant ewes (10, 15; 16, 22; 8, 10). The absence of nervous symptoms in ketotic animals (11, 12, 23) with blood glucose levels which fell within the normal range, indicated that the rate of glucose utilization by the cerebral tissues at these higher levels of circulating glucose was still sufficiently rapid to prevent dysfunction, and is thus in harmony with the above hypothesis.

Summary

Pregnancy disease was induced in well-conditioned Merino sheep by subjecting them to sudden changes of diet, short term starvation or oral dosing of Sulphadimidine.

The blood levels of ketones and glucose were followed daily throughout the preclinical, clinical and postclinical stages of this metabolic disorder.

Typical clinical symptoms of cerebral dysfunction were obtained in six pregnant ewes entering their last month of gestation, a non-pregnant ewe, and a wether.

No relation was found between clinical symptoms, minimum blood glucose or maximum blood ketone levels or the time at which these occurred, thus indicating that the nervous symptoms were not due to either the low levels of circulating blood glucose or the high levels of ketone bodies *per se*.

The appearance of clinical symptoms amongst the pregnant ewes was invariably preceded by both a persistent hypoglycaemia and a persistent ketosis; this was less clearly defined in the case of the non-pregnant ewe and the wether.

The possibility that the nervous symptoms could have been due to impaired glucose utilization by the cerebral cells, brought about by adrenal cortical hyperactivity is discussed.

Acknowledgements

The authors wish to thank Professor R. Clark for his help with this work, Thanks are also due to Miss A. Scholz and Mr. R. J. J. Briel for expert technical assistance.

REFERENCES

BASSETT, J. M., 1963. The influence of cortisol on feed intake and glucose metabolism in sheep *J. Endocrinol.* Vol. 26, p. 539.

FAZEKAS, J. F., 1958. Pathologic physiology of cerebral dysfunction. *Amer. J. Med.* Vol. 25, p. 89.

FRASER, A. H. H., GODDEN, W., SNOOK, L. C. & THOMSON, W., 1938. The influence of diet upon ketonaemia in pregnant ewes. *J. Physiol.* (Lond.), Vol. 94, p. 346.

GILL, J. C. & THOMSON, W., 1954. The production of ketonaemia and pregnancy toxaemia in ewes through manipulation of the feeding level. *J. Agric. Sci.*, Vol. 45, p. 229.

GROENEWALD, J. W., GRAF, H., BEKKER, P. M., MALAN, J. R. & CLARK, R., 1941. Domsiekte or pregnancy disease in sheep. *Onderstepoort J. Vet. Sci. Anim. Jnd.*, Vol. 17, p. 245.

HOLMES, J. R., 1953. Pregnancy toxaemia in grassland flocks. *Vet. Rec.*, Vol. 65, p. 299.
HOLMES, J. R., 1958. Ketosis in the ruminant. *Vet. Rec.*, Vol. 70, p. 1012.

JARRETT, I. G., JONES, G. B. & POTTER, B. J., 1964. Changes in glucose utilization during development of the lamb. *Biochem. J.*, Vol. 90, p. 189.

LEHMANN, H. & SILK, E., 1952. The prevention of colour fading in the Folin and Wu estimation of the blood sugar. *Biochem. J.*, Vol. 50, p. 31.

McCLYMONT, G. L. & SETCHELL, B. P., 1955. Ovine pregnancy toxaemia. *Aust. Vet. J.*, Vol. 31, p. 53.

MOSS, G., 1964. The contribution of the hexose monophosphate shunt to cerebral glucose metabolism. *Diabetes*, Vol. 13, p. 585.

PROCOS, J., 1961. Ovine Ketosis. I: The normal ketone body values. *Onderstepoort J. Vet. Res.*, Vol. 28, p. 557.

REID, R. L., 1950. Studies on the carbohydrate metabolism of sheep. I. The range of blood-sugar values under several conditions. *Aust. J. Agric. Res.*, Vol. 1, p. 182.

REID, R. L., 1953. Studies on the carbohydrate metabolism of sheep. VI. Interrelationships between changes in the distribution and levels of glucose and in the levels of volatile fatty acid in the blood of lambs. *Aust. J. Agric. Res.*, Vol. 4, p. 213.

REID, R. L., 1960. Studies on the carbohydrate metabolism of sheep. XI. The role of the adrenals in ovine pregnancy toxaemia. *Aust. J. Agric. Res.*, Vol. 11, p. 364.

REID, R. L. & HOGAN, J. P., 1959. Studies on the carbohydrate metabolism of sheep. VIII Hypoglycaemia and hyperketonaemia in undernourished and fasted pregnant ewes. *Aust. J. Agric. Res.*, Vol. 10, p. 81.

THIN, CHRISTIAN & ROBERTSON, A., 1952. The estimation of acetone bodies. *Biochem. J.* Vol. 51, p. 218.