J. Physiol. (1938) 94, 346–357 612.63:616.153.61

THE INFLUENCE OF DIET UPON KETONAEMIA IN PREGNANT EWES

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(*Received* 18 *August* 1938)

Introduction

An important cause of mortality in pregnant ewes is variously called "pregnancy disease", "pregnancy toxaemia", or "twin-lamb disease". Its exact aetiology is unknown, although there is general agreement that it is not infectious, that it occurs most frequently in multiple pregnancies, and that it is in some way predisposed to by dietary factors.

The symptomatology is reasonably characteristic. In an affected ewe the gait becomes uncertain and sight is often affected, some animals apparently becoming totally blind. Coma and death usually follow. If parturition occurs the ewe frequently recovers. Post-mortem, the most characteristic feature is pronounced fatty infiltration or degeneration of the liver.

The disease is associated with a marked ketosis, both blood and urine containing excessive amounts of ketone bodies. A strong odour of acetone can often be detected in the breath. Sampson & Hayden [1935] report that in four ewes with pregnancy disease the total ketones in the blood ranged from 36 to 49 mg./100 c.c., whereas in nine normal pregnant ewes the average value was only 3 mg. Allcroft & Green [1938] have found values up to 145·8 mg./100 c.c. in serum from ewes with pregnancy disease.

The ketonaemia is accompanied by a hypoglycaemia. Roderick & Harshfield [1932] found that many ewes with pregnancy disease had blood sugar levels below 30 mg./100 c.c., whereas the value in normal pregnant ewes ranged from 40 to 57 mg. Lyle Stewart [1938] states that in ewes with pregnancy disease, "the blood sugar was frequently, but not invariably, very low".

Among the various possible dietary factors suggested as predisposing to the disease, the classical theory is that of Gilruth [1899], who first described the condition. He held that it is due to overfatness and lack of exercise in pregnant ewes. His views have been supported by McFadyean [1924], Greig [1929], Belschner [1930] and Cameron [1937].

The precisely opposite view, namely, that pregnancy disease is due to under-nutrition of the pregnant ewe, has been supported by Leslie [1931, 1933], Dayus & Weighton [1931], Roderick & Harshfield [1932, 1937], Hopkirk [1934] and McLinden [1937].

A modification of this view was suggested as a result of a survey carried out by the North of Scotland College of Agriculture in its local area. From the replies to a questionnaire issued to farmers who had suffered losses from pregnancy disease, it appeared that protein deficiency in the rations fed might be a predisposing factor.

Finally, field observations made on the Duthie Experimental Stock Farm, attached to the Rowett Institute, suggested that sudden food deprivation, following snow storms, might predispose to the condition.

During the winter of 1937–8, an attempt was made at the Rowett Institute to assess the probable importance of these various suggested dietary factors in the experimental production of ketonaemia in pregnant ewes. Although ketonaemia was chiefly under consideration, observations were made also on the clinical condition of the ewes and on the state of the liver of those dying or killed during the course of the experiment.

The experiments were designed to test the various hypotheses as to the dietary factors predisposing to pregnancy disease which have been outlined above:

(1) The effect of gross over-fatness plus close confinement.

(2) The effect of sudden enforced fast on ewes grossly over-fat and closely confined.

(3) The effect of under-nutrition followed by sudden enforced fast.

(4) The effect of under-nutrition followed by liberal feeding during the last month of pregnancy.

(5) The effect of variation in the protein intake.

Methods

Grouping. Ninety aged Greyface ewes were used in these experiments. They were kept in pens indoors so that close control of their diet was possible. In October, after being accustomed to the pens, the ewes were mated with a Suffolk ram, and kept under observation throughout their pregnancy.

The ninety ewes were divided into six similar groups of fifteen. The numbering of the groups, their summarized treatment, and the starch and protein equivalents of their rations are shown in Table I.

TABLE I

Group	Summarized treatment	Daily intake: Starch equiv. lb.	Daily intake: Protein equiv. lb.
1	Fed to over-fatness	1·69	0·24
2	Fed to over-fatness. Periods of fast	1·69	0·24
3	Under-nourished. Period of fast	0·75	0·10
4	Under-nourished. Period of liberal feeding in last month of pregnancy	0·75 1·71	0·10 0·40
5	Low protein intake	0·80	0·086
6	Adequate protein intake	0·81	0·154

Blood sampling. In order to study the progressive changes in certain constituents of the blood as pregnancy advanced, the ewes were bled at repeated intervals. The blood was drawn from the jugular vein. Estimations were made soon after the blood was withdrawn. With two exceptions all routine bleedings were carried out between 8.45 and 9.15 a.m.

Estimations made on the blood. Because of the number of animals involved, routine estimations were restricted to those thought likely to be of major importance, namely total ketone bodies and blood sugar. At the commencement of the experiment, midway in pregnancy, and just before lambing, the serum inorganic P, Ca and phosphatase were estimated for each ewe. These were found to be normal for the particular stage in pregnancy.

Analytical methods

Total ketone bodies in blood. The gravimetric method, using Dénigès' reagent, as described by Peters & van Slyke [1931], was employed. From a series of tests made on blood from ketonaemic ewes, it appeared that the "ketone" present was mostly in the form of β-hydroxybutyric acid, as a negligible precipitate was obtained prior to oxidation of the blood filtrate with dichromate solution. For this reason, the figures for "total ketones" in terms of acetone, present in blood, were calculated on the assumption that all the acetone precipitated was obtained from β-hydroxybutyric acid, i.e. the mass of the precipitate in mg. was multiplied by $1\frac{1}{3}$.

Blood sugar. The Somogyi modification of the Shaffer-Hartmann method as described by Peters & van Slyke [1931] was used. Reagent no. 2 was used—this is sensitive to low concentrations of blood sugar.

Serum inorganic P, *serum* Ca, *and serum phosphatase.* The methods adopted at the Rowett Institute [Godden, 1937] were used.

Results

Group I. This group of 15 ewes was fed a ration of 6 lb. turnips, 2 lb. meals and ½ lb. hay per head daily. On this ration the ewes became grossly fat. The average body weight increased from 141 lb. on 27 October 1937 to 191 lb. on 2 March 1938. The blood sugar was consistently high (up to 68 mg./100 c.c.) and ketonaemia was completely absent. Three ewes died from complications due to pregnancy, but none showed any of the symptoms usually associated with pregnancy disease. The livers of the ewes which died appeared macroscopically normal, were shown by chemical analysis to contain the usual lipids in the normal ratios, and on microscopic examination showed no evidence of fatty infiltration. In short, the combination of over-fatness and confinement in these pregnant ewes produced none of the symptoms or pathological changes associated with pregnancy disease of sheep.

Group II. This group of fifteen ewes was treated precisely as Group I except that they were subjected to periods of food deprivation in late pregnancy.

Until the sheep in this group were fasted on 5 February, their history was precisely similar to that of Group I. Their body weight increased, their blood sugar remained at a high level; there was no vestige of ketonaemia. But towards the end of the second day's fast, the ewes became lethargic, and some were too weak to stand. After a 48 hr. fast the ewes were bled prior to refeeding, when it was found that the average figure for blood sugar had fallen by 25% (to 40 mg./100 c.c.) and that two ewes had developed slight ketonaemia. Quarter rations were then supplied but the ewes showed little desire for food, some of them eating practically nothing. On the next day (18 February) transient blindness in some ewes and weakness in all was noted. At 11 a.m. three sheep were in a semi-comatose condition. These animals, however, recovered. By 24 February all the sheep except one, appeared normal. Despite the improvement in appearance it was found that their blood contained a considerable amount of ketone bodies (nine averaged 28 mg./100 c.c.). When the full ration was again supplied the sheep continued to improve rapidly and on 5 March it was found that their blood contained no ketone bodies and that the blood sugar was as high as prior to starvation.

The individual response to fasting was very varied, some sheep within the group showing little change in appearance or in blood constituents, while in others marked abnormalities occurred. Moreover, there ap-

peared to be no close correlation between the clinical appearance of the sheep and ketonaemia or blood sugar level. Thus the ewe (no. 85) which appeared clinically to be most badly affected maintained a normal blood composition, even when semi-comatose; while in another ewe (no. 51) which appeared normal it was found that the blood contained 32 mg./100 c.c. of ketone bodies and that the blood sugar level had fallen from 56 to 37 mg./100 c.c. Ewe no. 85 subsequently lambed with assistance two lambs, which had been dead at least a week. She had to be slaughtered owing to excessive haemorrhage from a torn uterus. Ewe no. 51 lambed normally two good lambs.

A second period of fasting was imposed on 5 March. It was thought probable that the phenomena observed in the first fast would be repeated in an intensified form. The results, however, were different. The clinical symptoms of lethargy, weakness and semi-coma were not observed on this occasion. On the contrary, blood changes, not well marked as a result of the first fast, were much more pronounced. Thus, the average blood sugar level of the entire group fell by 50%, and four ewes had blood ketones of 13, 13, 15 and 19 mg./100 c.c. respectively. On restoration of the full ration the blood of all the ewes except one returned to normal and all the sheep appeared to be in good health.

Group III. This group of fifteen ewes was fed on a ration of ½ lb. hay mixture, 2½ lb. turnips and 0·8 lb. meals per head per day. On this ration the ewes gained very slightly in weight during pregnancy. Since, under normal conditions, pregnant ewes gain considerably in weight in the latter part of pregnancy (Rowett Institute, unpublished observations), it may be accepted that this group was under-nourished. Moreover, the ewes, towards the end of the experiment were obviously lean and weak. The average body weight at the beginning of the experiment was 142 lb., and at its termination 148 lb.

In contrast to Group I, which was fed to repletion and which showed no ketonaemia, ten out of the fifteen under-nourished ewes in Group III developed ketonaemia of varying degree. This ketonaemia was associated with a hypoglycaemia.

On 25 February a 48 hr. fast was imposed on this group. The first effect was noticed at the 34th hr. of the fast, when one ewe (no. 37) appeared to be blind. By next morning this ewe was unsteady and swayed when made to walk. Two other ewes (nos. 56 and 25) had by this time become blind and weak, one swaying when walking. Other five ewes (nos. 21, 6, 26, 62 and 70) were dull and stood with drooping heads and limp ears. After the fast was ended, the affected ewes recovered,

although they took some little time to regain appetite, and failed to regain the weight they had lost.

The period of fasting accentuated the abnormalities of the blood to a marked degree, the ketone bodies increasing by over 100% and the blood sugar dropping to 23 mg./100 c.c.

Subsequent to lambing, it was found that the earlier behaviour of the individual ewes in this group was correlated with the number of lambs they were carrying. Thus, one ewe proved barren. Although she lost 4 lb. in weight during the experimental period, at no time did she show any ketonaemia or hypoglycaemia. Moreover, ketonaemia did not occur except during fasting in the four ewes which bore single lambs. On the other hand, of ten ewes which bore twin lambs, all showed ketonaemia during the course of the experiment, before as well as during fasting. Some of the data for each individual sheep, which are of considerable interest, are shown in Table II.

TABLE II. Total ketones. Data for Group III

No. of ewe	No. of lambs	Total ketones in blood, mg./100 c.c.					
		29/10	26/1	17/2	25/2*	27/2	9/3
91	0	0	0	0	0	0	0
70	1	0	0	0	0	25	19
6	1	0	0	0	0	11	0
54	1	0	0	0	0	11	0
75	1	0	0	0	Tr.	41	0
25	2	0	0	Tr.	13	27	9
48	2	0	0	12	29	Lost	36
17	2	0	0	9	25	48	Tr.
26	2	0	0	9	15	35	Tr.
62	2	0	0	24	24	47	32
19	2	0	0	8	19	39	Tr.
58	2	0	0	14	29	55	20
37	2	0	0	19	25	43	28
21	2	0	0	15	21	43	49
64	2	0	0	35	44	56	Lambed

* Animals were bled at 9 a.m. on the morning of 25 February 1938 and then fasted until 9 a.m. on the morning of 27 February 1938.

Group IV. This group of fifteen ewes received exactly the same ration as Group III until about a month before lambing. During four-fifths of their pregnancy, therefore, they were fed a ration sufficient to maintain weight which, in pregnant ewes, presumes that they were under-nourished. In the last month of pregnancy they were fed a full ration consisting of 6 lb. of turnips, ½ lb. hay mixture and 2 lb. of the meals mixture fed to Group VI, on which they gained rapidly in weight. During the four months these sheep were on a maintenance diet the average gain in body weight was only 5 lb. As pregnancy progressed, ketonaemia and

hypoglycaemia became evident in six ewes. All these ewes eventually bore twins. During the first 14 days of being fully fed the average body weight increased by almost a pound a day; the average blood sugar figure increased from 36 to 53 mg./100 c.c. and all traces of blood ketones disappeared. The positive correlation of ketonaemia with twin pregnancy and its negative correlation with dietary improvement is shown in Table III.

TABLE III. Data for Group IV

Index no. of ewe	Change in bodyweight 28. x. 37–17. ii. 37 lb.	No. of lambs	Total ketones in blood, mg./100 c.c. 29/10	25/1	16/2*	2/3	10/3
43	+14	0	0	0	0	0	0
15	17	1	0	0	0	0	0
29	13	1	0	0	0	0	0
9	0	1	0	0	0	0	0
39	3	1	0	0	Tr.	0	0
87	30	2	0	0	Tr.	0	Lambed
78	22	2	0	0	9	0	0
30	17	2	0	0	0	0	0
80	12	2	0	0	Tr.	Tr.	Lambed
74	10	2	0	0	44	0	Lambed
47	1	2	0	0	0	0	0
100	2	2	0	0	31	0	0
50	− 4	2	0	0	19	0	0
57	−14	2	0	13	57	Tr.	0
90	−13	2	0	0	45	0	0

* Changed to production ration on 17 February.

Groups V and VI. These two groups, each of fifteen ewes, can be considered conveniently together. The only difference in their experimental treatment was that the protein equivalent of the ration of Group VI was almost double that of Group V, and this difference in experimental treatment made no marked difference to the results. On 19 October 1937 each group showed an average live weight of 139 lb. On 1 March 1938 the average live weights were 150 and 155 lb. for Groups V and VI respectively. The average figures for blood ketones in Group V at successive bleedings were as follows: 0, 0, 17, 23 mg./100 c.c.; in Group VI the corresponding figures were 0, 2, 17, 14 mg. The figures for blood sugar at the last three bleedings were in Group V 39, 40, 35 mg., and in Group VI 39, 39, 40 mg./100 c.c.

Illness occurred in several sheep in both groups. Loss of appetite, a dazed appearance in the mornings and temporary blindness were the main symptoms noted. The sick sheep tended to become worse until they lambed or were slaughtered. Two ewes in Group V (nos. 7 and 35) and one in Group VI (no. 3) were slaughtered when in a dying condition. At post-mortem all these ewes were found to contain two or more lambs and

their livers were pale and friable. Chemical and histological examination showed that these livers contained abnormal amounts of fat (58–65 % total lipids on a dry matter basis).

Ketonaemia in relation to changes in body weight of the ewe

From a consideration of the figures both for the averages of groups and for the individuals within groups, it is clear that an inverse correlation exists between ketonaemia on the one hand, and body weight and

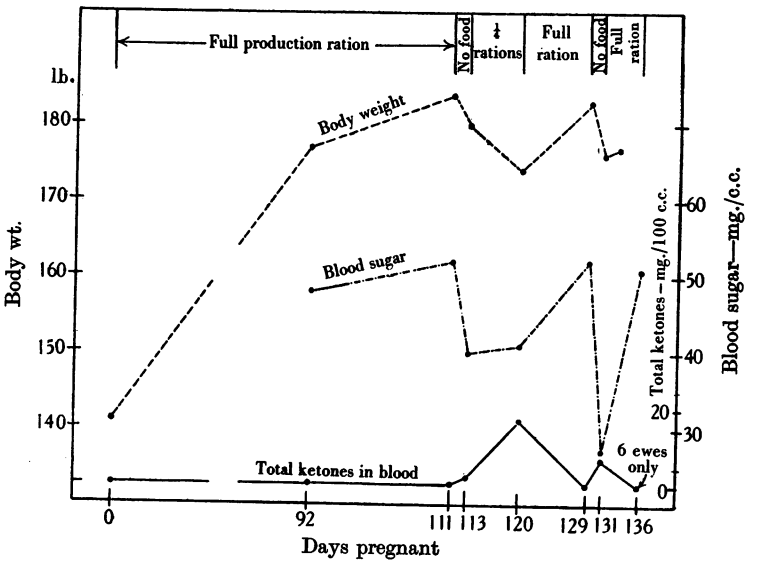

Fig. 1. Effect of starvation on well-fed pregnant ewes.

blood sugar on the other. Thus, with a fall in body weight there is observed a corresponding fall in the blood sugar level, while at the same time the ketones in the blood increase. This relationship is clearly illustrated in Fig. 1, which shows the average body weight, blood sugar, and blood ketones in Group II plotted against time expressed as the duration of the experiment in days.

The same relationship is illustrated in Fig. 2 which is taken from the data of Group III (maintenance with one period of fast) and in Fig. 3 taken from the data of Group IV (maintenance followed by full feed).

It is evident from these figures that ketonaemia tends to increase with under-feeding, still more with fasting, and to decrease when food intake is increased.

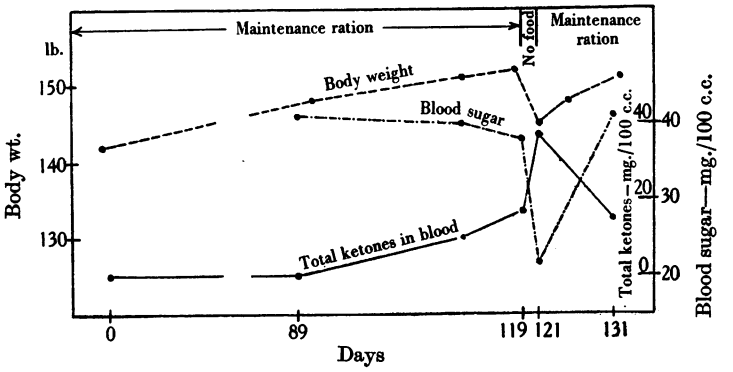

Fig. 2. Effect of starvation on poorly-fed pregnant ewes.

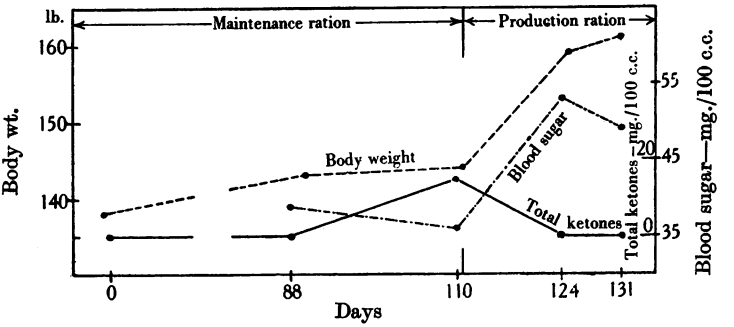

Fig. 3. Effect of increased food on poorly-fed pregnant ewes.

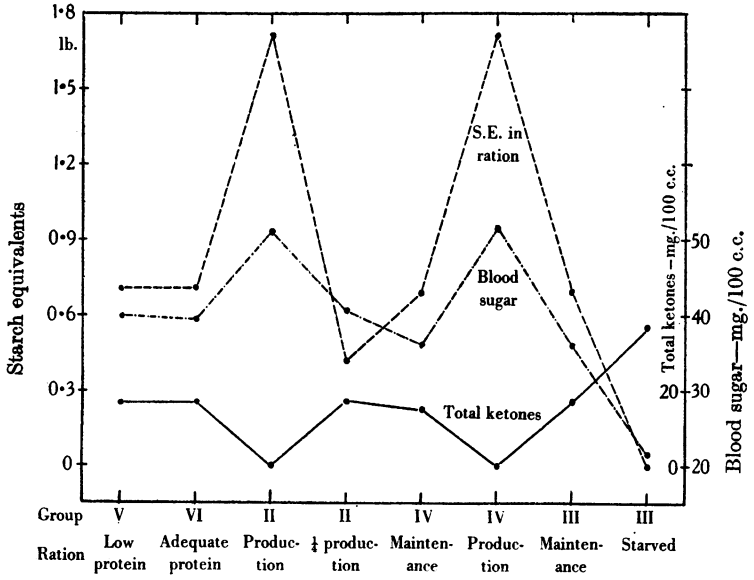

Fig. 4. Relation between food eaten blood sugar levels and ketonaemia.

Ketonaemia in relation to caloric value of the diet

The results of these experiments suggest that there is a definite relationship between the caloric value of the diet and the incidence of ketonaemia (and hypoglycaemia) in the pregnant ewe. This apparent relationship is illustrated in Fig. 4, where it is shown that in all groups the intensity of the ketonaemia varied inversely with the caloric value of the diet and with the level of sugar in the blood.

Discussion

In a general discussion of the results of the experiments described above, it is necessary to consider in the first place whether the ketonaemia observed is related to diet and to pregnancy, and in the second place whether the ketonaemia and other less easily measurable symptoms observed are identical with or related to those of pregnancy disease of sheep as it occurs in the field. We desire to emphasize that while we consider the evidence with regard to the first question as fairly clear and definite, we think that the evidence in regard to the second question is suggestive, but not conclusive.

Ketonaemia in relation to diet and pregnancy

It has been shown in these experiments that ketonaemia can be produced experimentally by a low level of feeding or by fasting, and that a ketonaemia so produced can be dispelled by an increased food intake or by terminating the fast. There is evidence to show that this effect of diet is directly related to its caloric value but not to its protein content. This result is in keeping with the known effect of starvation in producing ketosis. Moreover, in our experiments, the severity of the ketonaemia appeared to be correlated with the loss of body weight in the pregnant ewes.

The results of the experiments also show that ketonaemia is more readily produced in ewes bearing twin lambs than in those bearing singles, and that the ewes which were barren showed no ketonaemia at all. This finding is in keeping with the relationship found between the caloric value of the diet and the incidence of ketonaemia of pregnant sheep since it is known that there is a marked increase in the basal metabolic rate of the mother during the latter part of pregnancy [Harding, 1925], and consequently that the mother's caloric requirements must also increase. Further, Murlin [1910], working with a female bull terrier in successive pregnancies, found that the increase in basal metabolism was proportional to the weight of the offspring at birth. In pregnant ewes, Ritzman & Benedict [1931] have found that a rise in basal metabolism of about 18 % per unit of surface area occurs during the final three months of pregnancy.

The results of our experiments justify us in concluding that a caloric deficiency of the diet will produce ketonaemia in pregnant ewes more easily in multiple than in single pregnancies, and that the ketonaemia so produced can be dispelled by increasing the caloric value of the diet.

Pregnancy disease of sheep in relation to diet

In our introduction we discussed the various theories which have been advanced as to the dietary factors causing or predisposing to pregnancy disease of sheep. Our experimental results give support to some but not to others.

They are totally against the commonly held view that pregnancy disease is caused by over-fatness and lack of exercise. In two of our groups, thirty sheep in all, of which the majority bore twins, a degree of over-fatness was produced which is never equalled in commercial sheep. Moreover, the lack of exercise due to confinement in small pens was something much more severe than commercial sheep ever suffer. Yet, except where a period of fasting was deliberately imposed, no ketonaemia or any other symptom suggestive of pregnancy disease was observed.

Nor do our results support the view that protein deficiency is a causative or predisposing factor. Thus there was no apparent difference in the incidence of ketonaemia or other symptoms suggestive of pregnancy disease between the group with a ration of which the protein equivalent was 0·086 and that where it was 0·154. It must be admitted, however, that in the group of fat sheep where ketonaemia was totally absent the protein equivalent of the ration was still higher, namely, 0·24.

Our results do support the view that under-nutrition used in its wider sense, or caloric deficiency in its more restricted meaning, produces ketonaemia in pregnant ewes. They also show that it may produce some of the symptoms commonly held to be characteristic of pregnancy disease, such as temporary blindness and coma. Moreover, in certain ewes showing ketonaemia and which were slaughtered, the livers were found both by chemical and microscopical examination to contain an abnormal amount of fat. The data regarding the livers will be dealt with later in a separate communication. It must be admitted, however, that in individual ewes there was not always a correspondence between the clinical and chemical data. Some ewes with pronounced symptoms suggestive of pregnancy disease showed little or no ketonaemia, while other ewes with severe ketonaemia showed no obvious signs of ill-health. On the other hand, certain field cases of pregnancy disease which we were able to investigate showed a close correspondence in clinical appearance,

blood chemistry, and state of the liver to several of the cases of experimental ketonaemia we were able to produce by dietary measures.

Summary

1. With ninety mated ewes, divided into six groups of fifteen, kept in confinement, an attempt was made to produce ketonaemia experimentally, by over-feeding, under-feeding, and by protein deficiency.

2. Pregnant ewes fed to extreme fatness did not develop ketonaemia.

3. A wide variation in the protein content of the diet did not influence the frequency of occurrence or the severity of ketonaemia.

4. The caloric value of the diet was found to be inversely correlated with frequency of occurrence and severity of ketonaemia.

5. Ketonaemia produced by under-nutrition was more frequent and severe in multiple than in single pregnancies.

6. Certain of the symptoms and pathological changes generally considered as characteristic of pregnancy disease were observed in pregnant ewes that were under-nourished or fasted.

7. The relation of experimental ketonaemia produced by under-feeding of pregnant ewes to pregnancy disease of sheep is discussed.

We desire to acknowledge our indebtedness to Messrs A. R. Wannop and A. Macaulay of the North of Scotland College of Agriculture for drawing our attention to field cases. One of us (L. C. S.) is indebted to the University of Western Australia for a Hackett Studentship.

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