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Domsiekte or Pregnancy Disease in Sheep—II.

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CONTENTS.

SECTION A.—EXPERIMENTAL PROCEDURE AND RESULTS.

(CONTRIBUTED BY J. W. GROENEWALD.)

INTRODUCTION.

A STUDY of the literature, as indicated in Part I of this paper, indicates that, although much that has been written appears to be vague and even contradictory, certain very definite facts can be deduced. There is no doubt that the disease is primarily one of carbohydrate metabolism. Attempts to produce the condition artificially had not been successful until in 1934 Hopkirk reported acetonaemia and fatty livers in fasted fat ewes, and made the suggestion that a liver block, due to a sudden starvation, may be the cause of the condition. Similar experiments were carried out by Fraser *et al.* (1938 and 1939). It was, therefore, decided to attempt to produce the condition known to us as " Domsiekte " by this method.

EXPERIMENTAL PROCEDURE.

The chief objects of the present investigation were to produce clearcut- clinical cases of pregnancy disease. It was felt that, when once the condition could be produced, remedial measures could be studied in further work, which is in progress at the present time.

A record was kept on monthly weights, blood analyses, as well as urine analyses carried out periodically. Clinical symptoms and the pathological findings were carefully noted.

Thirty-four good-conditioned, 4-tooth merino ewes were selected for this work. Although the sheep were divided into five groups of seven sheep each, they all received the same basal ration during the first four months pregnancy period. The ration consisted of a concentrate mixture fed *ad lib.*, a record being kept of the amount consumed by each sheep. The mixture contained 80 parts by weight of crushed yellow mealies and 20 parts by weight of meat and bonemeal. In addition to the concentrate mixture, which was fed in the individual feeding boxes, each ewe received half a pound of cut-up greenfeed. The ewes were put into their individual feeding pens during the early afternoon and remained there until the following morning, when they were allowed to go into a small ground-floored paddock. The paddock was equipped with a hay rack in which lucerne hay was always available. It was, therefore, possible to measure the food consumption of each sheep separately, although the animal had as much as it wished to eat.

Similarly a record was kept of the water consumed by each ewe during the last month of pregnancy. The water was measured into a small trough in each feeding pen and the amount left over, measured back daily.

A plan of the group treatments during the last month of pregnancy may be given as follows:—

Group 1.—Control group, to continue receiving their ordinary ration.

Group 2.—The basal ration to be replaced by poor quality veld hay + 1 ounce of cane sugar each, daily.

Group 3.—The basal ration to be deleted and poor quality veld hay substituted + 1 ounce meatmeal (80 per cent. protein) each, daily.

Group 4.—The basal ration to be deleted and poor quality veld hay substituted.

Group 5.—The same treatment as in the case of group 4, but the ewes to remain non-pregnant.

Whenever the hay ration in the case of a particular ewe in group 4 was substituted for the ordinary ration as a result of the animal having reached the fourth month pregnancy stage, one ewe in the non-pregnant group 5, also had her ration changed. The sugar and meatmeal supplements were dosed to each ewe daily in order to make sure that the ewe actually received the correct quantity. All sheep were housed in the open, a fact which occasionally interfered with their normal feed consumption on account of rain.

Results.

During the first four months of pregnancy the average daily concentrate consumption for each sheep in the different groups, was as follows:—

Group	1:	Grain consumption		1·6 lb.	each per day.
,,	2:	,,	,,	1·9 lb.	,,
,,	3:	,,	,,	1·6 lb.	,,
,,	4:	,,	,,	1·2 lb.	,,
,,	5:	,,	,,	1·1 lb.	,,

In addition to grain each sheep consumed approximately 2 lb. of lucerne hay per day. The daily average water consumption per sheep in each group was:—2·45 litres, 2·26 litres, 1·99 litres, 2·16 litres and 2·46 litres for groups 1, 2, 3, 4 and 5, respectively. Considerable variation occurred in regard to the amount of water consumed by individuals within the same group. However, as soon as a sheep showed symptoms of pregnancy disease, it immediately ceased to drink water.

The energy value of the pre-experimental ration, which was the ration consumed by the control group throughout the experiment, was 2·11 Therms and conforms to the Morrison energy requirement for pregnant ewes. However, when the ration was changed to that of old veld hay in the case of groups 2, 3, 4 and 5, the ewes practically refused all feed. Assuming that an average of at least ½ lb. of hay was consumed, the energy intake would then be 0·125 Therms. In addition to the hay groups 2 and 3 received an ounce of cane sugar and an ounce of pure meatmeal each respectively. Although the quantities of sugar and meatmeal fell far short of providing the energy requirements of the ewes, it was felt that by elevating the energy intake, some degree of milder affection may become manifest in such groups. Such results would then give a useful indication for future work. The calorific value of the sugar and meatmeal was approximately equal, but the oral administration of more than an ounce would have entailed a considerable amount of difficulty and possibly other complications as a stomach tube would have had to be used.

The monthly average group weights are illustrated, for all ewes at the same stage of pregnancy in Graph I.*

From the curves indicating the average group weights, it may be seen that a depression occurred in weight gain during the third and fourth month pregnancy period. This weight depression, which is evident in all but group 4, may be attributed to unfavourable weather conditions. However, attention should be drawn to the fact that the peak weights all vary within a range of four pounds and may not indicate any significance. The non-pregnant group remained consistently lighter. Of course the higher the peak weight reached, for example group 4, the faster was the drop in weight during the last month when the ewes were receiving veld hay and in the case of groups 2 and 3, an ounce each daily of sugar and meatmeal respectively.

Certain ewes in groups 1 (control) and group 5 (non-pregnant) were affected adversely by the unfavourable weather conditions. They developed a diarrhoea, lost appetite and consequently weight, as may be seen in Graph II.*

Instead of the expected gradual gain in weight, these ewes lost weight and eventually aborted. No. 2 developed slight symptoms of pregnancy disease spontaneously.

The individual behaviour of each ewe, in regard to gain or loss in weight on onset of disease, is illustrated in Graph III.*

From Graph III, it will be seen that fewer sheep appear in group 1 because two ewes are already accounted for in Graph II. Number 12 in this group was actually losing weight when she was sacrificed as a control for pathological comparisons. In group 2, three cases of pregnancy disease occurred at periods

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* See end of this section.

of 16, 18 and 9 days after commencement of the starvation diet. In group 3 there were six cases which occurred on the 4th, 7th, 12th, 14th, 15th and 16th day, respectively. In group 4 there were seven cases in periods of 3, 6, 6, 5, 7, 7 and 5 days after veld hay ration was given, and in group 5 four cases occurred in periods of 36, 24, 40 and 48 days. The average time taken in the different groups before symptoms were shown is, therefore, 14⅓, 10⅓, 5⁴/₇ and 37 days respectively.

The average period taken by the different groups before the onset of symptoms, may be correlated with the rate of weight lost by the ewes in the groups. By comparing the angle at peak weight with the degree of weight lost, it will be seen that the angle is most acute in the majority of the ewes in group 4, where the pregnant ewes received veld hay only. The degree of weight lost is wider in group 3 where an ounce of protein supplement was given, and wider still in group 2 where the ewes received an ounce of sugar each daily in addition to the veld hay which was given to all groups excepting the normal pregnant control. The non-pregnant group 5, showed the least tendency to lose weight rapidly.

Symptoms.

One of the first symptoms generally shown by an affected ewe appears to be a rather stiff gait and the head held high. At this early stage there is a general expression of nervousness, eyes large and ears often drawn back or showing slight twitching. Affected ewes will refuse feed and water. The rate of respiration is increased, but the temperature remains normal.

In a flock, cases are not noticed, as a rule, before they lag behind. At this stage knuckling over of the hind fetlocks may occur, stiff hocks and an erratic gait. Visibility is frequently impaired and the ewe may become blind in one or both eyes. If excited the ewe will frequently stagger in circles and fall down. Later a droopy attitude is assumed and the head hangs with the nose practically touching the ground.

During the last stage the ewe lies more or less in the posture of a milk fever case in the cow, head to one side and forelegs often stretched out in front of her. In many cases the nose actually rests on the ground. A state of complete coma soon sets in. A full term lamb may be born from an affected ewe, in which case the ewe may recover. However, experience shows that spontaneous recovery during the latter stages of the condition, is extremely rare.

As far as may be judged, the periods of hypernervousness and coma vary considerably in duration. In fact certain outbreaks show the condition to be present is the hypersensitive state only, whereas in other cases the ewes go into a coma almost immediately which may last for a few days.

Details in regard to service, reproduction, haematological and chemical observations and onset and intensity of symptoms, have been tabulated and are presented in Tables 8 to 12, at the end of this article.

A study of the tables show clearly that not only was the percentage of ewes affected large, in group 4, but the symptoms were more marked. Although a larger number of ewes became affected in the group of ewes that received a protein supplement than in the case of those that received a sugar supplement, the symptoms were more marked in the latter group.

The symptoms were slight in the case of the non-pregnant group and the ewes soon became comatose. However, these ewes carried less condition than those in the other groups.

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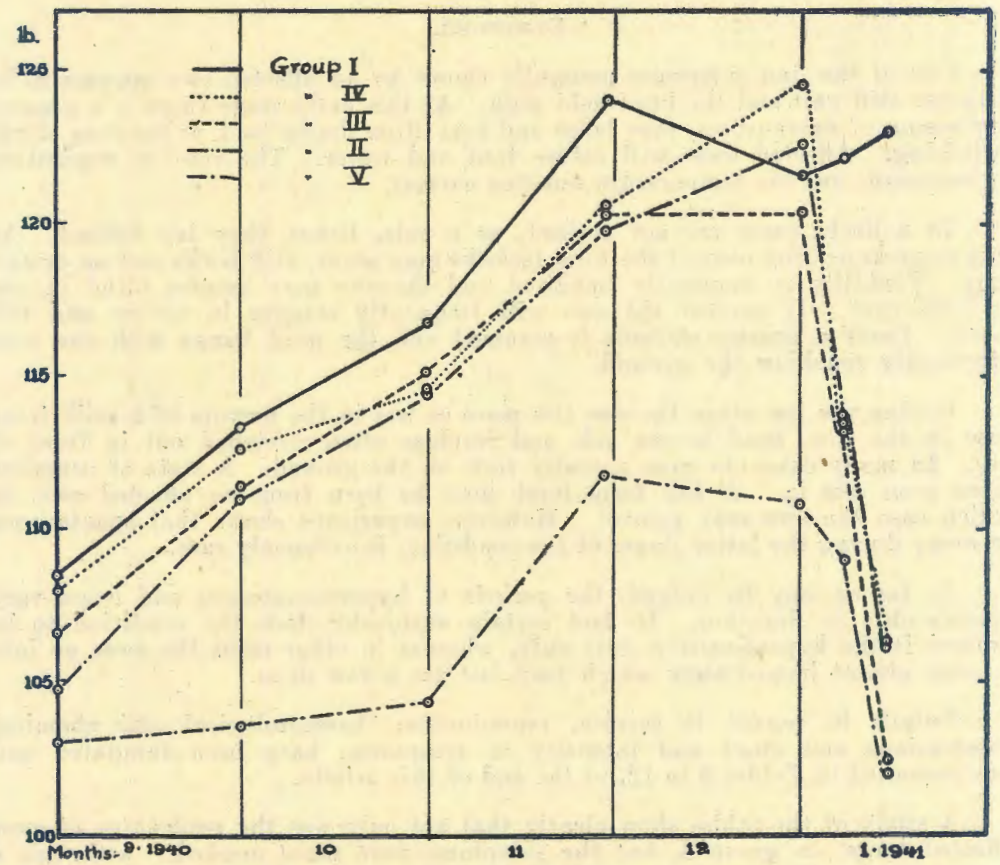
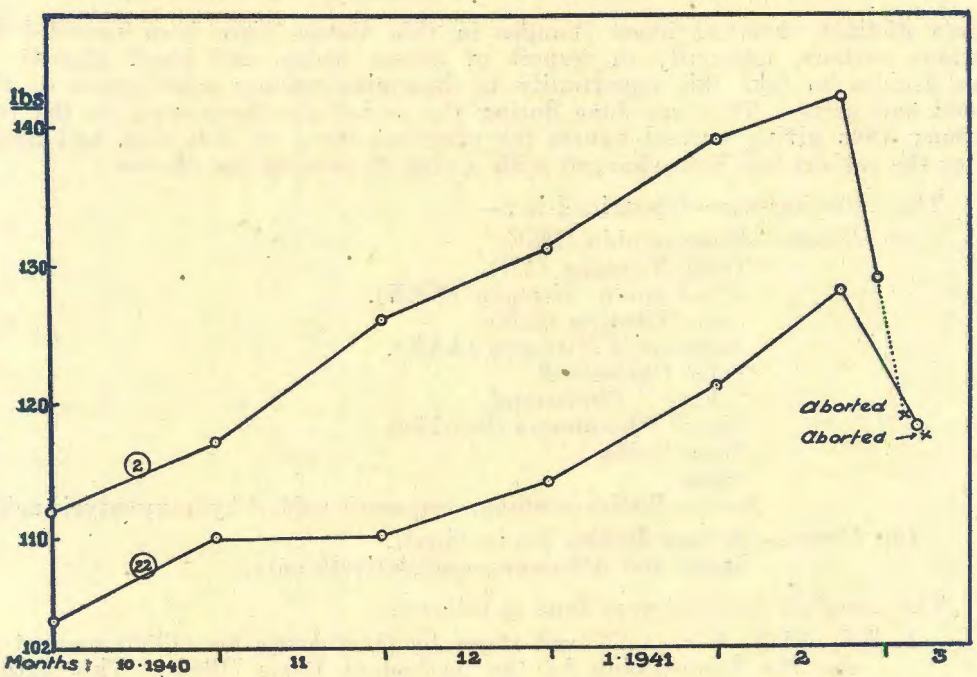
GRAPH I.—Average Group Weights.



GRAPH II.—Individual Loss in Weight.



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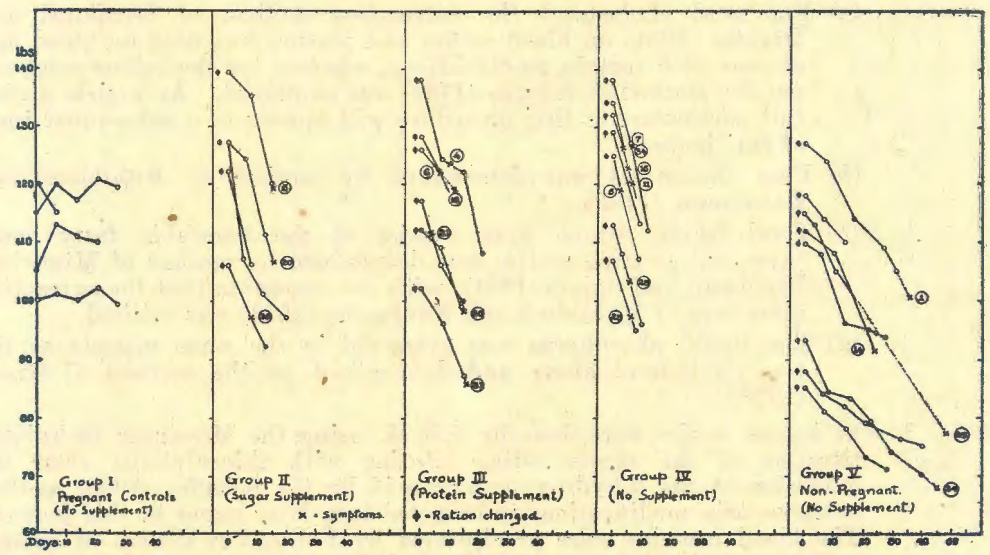

SECTION B.—CHEMICAL ANALYSES OF BLOOD AND URINE.

(CONTRIBUTED BY H. GRAF, P. M. BEKKER AND J. R. MALAN.)

SINCE distinct chemical blood changes in this disease have been recorded by various authors, especially in respect of ketone bodies and blood glucose, it was decided to take this opportunity to determine various constituents of the blood and urine. This was done during the period the sheep were on the full ration; thus giving normal figures for pregnant sheep on that diet, and again after the rations had been changed with a view to causing the disease.

The following were determined in:—

(*a*) *Blood*:—Haemoglobin (Hb).
Total Nitrogen (TN).
Non-Protein Nitrogen (NPN).
Urea Nitrogen (UN).
Amino-acid Nitrogen (AAN).
Total Cholesterol.
" Free " Cholesterol.
Lipoid Phosphorus (lecithin).
Total lipids.
Sugar.
Ketone Bodies (acetone, acetoacetic acid, β-hydroxybutyric acid).

(*b*) *Urine*:—Ketone Bodies (as in blood).
Sugar and Albumen—qualitatively only.

The chemical analyses were done as follows:—

1. TN, NPN, UN, AAN and sugar by Graf using his (1933) method as also the haemoglobin by the method of Roets (1940). This author wishes to express his indebtedness to J. M. Scholtz for the bleeding of the sheep and for assistance in the above determinations.

2. The blood lipids by P.M.B. as follows:—

   (*a*) For total cholesterol the extractiom method of Bernhard and Drekter (1931) on blood serum and plasma was used on blood and plasma with certain modifications, whereas for the colour comparison the method of Schube (1932) was employed. An article giving full particulars of this procedure will appear in a subsequent issue of this journal.

   (*b*) Free cholesterol was determined by method of Mühlblock and Kaufmann (1931).

   (*c*) Total lipids, which here consist of the titratable fatty acids expressed as tripalmatin, was determined by method of Mimwich, Friedman and Spiers (1931), with the exception that the extraction procedure of Mühlblock and Kaufmann (1931) was utilised.

   (*d*) The lipoid phosphorus was extracted in the same manner as the free cholesterol above and determined by the method of Green (1928).

3. The ketone bodies were done by J.R.M. using the Messinger technique, titration of the excess iodine solution with thiosulphate, after the oxidation of the β-hydroxybutyric acid by the Schaffer (1908) method using certain modifications to be noted in a later paper in this journal. The blood proteins were precipitated by Folin-Wur system of sodium tungstate and sulphuric acid (Graf, 1933).

In all cases blood was collected in bottles containing 1 per cent. of a 20 per cent. potassium oxalate solution and the analyses started within 30 minutes of bleeding. To minimise physiological variations due to withdrawing blood at varying periods after meals, bleeding was always done betweeen 8.30-9 a.m. or approximately 1½-2 hours after the morning feed.

Analytical Data—Normal Figures. (See Table 1, 1a and 1b.)

These data are derived from five pregnant ewes kept on the full ration throughout the period of the experiment and serve therefore as control or normal figures for the ration used. In all of the following tables, however, further normal blood figures are incorporated, since in most of the animals utilised blood analyses were undertaken during the preliminary period, during which they were on a normally sufficient and balanced diet. These figures are indicated in the tables by being placed above the black dotted line in the figures recorded for each animal.

To serve as a basis for the evaluation of the data obtained in cases of Domsiekte, the minimum and maximum figures for all the constituents determined, both in blood and urine, have been given. Normal variations found in individuals from analysis to analysis or amongst different individuals in the same group are not discussed since they do not directly bear on the subject under discussion.

Table 1.

Summary of Normal Data of Various Blood Constituents from Sheep on a Satisfactory Ration.

Constituent.	Minimum.	Maximum.	Narrower Range.	Average of all Figures.	Remarks.
Haemoglobin (Hb), grams per 100 c.c.	9·3	17·6	12–15	13·7 (51 determs)	—
Total Nitrogen (T.N.) (whole blood), grams per 100 c.c.	2·2	3·4	75 % between 2·8–3·3	2·9 (51 determs)	—
Non-protein Nitrogen (NPN), mgms. N per 100 c.c.	21·0	37·4	25–35	27·8 (51 determs)	Increases in Domsiekte.
Urea Nitrogen (U.N.), mgms. per 100 c.c.	7·1	19·0	12–15	12·5 (48 determs)	Increases in Domsiekte.
Amino Acid Nitrogen (A.A.N.), mgms. per 100 c.c.	4·8	8·2	84% between 5·5–7·5	6·9 (50 determs)	—
Sugar (S), mgms. glucose per 100 c.c.	39·7	67·6	45–55	49·2 (50 determs)	Hypoglycaemia in Domsiekte.
Total Cholesterol, mgms. per 100 c.c.	71·0	152·0	85% between 90–130	106·7 (48 determs)	—
Free Cholesterol, mgms, per 100 c.c.	42·0	88·0	80% between 60–80	67·3 (48 determs)	—
Lipoid Phosphorus, mgms. per 100 c.c.	6·5	9·8	80% between 7–9	8·4 (48 determs)	—
Total Lipids, i.e. Titratable Fatty Acids, mgms, per 100 c.c.	276	509	80% between 300–450	380·2 (48 determs)	—

Acetone Bodies (Acetone, Acetoacetic Acid and β-hydroxybutyric Acid).

The acetone and acetoacetic acid were determined together and the β-hydroxybutyric acid separately, but for the sake of convenience the latter is also expressed in terms of acetone (to recalculate as β-hydroxy. multiply the acetone figure by 1·78). "Total" acetone—comprises all three fractions all expressed in terms of mgms. acetone per 100 c.c. blood.

TABLE 1A.

Acetone Bodies in Blood—Normal Figures.

Constituent.	Minimum.	Maximum.	Narrower Range.	Average of all Figures.	Remarks.
Acetone and acetoacetic acid (mgms, acetone per 100 c.c.)	·32	2·90	70% between ·3–1·0	·95 (42 determs)	Marked increase in Domsiekte.
β-hydroxybutyric acid (as acetone)	·61	4·51	80% between ·6–3·0	2·02 (42 determs)	Marked increase in Domsiekte.
"Total" acetone mgms per 100 c.c.	·96	7·15	86 % below 5 mgms. 34 % below 2 mgms.	3·0 (44 determs)	Marked increase in Domsiekte.

Urine Analyses.

The urine was collected in specially constructed metabolism cages over a 24 hour period (from 7 a.m.-7 a.m.). During this period the sheep received their specific rations plus water *ad lib.* The 24 hour output of urine collected was measured, well mixed and aliquots taken to determine the same acetone bodies as in the case of blood, a sample of which was taken between 8.30-9 a.m. of the day on which the sheep were caged.

Quantity Excreted.—As was to be expected this varied very considerably, not only amongst sheep of the same group, i.e. on the same diet but also in the same sheep on the different days of sampling. From normal sheep as low as 68 c.c. and as high as 2,450 c.c. were obtained per 24 hours. Although the various tables give both the acetone bodies expressed "per 100 c.c. of urine" and the total 24 hour excretion, the latter is the more significant. All fractions are expressed in terms of acetone.

Sugar in Urine.—Qualitative tests for sugar in urine were done on a large number of samples, but these always proved negative. The hypoglycaemia encountered in this disease is thus not due to a glycosuria or draining of sugar via the kidneys.

Albumen in Urine.—Qualitative tests for albumen on a few urine samples were mostly negative, occasionally a slight turbidity was encountered.

CHEMICAL DATA OBTAINED FROM THE VARIOUS GROUPS OF SHEEP PLACED ON DIFFERENT RATIONS.

The data available for each group is given in tabular form and attention drawn here only to significant alterations noted after the change in rations had been made.

TABLE 1B.

Urinary Acetone Bodies—Normal Figures.

Constituents.	24 hour Excretion mgms.	Mgms. per 100 c.c. Urine.
Acetone, plus Acetoacetic Acid.	3·26 to 118·33 mgms. 92% under 24 mgms. 50% under 10 mgms.	·97 to 22·24 mgms. 58% under 5 mgms. 80% under 10 mgms. *Average* of 26 determs = 6·1 mgms.
β-Hydroxybutyric Acid.	3·4 to 26·4 mgms. 42% under 10 mgms. 61% under 15 mgms. 81% under 20 mgms. *Average* of 26 determs = 12·4 mgms.	·44 to 9·38 mgms. 58% under 5 mgms. *Average* of 26 determs = 4·9 mgms.
"Total" Acetone Bodies.	9·37 to 129·36 mgms.* 34% under 20 mgms. 87% under 40 mgms. *Average* of 31 determs = 29·3 mgms.	2·3 to 25·7 mgms. 22% under 5 mgms. 53% under 10 mgms. 82% under 15 mgms. *Average* of 28 determs = 9·7 mgms.

* See footnote, Table 4.

Group 1.—*Pregnant Ewes—Normal Ration* (*see Table* 8).

This was introduced as a control group, and to provide the chemical data to serve as the basis for evaluating any changes in the blood or urine composition of sheep in the other groups provided for in this experiment. The normal figures recorded in the previous pages comprise those obtained from this group plus data from other sheep collected during the period when all the animals were on the same ration that this group received throughout the experiment.

Group 2.—*Pregnant Ewes. Poor Quality Veld Hay Plus Cane Sugar Supplement.* (*See Table* 9.)

The cane sugar supplement of one ounce per day did not suffice to prevent the disease from appearing in this group, although there was a distinct decline in the severity of the clinical symptoms and an increase in the time interval between change of ration and onset of symptoms.

The chemical data also reflects this intermediary position, three out of the five sheep (Nos. 3, 20, 35) showing a hypoglycaemia, acetonaemia and acetonuria of moderate degree.

Sheep No. 25 presents a conflicting picture and in our opinion is not a true case of domsiekte. The symptomatology and post-mortem suggests this diagnosis but the chemical analyses reveals—

(*a*) a markedly increased NPN on the day of death (150 mgms. N per cent.);

(*b*) no hypoglycaemia but a distinct hyperglycaemia (100 mgms. glucose per cent.);

(*c*) no acetonaemia. (Urine not available.)

Group 3.—*Pregnant Ewes: Poor Quality Veld Hay* ad lib. *plus One Ounce per Day of Meatmeal* (80 *Per Cent. Protein*). (*See Table* 10.)

In this group all the sheep showed clinical symptoms from 4 to 16 days after the change-over of rations. The blood and urine changes are very striking, especially the ketosis, where in one case (No. 21) over 2 per cent. of acetone bodies were found in the urine. The N.P.N. in 4 cases (Nos. 4, 6, 16 and 21) is increased showing in case 4 a gradual progressive rise up to 158 mgms., but in the others only shortly before death, and then, although increased beyond the normal physiological limits, only of moderate degree. The increase in N.P.N. is essentially due to an increased U.N. although in sheep No. 4 there is a high " undetermined " nitrogen fraction. The A.A.N. in 2 cases (Nos. 4 and 16) shows a tendency to rise. The hypoglycaemia is marked in four cases (4, 16, 21 and 30) but a peculiar feature is a distinct rise in the bloodsugar level shortly before death, rising in one case (6) to even beyond the physiological limit. Sheep 31 viewed in the light of the available chemical data is not a case of domsiekte.

Acetonuria and acetonaemia is shown by all excepting No. 31.

Group 4.—*Pregnant Ewes: Only Poor Quality Veld Hay* ad lib. (*See Table* 11.)

As the sheep only reluctantly ate the poor quality hay proffered and received no supplement of any kind this group may be regarded as showing the direct effects of sudden marked semi-starvation on heavily pregnant ewes.

Seven ewes showed clinical symptoms and all eight developed hypoglycaemia, acetonaemia and acetonuria of a high degree. The bloodsugar dropped as low as 21·5 mgms. per cent. (No. 11) and the " total acetone bodies " excretion over 24 hours reached a peak of 2,785·5 mgms. or close on 3 per cent. (No. 34). In three cases the N.P.N. shows rises e.g. in No. 7 up to 140 mgms. N per cent., No. 11 up to 62·6 mgms. and No. 28 up to 80·5 mgms. The dangerous effect of sudden semi-starvation on heavily pregnant sheep becomes thus very apparent. Out of eight sheep three died before term and five aborted so that not a single live lamb was born in this group. The first symptoms appeared within three days and at the latest within 7 days and the total mortality was five out of eight (63 per cent.).

Group 5.—*Non-pregnant Ewes: Only Poor Quality Veld Hay* ad lib. (*See Table* 12.)

This group was introduced to note the effect of a very poor ration on normal *non-pregnant* ewes and may therefore be regarded as being the exact counterpart to group 4. The conditions were the same in both groups in every respect except that in group 5 the complicating factor of pregnancy was absent. The results are of great interest, cases occurring in this group of non-pregnant ewes, resembling pregnancy disease in all respects, i.e. as regards symptoms, pathological anatomy and chemical blood and urine findings. There were, however, differences, e.g. the time interval between the change-over in rations and the appearance of symptoms in group 4 was from 3 to 7 days whereas in this group from 24 to 48 days; the symptoms were less severe and the degree of hypoglycaemia and acetonuria less marked. A close analysis of the data shows (*a*) four of

the sheep showed clinical symptoms (Nos. 1, 14, 24 and 29); (*b*) four died (Nos. 14, 18, 19 and 20). Two animals (Nos. 1 and 24) were slaughtered for the purpose of a post-mortem examination, both being in a moribund condition when killed. All these animals showed fatty changes of the liver and nephrosis. (*c*) Hypoglycaemia was present in six cases (Nos. 1, 14, 25, 27 and 29). Acetonuria was present in five cases (1, 14, 19, 24 and 29).

It would thus appear that pregnancy *per se* is not an etiological factor, but its presence undoubtedly aggravates the position, greatly accelerating the condition and exacerbating its severity. Pregnancy therefore does not act as a cause, but rather represents an additional burden—the proverbial " last straw " —superimposed on the already severe metabolistic disturbances produced by a sudden change to poor diet, especially in the direction of a decreased calorific intake. Admittedly the condition produced in group 5 would not be recognised as " pregnancy disease " under field conditions, but fundamentally there appears to be no difference, one being " acute " or " fulminant ", the other " chronic " or " delayed ".

Group 5A.—*Non-pregnant Ewe: Full Ration Throughout Experiment.* (*Table* 10A.)

In conclusion, data are submitted in respect of a *non-pregnant* ewe kept throughout on the full normal ration and this sheep (No. 33) thus serves as a control both for group 1 (Table 8) and group 5 (Table 12). It was bled frequently throughout the final stages of the experiment and it is of interest to note—

(*a*) the normal range of bloodsugar (never below 40 mgms. per cent.); and

(*b*) that acetone bodies are excreted in quite appreciable amounts even in normal sheep (see also Table 2).

Summary of Chemical Data and Discussions.

I. *Haemoglobin*—No significant changes.

II. *Total Nitrogen* (T.N.).—This comprises essentially the blood proteins (haemoglobin, albumin, globulin, fibrin, etc.), the N.P.N. in relation to the total being insignificant, and in view of the marked fatty changes of the liver and the role this organ plays in the synthesis of some of these proteins, it was deemed advisable to include this determination. There are no constant alteration in a specific direction although some sheep, e.g. 4, 16, 30 and 31 in the protein supplement group, show a tendency to an increase, whereas in groups 4 and 5, receiving no supplement of any kind. slight decreases are noted, e.g. 5, 14, 15, 19, 24, 27, 28 and especially 29. There is, however, a possibility that apart from actual changes in the total amount of the blood proteins, changes in the relative proportions of the various proteins may occur.

III. *Non-protein Nitrogen and Urea N.*—An interpretation of the available data is rather difficult since the position in many cases is complicated by intercurrent conditions which themselves are responsible for increases in the N.P.N. e.g. abortions, retained after-birth and metritis. An analysis of the available figures shows the following:—

TABLE 2.

No. of Sheep.	N.P.N. Mgms. %	Days after Changeover in Rations.	Remarks.
26	50	Normal Ration	Dystochia.
20	40·8	10	Chronic metritis.
25	150	40	—
31	42·2	10	Aborted.
30	40	15	Aborted 17 days after change in ration. Domsiekte ?
4	158	17	Abortion and septic metritis.
21	60	19	—
6	78	14	Died before term.
16	55	15	Slaughtered before term.
7	140	18	Septic metritis.
5	46·2	9	Slight metritis.
28	80·5	8	—
23	40	16	—
11	62·6	17	—
1	65·2	36	Non-pregnant.
24	137·5	39	Non-pregnant.
29	81·9	48	Non-pregnant.

Data obtained in this laboratory show that in case of abortion, and particularly with retained afterbirth, and in cases of metritis very high N.P.N. figures are found. This undoubtedly complicates matters in the present experiment. That these conditions, however, are not solely responsible for the increased N.P.N. emerges from a study of group 5 where three out of nine sheep (1, 24, 29) showed marked increases, although none of them were even pregnant.

Sampson, Gonzaga and Hayden (1933), also record increases up to 140 mgms. N per cent. which they regard as " evidence of marked oligouria, that culminates in a fatal uraemia " and " in fatal cases a complete anuria is observed 1-4 days before death ". With these views we cannot fully agree. There are undoubtedly a number of our cases, which show an N.P.N. retention not associated with disorders of the genital tract, but we do not feel convinced that this retention is solely or even largely due to anuria. For instance No. 29 excreted 600 c.c. of urine on the day of its highest N.P.N. level; No. 24, 250 c.c.; and No. 28, 240 c.c. Two cases showed a very low urinary excretion, e.g. No. 16 only 86 c.c. urine and No. 7 only 20 c.c.

Clinically no anuria was observed but unless special attention is paid this symptom can easily be overlooked.

On pathological grounds a complete anuria would be difficult to explain. In the absence of a mechanical obstruction of the urinary tract due to any cause, or a ruptured bladder, anuria is generally only encountered with acute diffuse bilateral nephritis. No reference to nephritis in pregnancy disease has been found, although a nephrosis does occur. The renal pathological changes described do not give the impression of being of so severe a type as to completely destroy excretory function.

The question as to what is responsible for the hyperuraemia in this condition is thus still open, but possibly the following may have a bearing on the matter. In quite a number of cases one notices a rise in the bloodsugar level after the

period of hypoglycaemia. Analyses by Roderick, Harshfield and Merchant (1933) show that the glycogen content of the liver is much reduced and it is thus difficult to understand how the bloodsugar should be augmented, in some cases even considerably beyond the normal level (e.g. No. 21). Glucose can be derived from proteins (amino-acids) and the suggestion is put forward by Fraser *et al.* (1938), that the increased N.P.N. is an expression of an increased protein catabolism in an endeavour to supply the body with the sadly lacking bloodsugar. The increased diamination would be associated with an increased urea production. The nephrosis may to some extent interfere with excretion, accentuating thereby the hyper-uraemia.

IV. *Amino-Acid Nitrogen.*—This does not show any very striking changes, although in quite a number of cases (4, 7, 9, 11, 16, 18) there is a tendency to a rise in the later stages of the disease. In view of the large margin of safety of liver function, only very gross liver lesions will so seriously interfere with diamination as to lead to a significant rise in the blood A.A.N. level. The slight increases recorded here may be associated with the suggested increase in protein catabolism in an endeavour to make up the glucose deficiency. Sheep No. 1, a non-pregnant ewe on a starvation diet, showed a very high A.A.N. (over 22 mgms.). This animal, in a comatose condition, was killed for a post-mortem examination. It showed gross hepatic fatty changes.

V. *" Free " Cholesterol*—No significant changes. " Total " cholesterol—does not rise significantly above the upper physiological limits and confirms the findings of Roderick, Harshfield, Merchant (1933) that hypercholesterolaemia does not occur.

VI. *Lipoid Phosphorus*—No changes.

VII. *Total Lipids* (excluding lipoids).—With the exception of sheep 29 which showed a content of 841 mgms. per cent. lipids 24 hours before death, the blood fat moved within normal limits, in a few cases slight rises being observed, more freqeuntly slight decreases. Turbidity of the plasma, as noted in lipaemia, was never escountered. These were unexpected findings in view of the intense metabolism of fats as reflected by the heavy acetonuria, a pathognomic feature of the disease. In diabetes, for instance, lipaemia is of frequent occurrence. From a biochemical point of view the etiology of acetonuria in diabetes and in pregnancy disease is closely related, in so far as in both diseases there is a disturbance in the normal carbohydrate metabolism—viz. in diabetes due to the inability of body to oxidise the glucose in the absence of sufficient insulin (leading to hyperglycaemia) and in Domsiekte a glucose deficiency (insufficient intake and exhaustion of liver glycogen leading to a hypoglycaemia). In both instances the bodyfat is drawn on in a larger measure to cover the energy requirements and in severe diabetes this is reflected by the marked lipaemia often noted.

In pregnancy disease there is furthermore a heavy deposition of fat in the liver, for instance Roderick, Harshfield and Merchant (1933) have found an average crude fat content (on the dry basis) of 60·11 per cent. as against 7·28 per cent. for normal sheep liver. When one considers that the disease normally may have a very short " incubation " period (in our experiments as short as three days) the absence of lipaemia is still more puzzling. How does this deposition of fat take place without increasing the lipid content of the blood, the normal transport medium?

VIII. *Bloodsugar.*—Hypoglycaemia is a common finding, levels below 20 mgms. being recorded, thereby conforming with the data obtained by Sampson, Gonzaga and Hayden (1933), Roderick and Harshfield (1932), Cameron and Goss (1940) and others. The animal does not, however, directly succumb to the hypoglycaemia *per se* since in most cases the bloodsugar again rises, fairly often even above the initial level and in some cases even above the upper physiological limits, i.e. a state of hyperglycaemia supervenes. In the absence of a sufficient carbohydrate intake and the depletion of especially liver glycogen the origin of this increased bloodglucose is obscure. The suggestion has been made that it is possibly derived from body proteins (see Non-protein Nitrogen). This increase is associated with a decrease in the acetonaemia, without, however, leading necessarily to recovery. A hyperglycaemia in the initial stages of the disease was not observed.

For sheep in group 2 (Table 3) an ounce of cane-sugar per day to supplement the poor veld hay diet was not sufficient to prevent hypoglycaemia completely, although it was of some preventive value in reducing the degree of both hypoglycaemia and acetonaemia. Numerous qualitative tests for sugar in urine were always negative.

IX. *Ketone Bodies.*—Although quite a considerable amount of data is available for " total " ketones in the blood of sheep, no references could be found dealing with the proportions of acetone, acetoacetic acid and β-hydroxybutyric acid as found in the blood and in urine of ewes, nor the proportions and total amounts excreted over 24 hours. As this information seemed of general interest and of possible value in obtaining a clearer insight into the etiology of this disease we decided to investigate this aspect more closely. Analyses were, therefore, made of acetone plus acetoacetic acid and of β-hydroxybutyric acid separately, both in blood and in urine.

For the sake of convenience of comparison the normal data previously given and the findings in Domsiekte have been summarised in Tables 3 and 4. All the figures are expressed in terms of " mgms. acetone per 100 c.c."

TABLE 3.

Acetone Bodies.

Distribution per 100 *c.c., Blood and Urine.*

Constituents.	Blood.		Urine.	
	Normal.	Domsiekte.	Normal.	Domsiekte.
Acetone, plus Acetoacetic Acid, mgms. per 100 c.c.	·32 to 2·90 Average ·95	Up to 33·36	·97 to 22·24 Average 6·14	Up to 365·5
β-Hydroxybutyric Acid, mgms. per 100 c.c.	·61 to 4·51 Average 2·02	Up to 37·4	·44 to 9·38 Average 4·93	Up to 68·7
"Total" Acetone Bodies, mgms. per 100 c.c.	·96 to 7·15 Average 3·0	Up to 58·4	2·3 to 25·7 Average 9·7	Up to 416·8

TABLE 4.

Acetone Bodies.

Urinary Excretion per 24 Hours.

Constituent.	Normal.	Domsiekte.
Acetone, plus Acetoacetic Acid, mgms.....	3·26 to 118·30 (25)* Average of 25 = 14·9 Average of 24 = 10·5	Up to 2511·5
β-Hydroxybutyric Acid, mgms	3·39 to 26·4 Average of 26 = 12·4	Up to 312·8
"Total" Acetone Bodies, mgms.....	9·4 to 129·4 (84·4)* Average of 29 = 29·2 Average of 28 = 25·7	Up to 2735·8

* Too high, due to one figure of 118·3 mgms. obtained from sheep No. 10 which secreted on one day 2450 c.c of urine (polyuria ?),

Omitting this figure the maximum range is below 25 mgms. and the average of 24 determinations only 10·5 mgms. and for the "total" acetone maximum would be 84·4 and the average 25·7 mgms.

Averaging all available data we find the proportion of acetone plus acetoacetic acid to β-hydroxybutyric acid to be approximately as 1 : 2 for blood and as 6 : 5 or about in equal proportions in the urine, though wide variations appear if individual data alone are considered. During the period of the hyperketonaemia there is a tendency in many cases for these two fractions to be present in approximately the same proportions, although the quantity per 100 c.c. is greatly increased above the normal, viz. from a normal average of 1 mgm. per cent. acetone plus acetoacetic acid up to a maximum of 33·36 mgms. and from a normal of 2 mgms. per cent. β-hydroxybutyric acid up to 37·4 mgms. or in the case of "total" ketones from a normal average of 3 mgms. per cent. up to 58·4 mgms. per cent. In the urine, however, although normally the two fractions are excreted in about equal amounts, the position during hyperketonaemia alters radically, the excretion of acetone plus acetoacetic acid being greatly increased beyond that of β-hydroxybutyric acid, e.g. up to a maximum of 365 mgms. per cent. of the former to only up to 68·7 mgms. of the latter. If the total 24 hour urinary excretion is considered this shifting in the proportions of acetone plus acetoacetic acid to β-hydroxybutyric is still further emphasised (see Table 4). Although undoubtedly the amount of total ketones excreted per 100 c.c. of urine [data obtained from examining only an aliquot of urine collected at one urination and conventionally expressed in mgms. per 100 c.c. (mgms. per cent.)] gives an indication of the degree of hyperketonuria, the quantity excreted per day is a much more accurate measure of the intensity of the fat catabolism taking place; e.g. sheep 7 gave a figure of 416·8 mgms. per cent. with a twenty-four hour output of 1,542 mgms., whereas sheep 23 with a "total" acetone of only 155·7 mgms. per cent. eliminated via the urine during 24 hours 2,397·6 mgms. of acetone. This is of course due to the influence of the quantity of urine excreted over 24 hours and this factor is very variable, e.g. in the normal non-pregnant sheep 33 the urinary output varied from 68 c.c. to 320 c.c. (eleven collections during 11/4 to 30/5/40) and in the normal pregnant group (Table 8)

volumes up to 2,450 c.c. were obtained (sheep 10). The available data does not support the contention of Sampson, Gonzaga and Hayden (1933) that anuria is a feature of the disease (except possibly ewes 7 and 16) nor Roderich and Harshfield's (1932) suggestion as regards a polyuria.

The pathogenesis of hyperacetonaemia, i.e. the increased utilisation of body fats in the absence of sufficient carbohydrate or any interference with its metabolism and the resultant large scale production of acetone, acetoacetic acid, etc., is well known and need not be discussed here. There are, however, several peculiar features which would warrant further intensive research and thereby contribute to a clearer understanding of the carbohydrate and fat metabolism of the ruminant generally. Reviewing the available evidence one can but agree with those workers [Roderick, Harshfield, Hawn (1937), and others] who hold that the etiology of the disease is intimately associated with the carbohydrate metabolism in the sense of a decreased supply and exhaustion of the liver glycogen. Pregnancy itself plays only a secondary rôle, it represents, as it were, the proverbial last straw, twin pregnancies still further increasing the strain. This point of view receives distinct support from the data recorded in Table 6 which shows that non-pregnant ewes, placed on a semi-starvation diet develop what may be termed " sub-clinical " Domsiekte, there being no clinical symptoms but chemically determinable hypoglycaemia, hyperacetonaemia and hyperacetonuria. The demands for carbohydrate being less than in pregnancy, the stored liver glycogen suffices for a longer period, hence the greatly extended " incubation " period or time interval between decreased intake and the appearance of hypoglycaemia, etc. This would also enable the body to bring into action physiological means of adaptation to a decreased food intake such as possibly the production of sugars from body proteins. Under normal conditions farmers would rarely permit their flocks to endure semi-starvation for such long periods and hence the apparent confinement of Domsiekte to pregnant ewes only and the implication that the etiology is intimately associated with pregnancy.

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SECTION C.—PATHOLOGY OF DOMSIEKTE.

(CONTRIBUTED BY R. CLARK.)

IN studying the pathology of Domsiekte we are faced with the difficulty that there is no known specific pathognomic lesion, or group of lesions, by which a definite diagnosis can be made. In other words we still have to answer the question, what is domsiekte? Actually the only criterion we can go on is the presence or absence of clinical symptoms before death. This is naturally an unsatisfactory position as we must suppose the occurrence of subclinical cases.

The literature on the pathological chances is also very insufficient and vague. The only point on which there appears to be unanimity is that there are always fatty changes in the liver. This, in fact, is the only pathological finding noted in most articles. Fatty changes in the epithelium of the renal tubules are also mentioned by de Kock (1928) and Bosworth (1929) but neither of these authors appears to consider this constant.

Roderick and Harshfield (1932) describe a nephrosis with fatty changes exactly similar to those seen in the present studies. They consider it " the result, rather than the cause of the disease ".

It was decided, therefore, to study first the macro- and microscopical changes in the organs of those sheep which died while showing, or very soon after showing, the clinical symptoms of Domsiekte.

For this purpose the following sheep have been selected:—

No. 35—Group 2—Showed marked symptoms six days before death, lamb still in utero at death.

,, 6—Group 3—Showed marked symptoms for five days prior to death, lamb still in utero.

,, 16—Group 3—Showed slight symptoms five days before death, lamb still in utero.

,, 13—Group 4—Showed marked symptoms for two days, three days before death, lamb still in utero.

,, 15—Group 4—Showed marked symptoms for five days up till death, lamb still in utero.

,, 34—Group4—Showed marked symptoms for eight days before death, lamb still in utero.

,, 24—Group 5—Showed symptoms for one day five days prior to death, non-pregnant.

The occurrence of abortion, parturition and complications such as retained afterbirth with septic metritis and enteritis have caused many of the sheep to be discarded from this first study. The above sheep can all be expected to show the pathological changes associated with Domsiekte without any known complications.

Macroscopic Findings.

All these cases showed the same changes at post-mortem. The carcases were in very good condition, there being very large amounts of fat in all the depots.

The Fat.—The subcutaneous fat showed slight fat necrosis but this was very marked in the case of the omental fat while the perirenal fat was grossly affected. The coronary fat was very slightly or not at all affected. This fat necrosis was a very marked and absolutely constant change.

The Blood.—The blood stained well but was bluish in colour, there being a general cyanosis of the body. There was no abnormal collecting of fluid in the serous cavities.

The Lungs.—Apart from venous congestion the lungs were in all cases normal.

The Heart.—In some cases the myocard appeared to be slightly yellowish and friable, this change was in no case marked.

The Liver.—The most striking change was in all cases, in the liver. This organ constantly showed marked fatty changes, being a yellow khaki colour and very friable. On section it is often impossible to make out any lobulation and the fat can actually be seen glistening on the cut surface. This change was not always even throughout the liver. In some cases one half would appear not to be so badly affected, or the yellow colour would appear more marked over large patches, as big as one's hand.

The Pancreas.—No visible changes.

The Spleen.—No visible changes.

The Adrenals.—In all cases the adrenals were enlarged and exhibited marked degenerative changes. The organ was extremely friable, it often being almost impossible to extract it whole from the surrounding fat. On section the cortex was flabby and yellow.

The Kidneys.—In all cases the kidneys appeared yellow and friable, fatty changes being easily visible to the naked eye in the cortex. The capsule stripped easily in every case.

The Lymph Glands.—The Lymph glands were in all cases greatly diminished in size, it being extremely difficult to locate such glands as the prescapular, which was often not more than half an inch in length and deeply embedded in the massive fat deposit.

The Intestinal Tract.—In five of these eight typical cases there was a slight catarrhal enteritis present. The remainder showed no changes except a slight atrophy of the ruminal wall, judged by thinness and abnormal translucency.

The Uterus.—In the first seven cases normal, fully developed foetuses were present, the foetal membranes and uterine mucosa appeared normal. In the last case the sheep was non-pregnant, the uterus being normal.

Summary.—The post-mortem findings in these eight cases of undoubted Domsiekte were:—

> General cyanosis, fat necrosis especially of the perirenal and omental fat. Fatty changes of the liver, renal cortex and adrenal cortex and atrophy of the lymph glands.

Histopathology.

The Liver.

All the cases of undoubted Domsiekte showed marked fatty changes in the liver. In all ewes still pregnant at death this infiltration was peripheral in distribution although in one the entire lobule was so affected as to make it impossible to express any opinion on the intralobular distribution of the fat. In the case of the non-pregnant sheep which showed symptoms the infiltration was not nearly so marked and was centrally placed.

In speaking of peripheral or central infiltration it must be stated that in all cases the entire lobule was affected to some degree, but that the density and size of the fat droplets were greater towards one zone.

The intralobular distribution of the fat does not appear to be specific. Both de Kock (1928) and M'Fadyean (1924) state that it is "usually peripheral" but the latter author gives two microphotos, from different parts of the same liver, one showing peripheral and one central fatty changes.

In all cases the fat is laid down in very large droplets, often appearing to fill the entire cell and displacing the nucleus. There are no other changes present and the nucleus appears to be perfectly normal. In no instance was there a bile stasis, and Berlin-blue stained sections showed no abnormal presence of iron in the hepatic or Sterne cells.

Although this fatty infiltration of the liver is the most apparent change seen in Domsiekte and is probably constantly present, it cannot, of course, be considered specific. The degree of fatty infiltration does not show any relation

to the severity of the symptoms. In fact sheep that showed no symptoms presented fatty infiltration of the liver to the same extent as those that died in coma. Further, the normal pregnant sheep from group 1 which was slaughtered for the collection of comparative material showed a fatty infiltration equal to many of the Domsiekte cases.

As M'Fadyean (1924) has stated " It is generally recognised that towards the end of pregnancy, even in normal conditions, there is a tendency towards fatty infiltration of the liver that approaches the pathological ".

The Foetal Livers.

In all typical pregnant cases the foetal liver was sectioned and stained with Sudan III but no fatty changes were observed.

The Kidneys.

All the sheep that had been starved showed very marked regressive changes of the kidneys. This lesion was entirely absent from the control pregnant animal slaughtered, but showed no correlation to the presence or severity of the symptoms. The changes in the kidneys cannot, therefore, be looked upon as the direct cause of the symptom complex of domsiekte.

The distribution and nature of the changes seen were remarkably constant throughout. These changes consisted of a marked fatty degeneration and regressive changes.

Fatty Changes.

The fatty changes consisted in all cases of the inclusion in the epithelium of large droplets of fat staining bright red with Sudan 3. In a few cases there was also a fine powdering of small Sudan staining droplets present, but these two forms did not appear to have any direct connection with each other and appeared in entirely different portions of the organ. The large droplet degeneration will be dealt with first.

On examination of Sudan 3 stained sections under low power the distribution of the fat was most constant and striking. The typical picture consisted of a band of bright red tubules radiating outwards from the junctional zone and extending for a variable distance up the medullary rays. It was noticeable that not all the tubules of the ray were affected and the fat ended abruptly and at a practically constant level for all tubules towards the medulla. A variable number of tubules of the labyrinth of the cortex also showed large droplets of fat. A remarkable feature of this fat deposit was its all or none character, i.e. if a given tubule was affected it contained large droplets of fat, often displacing the nucleus, and practically all the cells were affected, while no fat at all could be seen in the unaffected tubules. In other words there was no evidence to suggest that the gross accumulations of fat arose through coalescence of fine droplets. Another noticeable feature was that where there were only a low percentage of convoluted tubules affected, these occcurred in groups in the section suggesting that the affected transections were portions of the same tubule.

These fat containing tubules were in all cases identified as proximal convoluted tubules. The fat changes in the medullary ray, beginning at the junctional zone were constant in all affected kidneys. As the fat changes became more extensive they progressed up the medullary ray and a greater percentage

of tubules in the labyrinth became affected. It would, therefore, appear that the fatty deposit takes place in the distal part of the proximal convolved tubule (spiral tubule) first and progresses up the tubule to later affect the convoluted portion. In no case were the glomeruli, Bowrian's capsule or the other tubules of the labyrinth affected in the same way.

When Sudan 3 stained sections were examined under higher power it was noted that the proximal convoluted tubules exhibited a marked foaminess and vacuolation of the cytoplasm of the epithelial cells, where there was no fat visible. As one approached an area of fat deposit the vacuoles became larger and appeared as hyaline droplets. Some of these then show a bright red rim, extending a variable distance into the droplet. All variations between large "Hyaline-like" droplets and completely stained fat droplets could be found. It would, therefore, appear that the droplets are formed first of a substance not staining with Sudan 3 and that this later metamorphoses into, or is replaced by, a Sudan staining fat.

It was noticeable that the cytoplasm showing these vacuoles stained a pinkish khaki colour as against the clear blue staining of the unaffected tubules.

When stained with Nile Blue the fatty deposit stained mauve to violet while the fat of the adipose tissue of the pelvis was pink. This would indicate that the fat in the affected epithelial cells contained a mixture of fatty acids and neutral fat. The same droplet and crescent or ring-staining reaction was seen in the Nile-Blue sections as in the Sudan 3 stained preparation. Staining with Nile Blue, therefore, did not give any clue as to the nature of the substance in the hyaline-like droplets or in the unstained centre of the ringed forms.

In kidneys showing very marked fatty changes there was often also a fine powdering in the cytoplasm of the tubules of the medulla and in the distal convoluted tubules. These changes were never marked and no preformed vacuoles could be seen.

Only in one case was the fat (1) confined to the basal portion of the cells, i.e. against the basal membrane. In other cases the fat droplets had no specific position, in fact they were often so large as to occupy the entire cell, with the nucleus displaced and pressed to one side.

In another instance (28) medium sized fatty droplets were seen in the nuclei themselves. This fat stained bright red with Sudan 3.

Together with these fatty changes there were marked regressive changes. As described above the cells of the proximal convoluted tubules show a foamy and droplet degeneration. This does not always pass over into fatty inclusions as described but often results in a cytolysis of the entire cell with karyolysis of the nucleus. Spherical agglutinations of hyaline cytoplasm, about the size of an erythrocyte, are often liberated in the lumen. The distal convoluted tubules were not so regularly or so markedly affected but sometimes showed catarrhal desquamation. Casts were not found in the typical cases but were often encountered, sometimes calcified, in the kidneys of sheep which had died some time after abortion.

These changes were not accompanied by inflammatory changes in the uncomplicated cases. There was sometimes a slight hyperaemia of the cortex and medullary rays but no cellular infiltration. In two cases complicated by septic metritis, following abortion, focal purulent nephritis was superimposed on the changes described.

Although it is admitted that the fatty accumulations in the cells and the degenerative changes are probably closely related, the two processes did not appear in combination at the same place. That is to say, the cytolysis and necrobiosis were usually seen where there was no fat present and the epithelium most affected with fatty changes (the spiral tubules) did not show marked desquamation. The desquamation was more marked in the more proximal coils of the Proximal Convoluted Tubules.

Two cases (24 and 21) showed a peculiar glomerular degeneration not seen in the sheep selected as typical for domsiekte. Both these sheep had shown symptoms 20 and 30 days prior to death but one had aborted and the other lambed soon after, and they had not shown symptoms since then. In both these kidneys a low percentage of renal corpuscles showed a filling of Bowman's capsule with a homogeneous substance stained pink with eosin and a shrinking and displacement of the glomerulus. Some of the glomeruli were completely disintegrated, appearing as cellular debris in the mass of pink staining material.

In another case (13) the glomeruli showed necrobiosis. This animal died of typical domsiekte with the lamb in utero, but had shown marked symptoms for a longer period than usual before death (4 days).

In all other cases of typical domsiekte the glomeruli showed no changes. It would appear, therefore, that the degeneration of the glomeruli, as well as the formation of casts, is a further stage in the changes of the kidney, but that the sheep usually die before they appear.

Berlin-blue sections of the kidneys showed very little or no iron present. Only in two cases was there haemosiderin in the tubular epithelium and this only affected a very low percentage of tubules. One of these cases was complicated with purulent metritis.

It will be seen that the kidney changes constitute a nephrosis in the generally accepted meaning of that term, and these findings confirm those of Roderick and Harshfield (1932) in all respects except that more advanced lesions were found, consisting of the formation of casts and the presence of glomerular degeneration.

It is of interest to note that the main site of fatty degeneration in the nephron of these sheep corresponds to that described by Dible and Hay (1940) in the kidneys of starved rabbits (spiral tubule). These authors claim that in their cases the increase in visible fat was directly proportionate to the increase in extractable fat and to the adiposity of the animal. They aver from this that the increased visible fat is due to a fat infiltration and not to fat phanerosis.

There is no reason to suppose that the same does not apply in domsiekte.

The Heart.

Many of the starved sheep, both clinical and non-clinical cases of Domsiekte, showed slight fatty degeneration of the myocard. The fat was in fine droplets throughout the fibres and was often patchily distributed. In no case was this degeneration very severe. No other changes were observed in the heart. The mildness of this degeneration coupled with the fact that it occurs in sheep killed for post-mortem as well as those which died, does not point to a degeneration of the myocard being an important factor of death in Domsiekte.

The Spleen.

The spleens of all the starved sheep showed a marked reduction in the size of the Malpighian corpuscles with the central artery appearing very prominent. At first glance the actual number of Malpighian bodies appeared to be greatly reduced but on closer examination it was found that this appearance was due to not observing extremely small bodies. Secondary follicles were entirely absent, the lymphoid tissue being represented solely by a narrow zone of small lymphocytes surrounding the central artery.

This reduction of lymphoid tissue, and of lymphopoietic activity, in the spleen, would appear to be in direct relationship to the lymphocytopenia noted in the circulating blood.

The spleens were also stained for fat (Sudan 3) and for free iron (Berlin-blue). No abnormal fat was noted and there was no increase in the amount of haemosiderin, many cases of domsiekte actually appeared to show less iron in the spleen than the normal controls.

In order to test out the correctness of the impression that there was a decrease in the size of the Malpighian bodies in the spleens of the starved sheep the following technique was applied. Sections were prepared from samples taken at random from the spleens of seven starved sheep. Seven control spleens from normal sheep were similarly treated. Using a low power objective and the camera lucida the outlines of trabeculae and Malpighian bodies were traced on graph paper. The edges of the spleen, where visible, and any holes in the section were also outlined. By a simple count of the graph squares the total area of trabeculae, red pulp and white pulp was obtained. From these figures the following were worked out:—

1. The number of Malpighian bodies per unit area (irrespective of the size).
2. The percentage of the total spleen area occupied by trabeculae.
3. The percentage of the total pulp area occupied by white pulp.

The following table gives a summary of the results:—

TABLE 5.

	Average Number of Malpighian Bodies per Unit Area.	Average Percentage of Total Area Occupied by Trabeculae.	Average Percentage of Total Pulp Occupied by White Pulp.
		Per Cent.	Per Cent.
Controls.....	22·6	6·3	7·5
Starved Animals.....	17·6	10·1	3·3

When the results were analysed statistically it was found that there was no significant difference between the numbers of Malpighian bodies in the two groups but that the other two differences shown were significant ($p = 0 \cdot 05$).

The increase in the percentage of space occupied by trabeculae would indicate a general atrophy of the splenic pulp. The significant drop in the area occupied by white pulp without any decrease in the number of centres present, is statistical proof of the decrease in size of the Malpighian bodies noted above.