Changes in the Liver Fat of the Pregnant Sheep at Different Levels of Nutrition and during Starvation

By N. L. FERGUSON

School of Agriculture, King's College, University of Durham

(*Received* 18 *January* 1954)

The importance of a study of the ovine fatty liver arises from the frequent reports of this condition in cases of pregnancy toxaemia occurring in the field or induced experimentally (Roderick & Harshfield, 1932; Frazer, Godden, Snook & Thomson, 1938–9, 1939–40; Groenewald, Graf, Bekker, Malan & Clark, 1941; Underwood, Curnow & Shier, 1943). According to Best & Ridout (1933, 1936), Best (1934), Best & Channon (1935), Best & Huntsman (1934–5), Best, Mawson, McHenry & Ridout (1936) and Channon & Wilkinson (1934), excessive fat accumulates in the liver of experimental animals, principally rats, under a variety of circumstances, e.g. when the diet is rich in fat, when cholesterol is given, after pancreatectomy and following injection of anterior-pituitary extracts.

In the pregnant animal, knowledge is uncertain about liver-fat changes, although it is of interest that Marshall (1922) quotes Mottram as finding an increasing 'physiological' infiltration of liver fat in woman during normal pregnancy. There are conflicting reports from the few instances of similar studies on animals. Coope & Mottram (1914) showed appreciable increases of liver fat in cats and rabbits, but Maclean (quoted by Best & Ridout, 1933) could not confirm this observation with rabbits. For the pregnant ewe, whether normal or suffering from pregnancy toxaemia, reports of fatty liver appear to have implied an infiltrated state, although there have been unsubstantiated claims that the presence of large quantities of fat renders the normal functioning of the liver cell impossible. There appears to be no certain evidence for considering this state of the ewe's liver to be a pathological one, although no attempts have been made to reverse the movement of fat into the liver of this species.

Snook (1939–40), reported 5–7% total fat for the liver of the normal pregnant ewe, claiming that fatty infiltration is not usual in normal gestation, but that it is a feature of pregnancy toxaemia. Dryerre & Robertson (1940–1), on the other hand, demonstrated a rise from 7 to nearly 12% total fat between the 2nd and 4th months of gestation in healthy ewes, and considered that pregnancy toxaemia accounted for only a slight increase in fat beyond this upper level, although they pointed out that they had examined only a few cases of the disease. It is significant that some of the same material used by Dryerre & Robertson as the basis of their researches was also examined by Snook.

Wallace (1948) found that there was little rise in the liver fat of ewes that were well nourished at the 144th day of pregnancy, even though low levels occurred at the 91st day. A considerable increase in liver fat occurred, however, when the quantity of food eaten was reduced over this period. The divergence of opinion between these workers represents the present state of knowledge of the 'fat status' of the ovine liver during pregnancy.

Starvation in its early stages has generally been assumed to result in the accumulation of liver fat, although in rats the results are variable (Best & Ridout, 1933). Roderick, Harshfield & Hawn (1937) found that the total liver fat in twelve experimentally starved ewes and lambs varied between 9·12 and 34·75%, and that this increase was proportional to the degree of inanition and to the period of starvation, although Best (1934) found that prolonged starvation may result in a decrease of liver fat. Ohta (1940) found that, whereas fasting in the rat caused large increases in liver fat at high summer temperatures, a decrease in fat occurred at low temperatures. Using rats and rabbits, Dible (1932) and Dible & Libman (1934) demonstrated marked increases in liver fat during starvation.

It seemed desirable to re-examine liver-fat changes in the pregnant ewe in relation to different levels of food intake accurately measured, and to study the effect of imposed periods of fast just before parturition when field cases of pregnancy toxaemia may be expected.

EXPERIMENTAL

Management of the ewes. Old 'half-bred' ewes (Border Leicester × Cheviot), which varied somewhat in type, were purchased for the experiment. They had the run of an old pasture and received no supplementary food for 5 weeks before the commencement of the experiment. On 10 November 1953, following individual treatment with 20 g phenothiazine, forty-four ewes were penned singly indoors, where they slept and were fed. They were released each day for from 5 to 6 h in an uncovered yard where water was allowed *ad lib.* Sawdust was used sparingly as bedding and the pens were cleaned daily.

Feeding of ewes. For the first 100 days of pregnancy the food consisted of the following mixture, fed together with hay and swedes to all ewes: crushed oats 30, dried brewer's grains 20, bran 20, linseed cake (crushed) 10, white-fish meal 10, dried sugar beet pulp (molassed) 8 and mineral mixture 2 parts by weight.

At the 100th day, the ewes were divided at random into two groups, which were maintained for the remainder of the experiment on high and low dietary planes respectively. The change to the low plane of nutrition was made gradually by withdrawing the mixture of concentrates over a period of 3 days, simultaneously allowing more hay and roots. Later, a mixture of hay and oat straw, and finally straw only was given together with roots to the low-plane group. High-plane ewes received no straw but, on a few occasions at an advanced stage in the experiment, mangolds were given to both groups when supplies of swedes were not available. Analyses of the foods are presented in Table 1. With the exception of the concentrate mixture the digestibility of the crude protein was determined by the method of Wedemeyer (1898) and the starch equivalent was estimated by the use of Hallsworth's (1949) equation

$$\text{starch equivalent} = 95{\cdot}11 - 1{\cdot}633 \times \text{crude fibre}.$$

The values for the concentrate mixture were obtained from Woodman (1948).

Table 1. *Percentage composition and feeding values of the foods consumed by ewes*

Constituent	Concentrates	Hay	Oat straw	Swedes	Mangolds*
Dry matter	86·8	85·3	88·6	11·5	12·0
Crude protein	19·0	5·8	1·9	1·3	1·0
Crude fibre	10·5	31·4	38·9	1·4	0·7
Ash	6·8	5·5	—	0·8	0·8
Digestible crude protein	15·8*	3·4	1·0	1·1	0·7
Estimated starch equivalent	60*	30	21	7·4	6·2

* Figures taken from Woodman (1948).

Throughout the experiment it was not possible to feed the ewes individually according to appetite, but a standard ration was allowed to all animals according to their group. The aim for the first 100 days of pregnancy for all animals, and for the high-plane group throughout, was to promote weight increases, and to allow losses of weight for the low-plane group in late pregnancy. It was accomplished by increasing or decreasing the ration periodically. Feeding times were 7.30 a.m., when the concentrated mixture was given, and 3.30 p.m., when the ewes received hay, roots and straw. The ewes of the low-plane group received all their roots at the morning feed. Records of daily food consumption were made beginning with the 5th day of feeding.

From the 138th day of pregnancy, food, but not water, was withheld for periods varying from 1 to 6 days from certain animals of both groups, which for this purpose were confined to their pens without access to the yard.

Weighing of ewes. The ewes were weighed at approximately 10.30 a.m. on Mondays and Thursdays throughout the experiment, but for the week before, and during the period of, fast the weighings were daily with the exception of Sundays.

Slaughter of ewes. Fig. 1 indicates the periods of gestation at which ewes were slaughtered.

On the 12th and 13th days of the experimental régime when all the ewes were feeding normally and were increasing in weight, five of them, selected at random, were slaughtered. A Suffolk ram was then allowed access to the remainder of the flock and conception dates were recorded as the day following service. From the 95th to the 102nd day of pregnancy a further group of six ewes, selected at random, was slaughtered. It was intended that from the 138th to the 144th day of pregnancy both fasted and unfasted ewes should be slaughtered at daily intervals. This was accomplished with the starved animals (seven on the high plane and eight on the low plane) but between the 119th and the 137th days of pregnancy it was necessary to slaughter seven low-plane ewes in deteriorating condition, so that only three ewes of this group remained to be killed in an unstarved condition from the 138th day onwards; in the high-plane group, eight unfasted animals were killed between the 138th and 143rd days of pregnancy. On the days they were killed the ewes that were allowed food received their morning meal and were quietly walked some 12 yd. to a site where they were shot and bled; a blood sample was taken from the slaughtered ewes for the determination of ketones. The livers were removed as quickly as possible and taken to the laboratory.

Analytical procedure. The liver fat was determined gravimetrically as the total ether-soluble fraction. The minced liver was homogenized and submitted to 4 h continuous hot extraction with 97% ethanol. The tissue residue and alcoholic extract were then continuously extracted with boiling diethyl ether for 2 h. The ether extract was dried to constant weight.

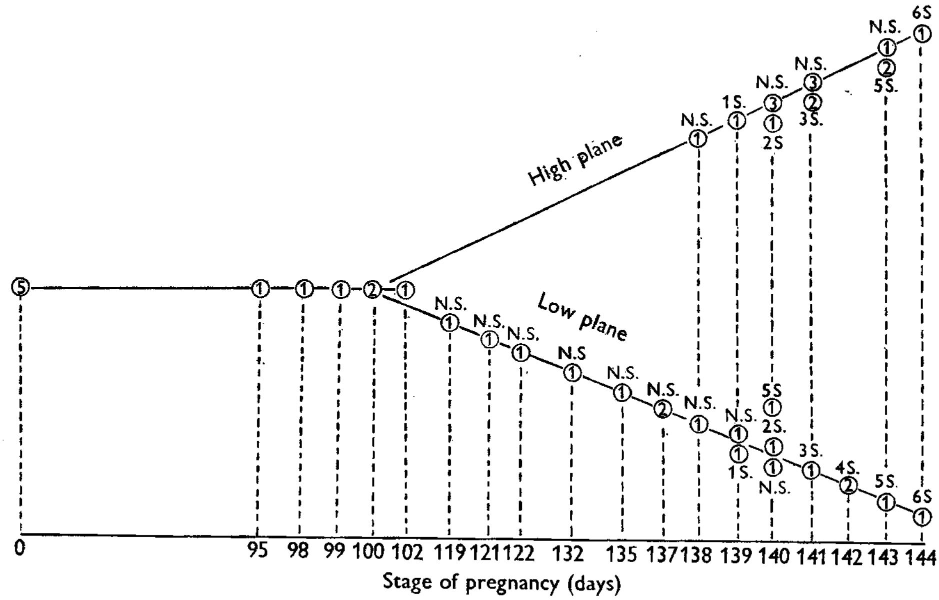

Fig. 1. Diagrammatic representation of the distribution of slaughterings over the pregnancy period. The number of ewes killed on any particular day is indicated by the figure in the circle. N.S., not starved; 1 S, ..., 6 S, number of days starved.

RESULTS

General progress of ewes

Although the area of each pen was only 5 × 2 ft. and the ewes were not able to turn round when confined, they appeared to experience no discomfort and had sufficient room in which to lie down. During the starvation period the ewes were not allowed access to the yard, but the space allotted to each animal was increased to 20 sq.ft.

When released from their pens, the ewes showed little inclination for exercise. With advancing pregnancy, the ewes on a high plane of nutrition spent most of their time lying down, being apparently contented; the ewes on the low plane of nutrition behaved in a similar way, because of weakness. When the first ewes of the low-plane group showed signs of pregnancy toxaemia they were gently exercised, but the exercise was not continued when it was found to be without beneficial effect.

Foot rot was prevalent and persisted despite frequent treatment. Ewe no. 31 (high-plane group, starved for 3 days) was lame for this reason and in pain for some time before her scheduled date of slaughter, but her appetite and weight were satisfactory. Ewe no. 57 (low-plane group, unstarved) was heavily infested with intestinal worms. A low-plane unstarved ewe, no. 46, died some 5–6 h before she was due to be slaughtered, and the liver was removed 3–4 h after death.

Food consumption

The mean food consumptions of the ewes for specimen weeks are presented in Table 2.*

Table 2. *Mean values (lb./week) for the food constituents, their starch equivalents and digestible crude-protein content eaten by the ewes*

Week of pregnancy	Hay	Straw	Swedes	Concentrates	Starch equivalent	Digestible crude protein
			All ewes			
1	10·3	—	7·0	7·0	7·76	1·54
5	10·3	—	7·0	7·7	8·20	1·65
9	10·0	—	7·0	10·2	9·56	2·04
14	8·2	—	17·5	12·2	11·07	2·38
			High-plane ewes			
16	8·2	—	21·1	12·9	11·20	2·41
19	7·5	—	26·8†	12·2	11·28	2·39
			Low-plane ewes			
16	—	8·0*	24·0	—	3·55	0·37
19	—	5·6	13·1†	—	1·86	0·14

* Including hay-straw mixture.
† Mangolds, including a few swedes.

It was felt desirable to restrict slightly the food eaten by the ewes until the 100th day of pregnancy, since beyond that time further increases in weight were expected for the high-plane ewes. The purpose was to avoid lack of appetite that might have followed overfeeding. This procedure had to be carefully controlled as the low-plane ewes had to be sufficiently advanced in condition by the 100th day of pregnancy to permit them to decline markedly in weight when their ration was restricted.

Up to the end of the 14th week of pregnancy, little difficulty was experienced in getting the animals to eat enough to maintain a steady increase in weight. From that time onwards the food consumption became more irregular in the high-plane group and the quantity of hay consumed dropped to 7·5 lb. by the 19th week. The weekly consumption of roots increased at this time to an average of 26·8 lb. and this quantity was eaten by all ewes of the high-plane group without any failure.

At a late stage in pregnancy it was necessary to change the supply of fish meal, as the original supply was finished. As the ewes of the high-plane group did not eat the new fish meal so readily the proportion of it in the concentrate mixture was reduced to 5% and remained at this level to the end of the experiment. The result was that the consumption of concentrates fell from 12·9 to 12·2 lb. between the 16th and 19th weeks of pregnancy, although during this interval the starch equivalent and digestible crude protein consumed were stabilized at approximately 11·2 and 2·4 lb./week respectively.

The food eaten by the low-plane ewes was severely reduced between the 14th and 16th weeks from the values of 11·07 lb. starch equivalent and 2·38 lb. digestible crude protein to 3·55 lb. starch equivalent and 0·37 lb. digestible crude protein. The weekly

* Records of the food consumption of every ewe and her weight changes are lodged in the Library of King's College, Newcastle upon Tyne, and are available for reference.

consumption of roots by this group at the latter date was 24 lb., but during the 19th week roots were being refused, and consumption dropped to the values of 1·86 lb. starch equivalent and 0·14 lb. digestible crude protein.

Weight changes

Fig. 2 shows the mean weekly weight changes of all ewes up to the 100th day of pregnancy, and of the unstarved high- and low-plane animals from that date until the 138th day. The weight for any one week is the mean of the weights recorded on Monday and Thursday of that week.

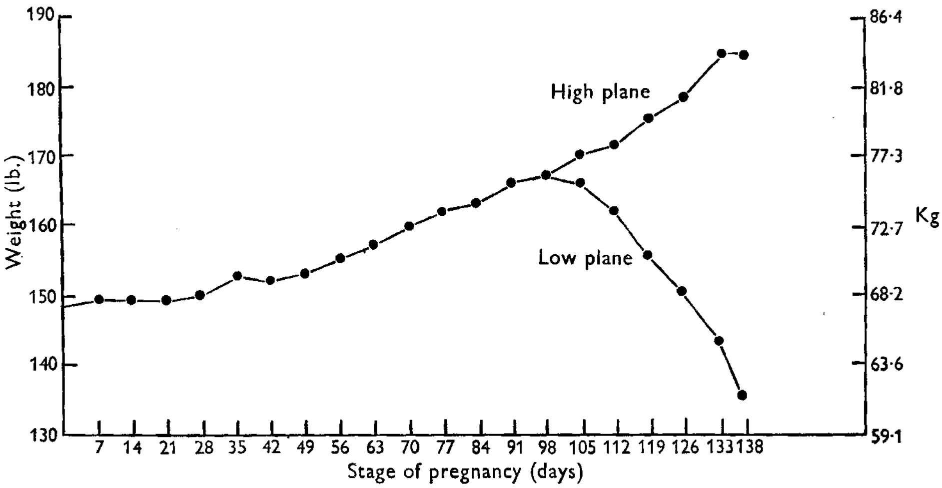

Fig. 2. The mean weekly changes in weight of unstarved ewes during pregnancy. The line represents thirty-nine ewes until the 98th day, then fifteen ewes in the high-plane group, and eighteen dropping to twelve ewes in the low-plane group.

The desired gradual rise in weight from the beginning of pregnancy (149 lb.) to the 100th day (168 lb.) was thus accomplished satisfactorily; the mean gains for this period varied between 4 and 31 lb. with a mean of 19 lb. The high-plane ewes gained on an average a further 17 lb. up to the 138th day of pregnancy. At this time (138th day) the low-plane ewes averaged 136 lb. in weight, or 49 lb. less than the high-plane group (185 lb.), a decrease of 13 lb. on the mean weight (149 lb.) for all ewes when non-pregnant. A single animal (no. 45) increased in weight by 2 lb. during the 38 days she was on the low plane of diet, and ewe no. 13 (high-plane) lost 11 lb. between the 133rd and 135th days, although some of this loss was recovered later. During the starvation period, the maximum loss in weight in the high-plane group of ewes was 31 lb. (ewe no. 9, starved for 6 days) and in the low-plane group 22 lb. (ewe no. 22, starved for 5 days).

Liver fat

Details of changes in total liver fat in the different groups of non-starved animals are given in Table 3. The ewes were weighed immediately before they were slaughtered and the weights shown refer to these figures and not to weekly average weights.

Table 3. *Changes in total liver fat of the ewes during pregnancy*

(Mean values with their standard errors)

	Non-pregnant	100th day of pregnancy	High-plane, not starved (138th–143rd day)	Low-plane, not starved (135th–140th day)
No. of animals	5	6	8	6
Weight (lb.)	170 ± 6	180 ± 2	182 ± 8	134 ± 5
Liver dry matter (g/100 g fresh liver)	31·4* ± 0·92	29·0 ± 0·45	30·0† ± 0·55	43·7 ± 1·41
Liver fat (g/100 g fresh liver)	8·80 ± 0·53	6·16 ± 0·31	8·87 ± 0·34	26·7 ± 1·99
Liver fat (mg/g body-weight)	1·02 ± 0·06	0·79 ± 0·05	1·08 ± 0·06	5·55 ± 0·55

* Mean for two ewes. † Mean for six ewes.

The percentage dry matter of the liver rose with the increase of fat in the livers. There was no apparent alteration in gross weight of the livers throughout the experiment, suggesting that fat infiltration occurred at the expense of water.

A slight decrease of fat from 8·8 to 6·1% occurred between the beginning and the 100th day of pregnancy, this difference being significant at $P=0{\cdot}05$, although when fat is expressed as mg/g body-weight, the difference only approaches border-line significance. An increase of the same order (6·1–8·8%) occurred between the 100th day of pregnancy and the 138th to 143rd day with the high-plane ewes, this difference being significant at $P=0{\cdot}05$. At this time the liver fat content of the high-plane animals was the same as before pregnancy (8·8%). There was a considerable accumulation of fat (26·7%) in the livers of ewes on the low plane of nutrition by the time they reached the 135th–140th day of pregnancy and this increase over all other groups was highly significant at $P=0{\cdot}001$; the increase was in fact three times that of the high-plane group at the 138th–143rd day of pregnancy and 20% more than the value for fat at the 100th day of pregnancy. When shown as a fraction of the body-weight, fat changes paralleled those expressed as a percentage of the fresh liver weight, but the marked loss in weight suffered by the low-plane animals accounted for a five-fold increase in their liver fat compared with that for the high-plane group.

The number of ewes starved in the high- and low-plane groups was seven and eight respectively, and the values for these animals are presented separately in Table 4, since variations in total liver fat between individuals were too great to permit mean values to be given for each group.

No marked change in the fat content of the liver occurred in the high-plane group of ewes with continued starvation after the 1st day, but the increase in fat in these animals was striking compared with the level attained in the unstarved animals of this group (8·8%, see Table 3). The general level of fat in the livers of the starved sheep was comparable with that (about 20%) attained by the low-plane animals in the 2nd, 3rd and 4th days of their fast. There was a tendency for fat to decrease in the liver by the 5th day of starvation in low-plane animals. An unexpectedly low figure for fat (5%) was obtained for ewe no. 42 (high-plane, starved for 1 day); it was out of keeping with values for the other ewes of this group and no explanation can be offered to account for it. It is also impossible to account for the high value (32%) for fat shown by ewe no. 45 (low-plane, starved for 1 day); ewe no. 19 (low-plane, starved for 6 days) had 32% of fat in the liver, an unexpected result as it is higher than the average for low-plane ewes before starvation (26·7%), but one which may be correlated with the marked activity and highly strung nature of this small animal.

A histological assessment of fat, to be given elsewhere, gave general agreement with the actual amount found by chemical analysis.

Table 4. *Changes in total liver fat in individual starved ewes, previously maintained on high and low planes of nutrition, at the end of pregnancy*

	High plane							
Ewe no.	42	13	11	31	15	12	9	
Weight (lb.)	204	180	178	174	152	166	164	
Period of starvation (days)	1	2	3	3	5	5	6	
Liver dry matter (g/100 g fresh liver)	27·5	37·5	41·0	42·0	—	35·0	31·7	
Liver fat (g/100 g fresh liver)	5·00	20·9	24·0	24·0	14·1	13·9	20·0	
Liver fat (mg/g body-weight)	0·63	2·69	3·20	3·54	2·30	1·95	2·67	
	Low plane							
Ewe no.	45	50	35	8	21	22	47	19
Weight (lb.)	165	110	110	119	128	170	122	100
Period of starvation (days)	1	2	3	4	4	5	5	6
Liver dry matter (g/100 g fresh liver)	51·0	42·9	45·1	38·0	34·0	—	39·2	42·1
Liver fat (g/100 g fresh liver)	32·0	21·9	23·9	21·9	14·0	10·9	20·0	32·0
Liver fat (mg/g body-weight)	5·39	3·37	3·95	3·86	2·86	1·45	3·48	7·99

Pregnancy toxaemia

Though the aim of the experiment was not the induction of pregnancy toxaemia, the appearance of this condition was anticipated, and opportunity was taken of studying it in relation to the level of diet and deprivation of food. Particulars of three cases observed are presented in Table 5.

Table 5. *Information about three cases of pregnancy toxaemia in unstarved low-plane ewes*

Ewe no.	4	16	17
Weight (lb.)	148	143	126
Stage of pregnancy at slaughter (days)	119	121	132
Liver dry matter (g/100 g fresh liver)	37·0	40·0	48·0
Liver fat (g/100 g fresh liver)	10·0	20·9	29·9
Liver fat (mg/g body-weight)	1·47	2·93	5·27

Though the livers of these animals contained more fat than those of the animals slaughtered at the 100th day, variations within the group were considerable. The circumstances of each case are considered below.

Ewe no. 4. On the 117th day of pregnancy the ewe did not rise and feed as usual at the morning feeding, and she trembled slightly when taken from her pen. She was able to walk, and consumed (approximately) $1\frac{1}{4}$ lb. of straw at the afternoon feed. On the 118th day she was unwilling to rise and again refused her food; she became dejected, although able to rise and to stand propped against the pen. There was little or no reaction to handling, and breathing was laboured. She ate 10 oz. straw in the afternoon. On the 119th day of pregnancy the ewe was scarcely able to stand and could not walk; she became semi-comatose although no smell of acetone was detected. She was slaughtered at 10.53 a.m. and was found to be bearing triplets, the combined weights of which were 14·19 lb. The concentration of ketone bodies in the blood was normal. The concentrations of calcium and magnesium in the serum lay within the normal physiological range.

Ewe no. 16. On the 109th day of pregnancy the ewe was found staring into space and did not react to handling. Her appetite declined and her vision became impaired. She separated herself from the others, seeking support when standing, and moving very slowly. Later she moved backwards with her head held downwards, and was *in extremis* shortly before slaughter on the 121st day of pregnancy. She was found to be bearing twin foetuses which together weighed 10·69 lb. The concentration of blood ketones was slightly higher than normal, although no smell of acetone was detected. The concentrations of calcium and magnesium in the serum lay within the normal physiological range.

Ewe no. 17. On the 125th day of pregnancy the ewe was found with her head pushed into a fence. She rose with some difficulty and moved with a sideways gait, and finally became semi-comatose and was unable to stand. Before slaughter on the 132nd day of pregnancy she made marked convulsive movements and extensor spasms of the hind-legs. On post-mortem she was found to be pregnant with twins, whose combined weight was 14·9 lb. The concentrations of calcium and magnesium in the serum lay within the normal physiological range. The concentration of ketone bodies in the blood was slightly greater than usual but no acetone was detected by smell.

All the low-plane ewes showed lassitude and unco-ordinated movements of varying degrees; some ground their teeth, but coma did not develop except in ewes nos. 4 and 16; no nervous symptoms were observed except in ewe no. 17. The high-plane ewes showed none of the marked abnormalities of the kinds described, but in the starved animals there was some weakness and slowness of movement after the longer periods of fast. With one unstarved low-plane ewe (no. 57) the dejected appearance was accentuated by a heavy intestinal worm burden.

DISCUSSION

The divergence of opinion between Dryerre & Robertson (1940–1), on the one hand, and Snook (1939–40) and Wallace (1948) on the other, about the fat content of the livers of well-nourished pregnant ewes, has emphasized the importance of an accurate knowledge of nutrient intake by experimental ewes. Snook apparently examined liver specimens from some of the ewes that were used in the experiments of Dryerre & Robertson and that are reported to have received daily 1·8 lb. starch equivalent and 0·25 lb. protein equivalent from oats, bran and hay, in addition to what they derived from good pasture. Frazer *et al.* (1938–9, 1939–40) provided ewes with 1·69 lb. starch equivalent and 0·25 lb. protein equivalent/day, and Wallace supplied 1·83 lb gross digestible energy and 0·36 lb. protein equivalent daily. In the present work, daily intake at the 100th day of pregnancy was 1·58 lb. starch equivalent and 0·34 lb. digestible crude protein (or a little less than this amount of protein equivalent), and with the high-plane ewes at the time of slaughter, 1·54 lb. starch equivalent and 0·34 lb. digestible crude protein. In the absence of any British standard for feeding ewes in late pregnancy, and in view of the fact that all the animals used in these experiments increased in weight and remained healthy, it can be assumed that the ewes were receiving a generous ration that was adequate in all respects.

Individual consumption was unfortunately not determined for the ewes studied by Dryerre & Robertson, and it is known that fat ewes, the type used in these workers' researches, are liable to decrease their food intake late in pregnancy. In the present work, for example, the daily consumption of ewe no. 13, 1·54 lb. starch equivalent and 0·32 lb. digestible crude protein on the 135th day of pregnancy, dropped on the 136th day to 0·54 lb. starch equivalent and 0·08 lb. digestible crude protein, and on the 137th day to 0·68 lb. starch equivalent and 0·10 lb. digestible crude protein. The present experiment has demonstrated the marked increase in liver fat in the low-plane ewes with food intakes lower than those quoted and, more dramatically, in the high-plane animals after a short period of fasting. There appear, therefore, to be grounds for Snook's contention that the appreciable rise in liver fat that he noticed in a few of his ewes could be attributed to variable food consumption.

The mean values for liver fat (8·9%) in well-nourished ewes found in the present study are higher than the values of 5·5% given by Snook (1939–40) and lower than those of Dryerre & Robertson (1940–1) (about 12%), but the findings support in general the views of Snook (1939–40) and Wallace (1948) that normal pregnancy is not accompanied by liver fat infiltration. Furthermore, they show clearly the effect of lowered intake of energy and protein in causing increases in fat, a finding at variance with that of Dryerre & Robertson (1940–1) who considered that excessive fatty infiltration was not due to dietetic factors.

In the experiments reported here the animals on the low dietary plane at the 119th day of pregnancy were receiving only straw and roots, and the daily food consumption dropped to an average of 0·39 lb. starch equivalent and 0·05 lb. digestible crude protein; by the 133rd day food consumption decreased to only 0·27 lb. starch equivalent and 0·02 lb. digestible crude protein, but further reductions still occurred in a few of the animals that remained after this date. Low-plane animals beginning their fast were thus in a state bordering upon starvation, so that withdrawal of food made no great difference to their metabolism, and the fat content of the liver changed little from earlier values for this group before starvation (26·7%).

Phillipson (1950) suggested that under such circumstances fat metabolism would be little altered by actual fasting and that ewes would experience little distress since they were already well accustomed to using their body fat. There were little or no body reserves of fat remaining at the end of the longer periods of starvation in the low-plane animals, indeed some of them were emaciated; if the liver in its infiltrated state represented the only appreciable body store of fat, it is understandable that liver fat should be reduced as starvation progressed.

Dryerre & Robertson (1940–1) reported lower values for fat (6·04%) than those obtained in the present work (8·8%), for non-pregnant ewes, but a striking difference between their results and those reported here is found at the 100th day of pregnancy. Further investigation of this point is required, for the decline to 6·1% fat reported here, compared with Dryerre & Robertson's figure of nearly 10% for the 100th day of pregnancy, may possibly be associated with the increased appetites of the ewes, and the increased efficiency of food utilization. Head (1953), however, has shown that pregnancy has no effect on the digestibility of the dry matter and nitrogenous constituents of a diet on which Cheviot ewes lost considerable weight, but that digestibility coefficients of dry matter and nitrogen fell considerably from November until the beginning of April in both pregnant and barren ewes. In the present work, however, the ewes were well nourished for the first 100 days of this period.

It is difficult to draw any conclusions about the significance of liver-fat changes in pregnancy toxaemia from the three cases of this disease reported here. Liver fat in these three ewes varied between 10 and 30% and the mean value of 20% is in fact lower than that encountered in the low-plane unstarved animals (26·7%) which showed no clinical signs of the disease. In field cases and in those experimentally induced, anorexia is invariably reported, so that, considering the main conclusions of the present experiment, the frequent finding of fatty liver could be ascribed to reduced food intake. Actual starvation did not lead to the disease in either the well-fed or poorly fed animals, although in the former it resulted in a marked rise in liver fat, which was also the result of the reduced food consumption of the low-plane group. Moreover, loss of condition *per se* late in pregnancy does not necessarily predispose to pregnancy toxaemia as Leslie (1933) suggested, since the animals, when starved, showed remarkable reductions in weight. It is interesting, however, to note that the cases of pregnancy toxaemia which did occur, and which could be considered of the nutritional type, were on the 119th, 121st and 132nd days of pregnancy, whereas starvation was not imposed until the 138th day.

SUMMARY

1. Total liver fat has been determined in a series of forty-four 'half-bred' pregnant ewes maintained on high and low levels of nutrition and following short periods of starvation.

2. Between the 138th and 143rd days of pregnancy ewes well nourished during the period of gestation had the same amount of liver fat (8·8%) as did non-pregnant animals, but at the 100th day there was slightly less.

3. Starvation imposed on well-fed ewes at the 138th day of pregnancy resulted in a pronounced rise in liver fat to an amount that changed little during the fasting period of 6 days.

4. Reduction of the plane of nutrition from a high level at the 100th day of pregnancy to one that approached starvation level, resulted in an increase in the fat content of the liver to 26·7% between the 137th and 140th days of pregnancy.

5. When at the 138th day of pregnancy starvation was imposed on ewes that had

been on a low plane of nutrition since the 100th day, there was an indication of a slight fall in liver fat as starvation progressed.

6. Pregnancy toxaemia, which occurred in three unstarved ewes on the low-plane diet, is considered in relation to these findings.

The present work was made possible by financial assistance from the Agricultural Research Council, and through facilities afforded at the University of Durham Agricultural Experiment Station. The day-to-day management of the ewes was in the hands of A. Jopling and later C. Ball, to whom thanks are due for this practical assistance. Acknowledgement is made of help received from the staff of the Veterinary Investigation Centre, Newcastle upon Tyne, in the preparation of liver homogenates, and especially from P. S. Pugh, who advised in planning the experiment and undertook the analyses for liver fat.

REFERENCES

Best, C. H. (1934). *Lancet*, **226**, 1155.
Best, C. H. & Channon, H. J. (1935). *Biochem. J.* **29**, 2651.
Best, C. H. & Huntsman, M. E. (1934–5). *J. Physiol.* **83**, 255.
Best, C. H., Mawson, M. E. H., McHenry, E. W. & Ridout, J. H. (1936). *J. Physiol.* **86**, 315.
Best, C. H. & Ridout, J. H. (1933). *J. Physiol.* **78**, 415.
Best, C. H. & Ridout, J. H. (1936). *J. Physiol.* **86**, 343.
Channon, H. J. & Wilkinson, H. (1934). *Biochem. J.* **28**, 2026.
Coope, R. & Mottram, V. H. (1914). *J. Physiol.* **49**, 23.
Dible, J. H. (1932). *J. Path. Bact.* **35**, 451.
Dible, J. H. & Libman, J. (1934). *J. Path. Bact.* **38**, 269.
Dryerre, H. & Robertson, A. (1940–1). *J. Physiol.* **99**, 443.
Frazer, A. H. H., Godden, W., Snook, L. C. & Thomson, W. (1938–9). *J. Physiol.* **94**, 346.
Frazer, A. H. H., Godden, W., Snook, L. C. & Thomson, W. (1939–40). *J. Physiol.* **97**, 120.
Groenewald, J. W., Graf, H., Bekker, P. M., Malan, J. R. & Clark, R. (1941). *Onderstepoort J. vet. Sci.* **17**, 245.
Hallsworth, E. G. (1949). *J. agric. Sci.* **39**, 254.
Head, M. J. (1953). *J. agric. Sci.* **43**, 214.
Leslie, A. (1933). *Aust. vet. J.* **9**, 9.
Marshall, F. H. A. (1922). *Physiology of Reproduction.* London: Longmans Green and Co.
Ohta, S. (1940). *J. Biochem., Tokyo*, **31**, 197. Quoted in *Nutr. Abstr. Rev.* (1940–1), **10**, 132.
Phillipson, A. T. (1950). In *Toxaemias of Pregnancy, Human and Veterinary. A Ciba Foundation Symposium.* [J. Hammond, F. J. Browne and G. E. W. Wolstenholme, editors.] London: J. and A. Churchill.
Roderick, L. M. & Harshfield, G. S. (1932). *Bull. N. Dak. agric. Exp. Sta.* no. 261.
Roderick, L. M., Harshfield, G. S. & Hawn, M. C. (1937). *J. Amer. vet. med. Ass.* **43**, 41.
Snook, L. C. (1939–40). *J. Physiol.* **97**, 238.
Underwood, E. J., Curnow, D. H. & Shier, F. L. (1943). *Aust. vet. J.* **19**, 164.
Wallace, L. R. (1948). *J. agric. Sci.* **38**, 93.
Wedemeyer, K. (1898). *Land Vers. Stat.* **51**, 375. See also *Agric. Progr.* (1945), **20**, 52.
Woodman, H. E. (1948). *Bull. Minist. Agric., Lond.*, no. 48, 11th ed.