

# Ovine White-Liver Disease (OWLD). Pathology

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**Ulvund M.J.: Ovine white-liver disease (OWLD). Pathology. Acta vet. scand. 1990, 31, 309-324.** – Microscopic liver changes could earliest be found after 1 month on OWLD pasture, and included extensive fatty change with large spherical vacuoles in hepatocytes, varying size of hepatocytes and nuclei, and formation of Councilman bodies. Later came ceroid deposits, biliary hyperplasia and mesenchymal proliferation. Changes occurred in all lambs which died or were killed due to OWLD, and altogether 83% of the lambs grazing OWLD pastures showed typical or suspect changes. Widespread haemosiderosis of the spleen was common. In severely affected lambs, sclerosis of the Peyer's patches and of the germinative centres of the intestinal lymph nodes were seen, as were neuronal atrophy and patchy microcavitation of areas in the brain stem. Four had polyvasculitis. Cobalt/vitamin B<sub>12</sub> supplemented lambs showed no specific changes. Lambs which grew well on other pastures (H lambs), but which were subclinically Co/B<sub>12</sub> deficient some years, showed no fulminant hepatic OWLD, but 15% developed some features seen in OWLD. They showed no extensive fatty change. Results indicate that OWLD is a manifestation of B<sub>12</sub> deficiency worsened by factors triggering early hepatic fatty change resulting in a more severe liver damage with loss of intracellular homeostasis rendering the hepatocytes vulnerable to other elements, like copper.

sheep; cobalt/vitamin B<sub>12</sub> deficiency.

## Introduction

Ovine white-liver disease (OWLD) has been characterized by fatty change, hepatocyte necrosis, ceroid deposits and bile duct proliferation (*Sutherland et al. 1979, Ulvund & Øverås 1980*). Status spongiosus of the brain (*Richards & Harrison 1981*) and polioencephalomalacia (*Sutherland et al. 1979*) have been reported in some lambs.

It has been unclear whether OWLD is a pure cobalt/vitamin B<sub>12</sub> deficiency, or whether coexistent factors are necessary for development of the disease. Our experiments indicate that although Co/B<sub>12</sub> deficiency is essential, cofactors play a part (*Ulvund 1990b, d, Ulvund & Pestalozzi 1990b*).

Pure Co deficiency results in fatty infiltration of the liver and deposition of haemosiderin in the spleen (*Filmer 1933*). In experimental Co deficiency, *MacPherson et al. (1976)* and *Fell (1981)* in fact found liver lesions similar to those seen in OWLD.

This article describes the pathology, especially the development of microscopic liver changes, in lambs with OWLD as compared with Co/B<sub>12</sub> supplemented lambs grazing the same pastures, and normally growing lambs on other pastures, some of which were subclinically B<sub>12</sub> deficient.

### Materials and methods

Experimental design has been described (Ulvund & Pestalozzi 1990a, Ulvund 1990c). Altogether 458 twin lambs of the Dala and Ry-gja breeds were included during a 6-year study (1981-86). Pasture outlet was in mid May each year, when the lambs were about 1 month old. The lambs grazed with their dams throughout the experimental period. Lambs designed S grazed OWLD pastures, which were either moderately (SCo) or heavily (SCo+) fertilized with Co, or not Co fertilized (S). H lambs grazed disease free control pastures, which some years had marginal to deficient Co contents. Prophylactic measures and symbols used are given in Table 1.

Development of the lesions was especially scrutinized during one year (1982). Altogether 16 lambs from 3 groups were killed after 11 and 22 days, and later after 1, 2, 3 and 5 months respectively on pasture (Table 2). Necropsy was performed in these, and in additional 17 lambs which died or were killed by intravenous injection of pentobarbital throughout grazing (Table 3). Based on the development of the liver lesions, criteria for typical (+) and suspect/indicative OWLD ( $\pm$ ) were put up.

In all 33 lambs which were necropsied (Table 3), liver (2 or 3 sections), ileum, spleen, jejunal lymph nodes, myocardium, lung and brain were examined. In the 16 lambs killed throughout grazing, following additional specimens were examined: Rumen, abomasum, duodenum, jejunum, caecum, colon, adrenals, kidney and muscles.

In addition, livers were examined from altogether 218 lambs from the various groups slaughtered at the end of the experimental period. Brains, ileum and jejunal lymph nodes were examined from altogether 39 lambs slaughtered in 1981, ileum and jejunal lymph nodes from 29 lambs slaughtered in 1983, and kidneys from 46 lambs slaughtered in 1985.

Tissues were fixed in 10% neutral buffered formalin (Carson *et al.* 1973) within a few minutes after killing, or about 1 h after slaughter. Tissues from the lambs found dead were fixed 1-18 h after death. Tissue samples were processed by conventional paraffin embedding, and routinely stained with haematoxylin eosin (HE). In 1982, additional stains were used for liver, spleen, lymph nodes and ileum: Periodic acid Schiff (PAS), van Gieson, Ziehl-Nielsen and Berlin (Culling 1963). Blood was drawn at killing

Table 1. Survey of group symbols used in the experiments.

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S	: Lambs grazing OWLD pastures
SCo	: Lambs grazing moderately cobalt fertilized OWLD pastures
SCo+	: Lambs grazing heavily cobalt fertilized OWLD pastures
SCoSO <sub>4</sub>	: Lambs dosed weekly with cobalt sulphate by mouth
SB <sub>12</sub>	: Lambs injected every two or three weeks with vitamin B <sub>12</sub>
SCuO	: Lambs dosed with copper oxide needles
SCopell	: Lambs dosed with cobalt pellets
SCoSeCu	: Lambs dosed with cobalt-selenium-copper glass boli
SSe	: Lambs dosed with selenium pellets
SColick	: Lambs having access to cobalt salt lick on OWLD pasture
H	: Lambs grazing disease-free H-pastures situated elsewhere Several H lambs were subclinically Co/B <sub>12</sub> deficient
HCo	: Lambs grazing Co fertilized H pastures
HCopell	: Lambs grazing H pastures, but dosed with cobalt pellets
HColick	: Lambs having access to cobalt salt lick on H pasture

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Table 2. Survey of lambs which were killed at various intervals throughout grazing, 1982. Time on pasture, lamb group and number, age (days) and live weight (lw, kg) at killing date, mean lw for the group, and weight increase (g/day) in each lamb during the 2 weeks preceding killing. Serum glutamate dehydrogenase (GLDH, U/l), plasma methylmalonic acid (MMA,  $\mu\text{mol/l}$ ) and vitamin B<sub>12</sub> (pmol/l) on the date of killing are also given. For group symbols, see Table 1.

Time on pasture	Group	Lamb number	Age	Lw	Mean lw	g/day	GLDH	MMA	B <sub>12</sub>
11 days	S	1	32	19	14	429	4	2	158
11 -	SB <sub>12</sub>	2	35	13	15	214	11	1	1168
11 -	H	3	41	13	13	429	12	84	41
22 days	S	4	52	15	17	286	13	3	52
22 -	SB <sub>12</sub>	5	59	21	17	286	14	-	3345
22 -	H	6	37	13	15	286	11	44	119
1 month	S	7	66	17	18	357	53	49	66
1 -	SB <sub>12</sub>	8	45	19	19	429	6	2	402
1 -	H	9	49	16	18	286	13	3	322
2 months	S	10*	82	11	26	÷ 71	887	24	90
2 -	S	11	100	15	26	÷ 143	168	53	-
	SB <sub>12</sub>	12	86	32	29	429	13	2	-
3 months	S	13	114	30	29	214	40	80	99
3 -	SB <sub>12</sub>	14	115	33	35	286	12	3	5540
3 -	H	15	120	41	41	429	8	3	143
5 months	S	16*	160	17	27	÷ 143	21	19	94

\* Killed because of severeness of the OWLD condition.

Table 3. Total number of lambs (n = 33) examined post mortem. Year of experiment, lamb group and number of weeks on pasture before dying (D) or killing (K) are given. For group symbols, see Table 1.

Year	Group	Weeks of pasture
1981	S	D: 2,9,15* K: 18*,20*,21*
	SCoSO <sub>4</sub>	D: 13, 21
1982	S	K: 1.5-21 (5 lambs) 7*,20*
	SB <sub>12</sub>	K: 1.5-21 (5 lambs)
	H	K: 1.5-13 (4 lambs)
1983	S	D: 6 K: 20* (4 lambs)
	S	K: 19*
1984	SB <sub>12</sub>	K: 19
	SCuO	K: 19* (2 lambs)

\* Lambs which died of OWLD, or were killed due to severeness of OWLD.

and examined for vitamin B<sub>12</sub>, methylmalonic acid (MMA), and glutamate dehydrogenase (GLDH, *Ulvund* 1990a, b).

## Results

Altogether 11 out of 60 unsupplemented lambs grazing the OWLD pastures (S lambs) died or were killed due to OWLD (Table 3). Additional 2 lambs which grazed the same pastures, but which were dosed with copper (SCuO lambs), also had to be killed due to severeness of disease. One lamb dosed with cobalt sulphate (SCoSO<sub>4</sub>) died of torsion of the gall bladder, while 1 SCoSO<sub>4</sub> lamb and 3 S lambs were found dead on pasture with signs of diarrhea (Table 3).

## Necropsy

The lambs which were killed after 11 and 22 days on pasture (Table 2) were normal. The

S lamb killed after 1 month had swollen, pale and soft liver only. The S lambs killed after 2, 3 and 5 months respectively had changes typical for OWLD, as reported below. No specific changes were found in the killed B<sub>12</sub> supplemented lambs (SB<sub>12</sub>) or the normally growing lambs grazing other pastures (H lambs).

All lambs which died or were killed due to OWLD were emaciated, had crusty ears, eye discharge, rough wool, and pale mucous membranes. The livers were pale, swollen and doughy with reduced texture, in some the surface had irregular raised paler areas (Fig. 1). Myocardium was soft, skeletal muscles appeared pale and poorly developed. The gastrointestinal organs had reduced size and fillness. The jejunal wall appeared thin, and the lymph nodes were moist and slightly swollen. A few lambs had moderate ascites.

Two OWLD lambs which died or were killed after 15 and 18 weeks on pasture (1981, Table 3) had numerous pin head sized yellowish foci in the liver, myocardium, kidney cortex and jejunal mucosa. One of them had visible thrombi in vessels of the mesenterium, intestinal wall, ovaries and ovarian ligaments.

The 2 SCuO lambs (Table 3) were icteric. Out of 48 livers from S lambs, 33 (69%) were pale/soft or soft only (Table 4).

#### *Development of microscopical liver changes*

After 11 and 22 days on pasture: All lambs had normal livers.

After 1 month: Lamb 7 (Table 2) had extensive centroacinar lipid accumulation shown as large distinct spherical vacuoles displacing the nucleus in the hepatocytes (Fig. 2&3). The hepatocytes varied in size, there were scattered swollen cells with enlarged nuclei, swollen nucleoli, and hyperchromatosis of nuclear walls (anisocytosis/-nuc-

leosis, Fig. 2C). Some swollen hepatocytes contained bright homogenous or foamy cytoplasm, and were dissociated (Fig. 2B). There were scattered ovoid masses of condensed acidophilic cytoplasm, some with pycnotic nuclear remnants, and sometimes these bodies were present within hepatocytes or macrophages (Councilman bodies, apoptosis, cytosegresosome formations, Fig. 2A, B). Foci of shrunken hepatocytes were found as well (Coagulative necrosis). There was slight dilation of the space of Disse.

After 2 months: Lamb 10 had extensive formation of large vacuoles in centroacinar hepatocytes, anisocytosis/-nucleosis, scattered swollen and dissociated single hepatocytes, and Councilman bodies (Fig. 3B, C). Some hepatocytes and macrophages contained reddishbrown deposits (HE) which were acid fast, PAS positive, autofluorescent, displayed no activity in polarized light, and contained no iron (ceroid). Biliary hyperplasia, sometimes beyond the terminal plate, and mesenchymal proliferation in portal areas were present (Fig. 3B). Scattered neutrophilic polymorphonuclear cells (PMNs) as well as focal aggregations in the sinusoids, and hypertrophic Kupffer cells (Kc) were seen. Lamb 11 had similar changes, but fatty change was periacinar, and, in addition, proliferation of intralobular biliary cells were seen (Fig. 2D).

After 3 months: Lamb 13 had diffuse fatty change, extensive biliary hyperplasia, proliferation of intralobular biliary cells, and changes as in lamb 10 (Fig. 2E, 3D).

After 5 months: Lamb 16 had diffuse fatty change, ceroid deposits, moderate biliary hyperplasia, lymphocytic infiltration in portal areas, and scattered PMNs in the sinusoids.

Table 4. Total number of lamb livers (n = 251) examined (the material of Table 3 included), numbers which were pale *and* soft or soft only, numbers with microscopic changes typical (+) or suspect/indicative of OWLD ( $\pm$ ), and ratio of lambs with typical or suspect OWLD histopathology of total number examined.

Lamb group	n	Macro		Micro		total number
		Pale/ soft	Soft only	OWLD+	OWLD $\pm$	
S	48*	29	4	27	14	41/48
SCo	45	0	7	0	5	5/45
SCo+	12	0	4	0	0	0/12
SCoSO <sub>4</sub>	8	0	1	0	1	1/8
SB <sub>12</sub>	34	0	2	0	0	0/34
SCuO	6	6	0	5	0	5/6
SCopell	8	0	1	0	0	0/8
SCoSeCu	6	1**	0	0	1**	1/6
SSe	6	1	4	1	3	4/6
SColick	12	0	2	0	2	2/12
H	44	7	5	0	6	6/44
HCo	6	0	1	0	0	0/6
HCopell	10	0	0	0	0	0/10
HColick	6	0	1	0	0	0/6

\* Two lambs were killed (1982) before liver changes had developed.

\*\*In this lamb the CoSeCure pellet had been dissolved and absorbed.

In all S lambs, the liver changes were evenly distributed in all sections. All killed SB<sub>12</sub> and H lambs had normal livers with intact uniform hepatocytes.

#### Microscopic criteria for OWLD

Based on the results above, OWLD+ comprised: Fatty change (large spherical vacuoles with sharp borders), anisocytosis/-nucleosis, Councilman bodies, coagulative necrosis, ceroid deposits (Fig. 2F), biliary hyperplasia (Fig. 3F), mesenchymal proliferation in portal areas and infiltrations of mononuclear and PMN cells in sinusoids. In OWLD $\pm$ , changes were moderate, unspecific, and important features were lacking, like hepatocyte necrosis or biliary hyperplasia. Fatty change was not present, or only few scattered fat vacuoles could be detected.

#### Development of changes in other organs

No specific changes were found by microscopy of rumen, abomasum, duodenum, cae-

cum, colon, adrenals, myocardium, kidney, lung and muscles. After 2 months on OWLD pasture, the Peyer's patches (PP) of the ileum of lamb 10 showed lymphocyte depletion and appeared fibrosed, with ceroid deposits in reticuloendothelial cells (RE cells) located towards the periphery. In the S lambs killed after 2 and 3 months respectively on pasture, PP were also moderately fibrotic, but in lamb 16 these changes were pronounced, and RE cells contained ceroid (Fig. 4B, C). The SB<sub>12</sub>, SCo and H lambs had normal PP.

In the jejunal lymph nodes (LN), a similar development of changes took place. After 2-3 months on OWLD pasture, the germinative centres of the LN of lambs 10, 11 and 13 appeared hyalinized, and were mostly populated by large cells with enlarged nuclei, some containing ceroid. In the medulla, the RE cells were swollen and contained haemosiderin. The lymph nodes in the SB<sub>12</sub>, SCo and H lambs were largely normal.

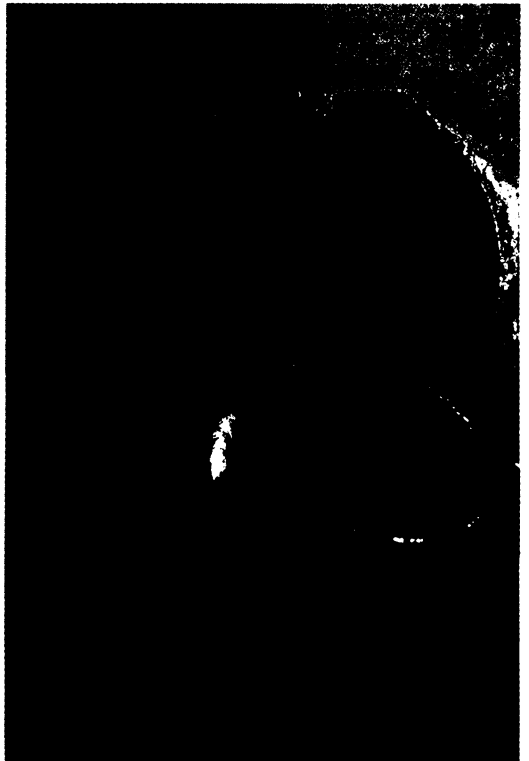


Figure 1.

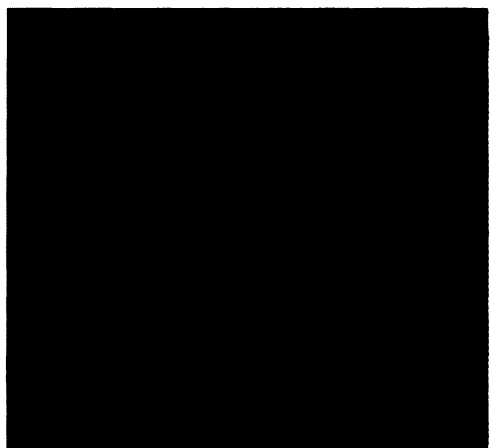
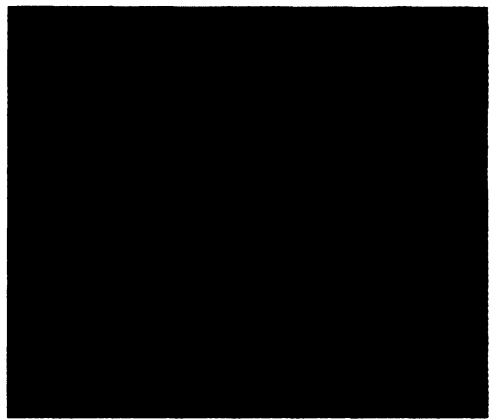
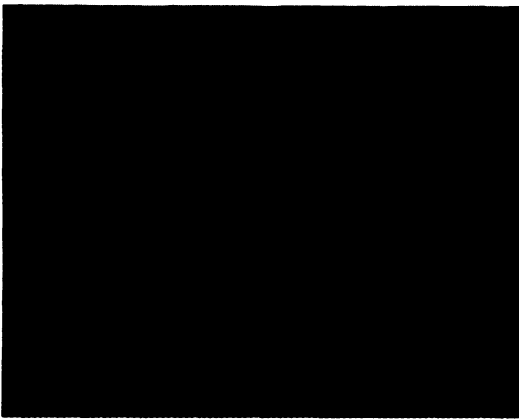
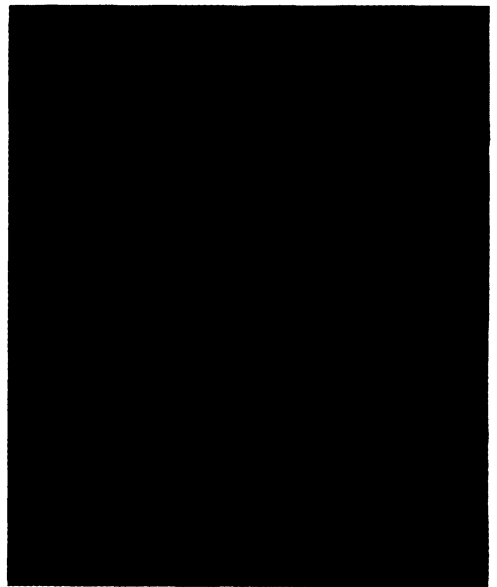
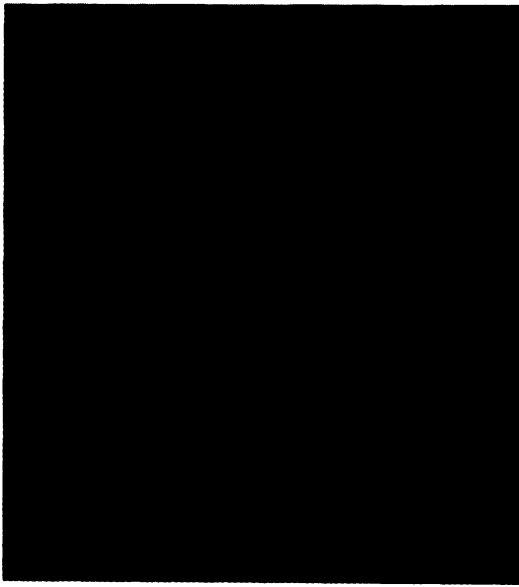


Figure 2.

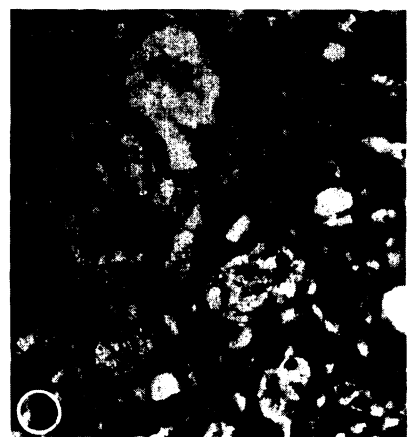
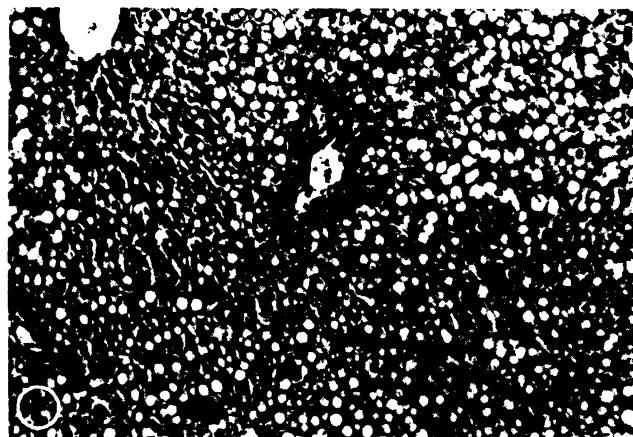
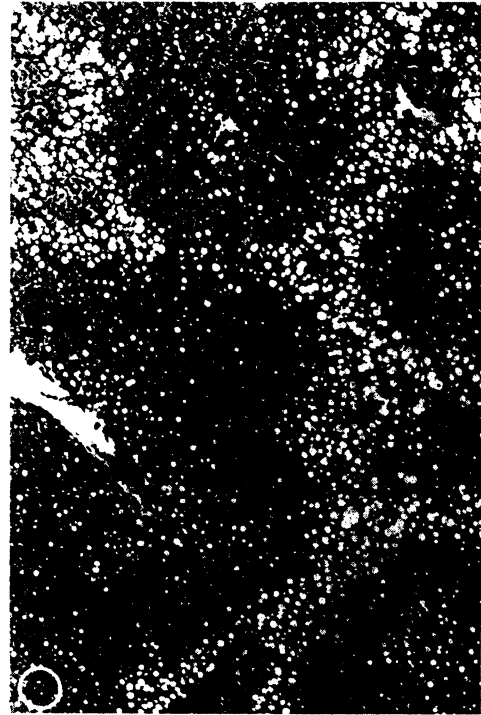
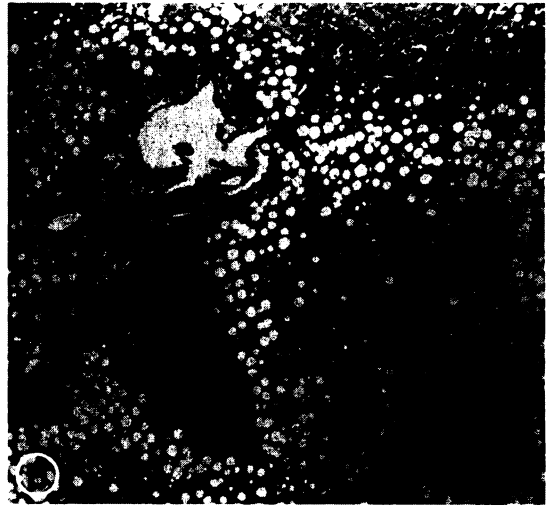
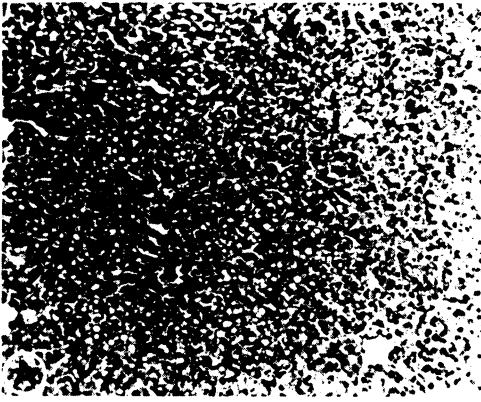


Figure 3.



Figure 1. Livers from lambs with ovine white-liver disease (OWLD). The livers were swollen, soft (A) or swollen, pale and soft (B, C). In some the surface had irregular, raised paler areas (D).

Figure 2. Photomicrographs (HE  $\times$  400) of livers from lambs killed at various intervals throughout grazing, 1982.

- A-C: Liver sections from lamb 7 killed after 1 month on OWLD pasture, showing fatty change, Councilman bodies (open arrows), swelling (dark arrows) and anisocytosis/-nucleosis (thin arrows).
- D: Liver section from lamb 11 killed after 2 months on OWLD pasture, showing proliferation of intralobular biliary cells.
- E: Liver section from lamb 13 killed after 3 months on OWLD pasture, showing accumulation of ceroid in degenerating hepatocyte.
- F: Liver section from an OWLD lamb slaughtered after 5 months on pasture (1982), showing ceroid in peri-acinar hepatocytes and phagocytes.

Figure 3. Photomicrographs of livers from lambs killed at various intervals throughout grazing, 1982.

- A: Liver section from lamb 11 killed after 2 months on OWLD pasture, showing fatty change. HE  $\times$  100.
- B: Liver section from lamb 7 killed after 2 months on OWLD pasture, showing centroacinar fatty change and biliary hyperplasia. HE  $\times$  100.
- C: Same as B, HE  $\times$  63.
- D: Liver section from lamb 13 killed after 3 months on OWLD pasture, showing diffuse fatty change and proliferation of inflammatory cells in parenchyma and perivascular stroma. HE  $\times$  63.
- E: Liver section from lamb 16 killed after 5 months on OWLD pasture, showing diffuse fatty change and moderate biliary hyperplasia. HE  $\times$  100.
- F: Liver section from an OWLD lamb slaughtered after 5 months on pasture (1982), showing biliary hyperplasia. HE  $\times$  400.

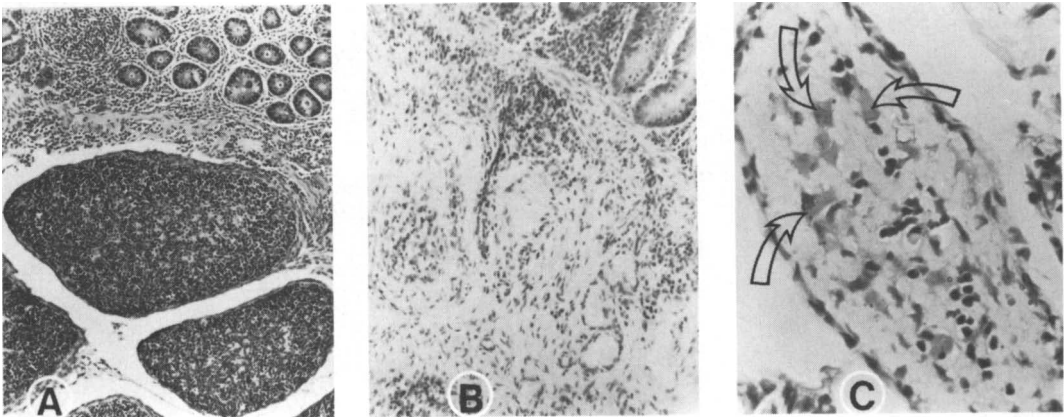


Figure 4.

- A: Photomicrograph of ileum from lamb injected every 3 weeks with vitamin B<sub>12</sub> and slaughtered after 5 months on OWLD pasture, showing normal Peyer's patches (PP). HE  $\times$  63.
- B: Photomicrograph of ileum from lamb 16 killed after 5 months on OWLD pasture, showing fibrotic PP with lymphocyte depletion. HE  $\times$  63.
- C: Same as B, showing ceroid deposits in reticuloendothelial cells of PP (arrows). HE  $\times$  400.

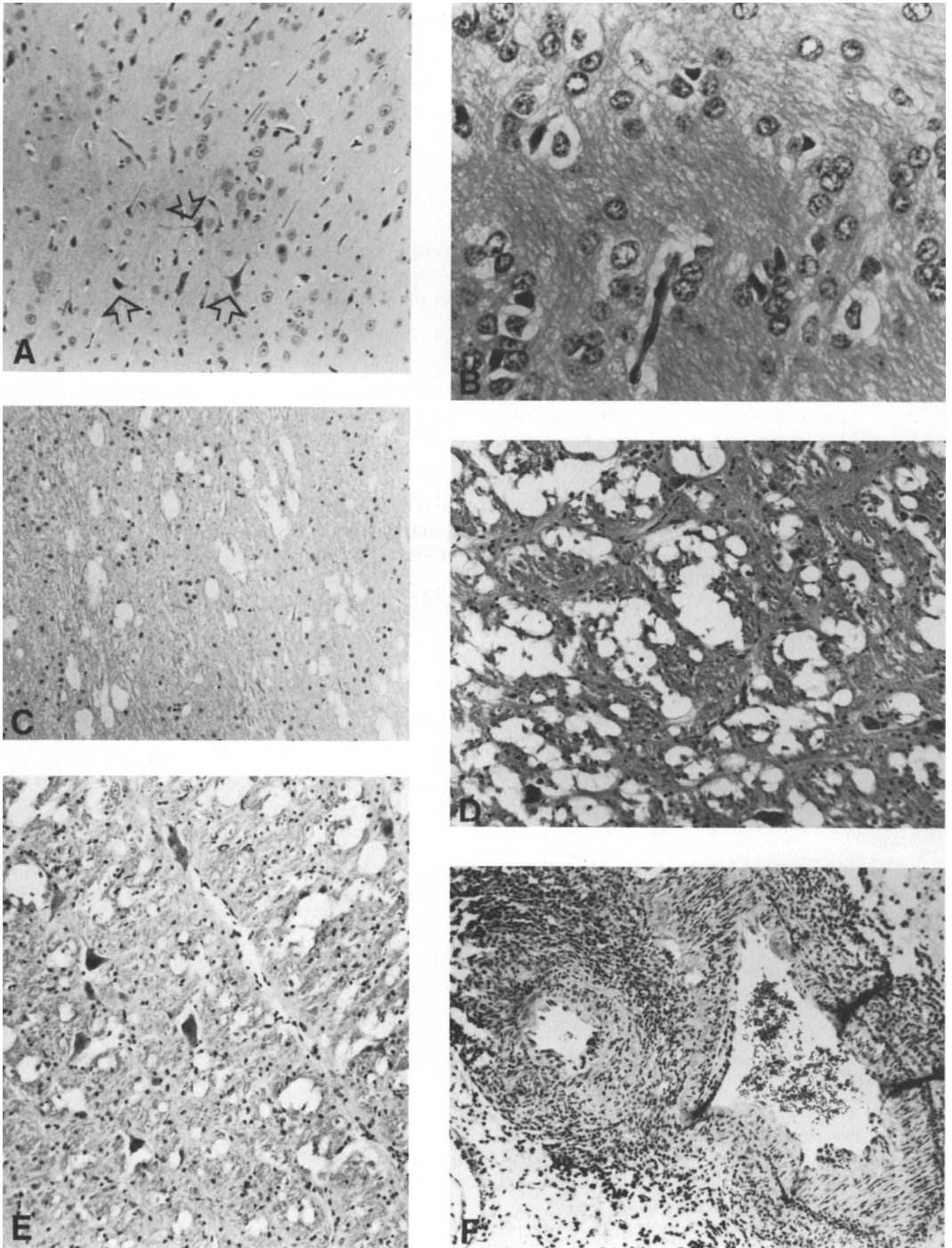


Figure 5.

In the spleen, increased amounts of haemosiderin was found in the red pulp after 2 months on OWLD pasture, and in all S lambs examined later. In the jejunum of some OWLD lambs, focal areas of epithelial necrosis and hypertrophy, and infiltrations of mononuclear cells were seen.

Microscopy of all brains of OWLD lambs which died or were killed, revealed scattered atrophic and pycnotic neurons (Fig. 5A), as well as an increase of pale swollen astrocytic cells with irregular indented outlines and prominent nucleoli (Alzheimer type II cells, Fig. 5B), especially in the deeper cortex layers and brain stem. There were also patchy areas of microcavitation of white and grey matter in deeper areas of cerebral cortex and throughout the brain stem (Fig. 5, C-E).

Two OWLD lambs which were necropsied after 15 and 18 weeks respectively on pasture (1981) had intramural hyaline necroses of arterioles, endothelial cell hypertrophy, and infiltrations of mononuclear inflammatory cells, sometimes with involvement of PMNs, in the vessel wall. Such vascular changes were found in the myocardium, adrenals, kidneys, lymph nodes, intestinal wall, mesenterium (Fig. 5F), and lung. A moderate polyvasculitis of arterioles in the intestinal wall was seen in an additional 2 OWLD lambs (1981).

#### *Occurrence of microscopic lesions*

All lambs which died or were killed due to OWLD had typical hepatic changes, as well as splenic haemosiderosis. About 70% had

varying degree of sclerotizing of PP, and 60% had similar LN changes. Of the 48 livers examined from S lambs, 56% were classified as OWLD+, while 83% either were OWLD+ or OWLD± (Table 4). All S lambs examined had varying degrees of neuronal atrophy and microcavitations of the brain.

Five out of 6 SCuO lambs had liver changes which could not be discerned from typical OWLD (Table 4). These 5 had sclerotic PP, as well as moderate neuronal atrophy and vacuolizations in the brain.

Only 1 out of 6 SSe lambs was classified as OWLD+. No lambs from any other group had fulminant OWLD. Three and 4 years respectively after Co fertilization of the OWLD pastures, 33 and 50% of the SCo lambs showed suspect liver histopathology with some few large fat vacuoles in hepatocytes, ceroid deposits, and few Councilman bodies. In the 2 SColick lambs classified as OWLD±, scattered large fat vacuoles were found, as well as some Councilman bodies. The SB<sub>12</sub> and SCoSO<sub>4</sub> lambs had normal livers, except for 1 SCoSO<sub>4</sub> lambs which was classified as OWLD±.

None of the H lambs had fulminant OWLD, but altogether 6 out of 44 had changes classified as suspect. This ratio was largest in 1986 (3/7). General fatty change never occurred.

#### *Blood samples*

All OWLD+ lambs had elevated serum GLDH and plasma MMA, together with plasma B<sub>12</sub> values below 100 pmol/l (Table 2).

Figure 5.

A-E: Photomicrographs of brain tissue from lambs which died or were killed due to OWLD.

A: Scattered atrophic and pycnotic neurons in cerebral cortex. HE × 100.

B: Increase of Alzheimer type II astrocytes in mesencephalon. HE × 400.

C-E: Varying degrees of patchy microcavitation throughout brain stem. HE × 100.

F: Photomicrograph of vessels of the mesenterium showing vasculitis with infiltrations of inflammatory cells throughout vessel wall. HE × 100.

## Discussion

The percentage of pale, soft livers among our S lambs (69%) was slightly higher than that found by *McLoughlin et al.* (1986) in a flock with OWLD (61%). *Sutherland et al.* (1979) reported that paleness and softness were typical in the acute phase, but less apparent in the chronic.

### *Microscopic liver changes*

Details of development of hepatic changes in simple Co deficiency have been difficult to find. The changes found in our lambs with clinical OWLD largely correspond to earlier descriptions (*Sutherland et al.* 1979, *Mitchell et al.* 1982). In our case, the fatty change appeared rather suddenly and extensively, and in the centroacinar zone first, while *Sutherland et al.* (1979) and *McLoughlin et al.* (1986) reported that it first appeared periacyary. Development of biliary hyperplasia came a few weeks later, as was also noted by *McLoughlin et al.* (1986).

To be able to discriminate between early fatty change and hydropic degeneration, specific fat staining is necessary (*Kelly* 1985). Due to lack of freeze sectioning facilities, this could not be performed. However, there is little doubt that the distinct vacuoles contained fat. In the severely affected lambs, the liver pieces floated in fixative, the least equivocal evidence of severe fatty change (*Kelly* 1985).

The fatty change appeared at a time when the lambs grew well (Table 2, *Ulvund & Pestalozzi* 1990a). Fatty change as a consequence of starving (*Kelly* 1985) is therefore excluded. The size and distribution of the vacuoles also differed from what we usually see in late pregnancy, ketosis or lactation, when hepatocytes often contain multiple vacuoles.

Although no morphometric examinations were performed, the appearance of hepato-

cytes of very varying size, together with varying size and appearance of the nuclei, led to classification of the changes as anisocytosis/-nucleosis. The eosinophilic structures seen in the hepatocytes were similar to the Councilman bodies described by *Wensvoort & Herweijer* (1975) in chronic hepatitis. The structures reflect sublethal hepatocyte injury caused by various insults, and synonymous names may be cytosegregosome formations or acidophilic bodies (*Kelly* 1985).

There was good correlation between liver changes and live weight (lw) gain. The few S lambs with normal livers at slaughter gained 6 (1981) and 17 kg (1984) respectively more on pasture than those classified as OWLD+. Generally there was no difference between the S lambs classified as OWLD+ and OWLD±. The SCuO and SSE lambs with normal livers gained 8 and 2 kg more than the others within their groups.

The appearance of OWLD suspect changes in SCo lambs three and four years after last Co fertilization correlated with reduced growth during those years (*Ulvund & Pestalozzi* 1990a), as well as reduced plasma B<sub>12</sub>, increased MMA (*Ulvund* 1990b), and reduced grass Co (*Ulvund & Pestalozzi* 1990 b).

In the H lambs the hepatocytes were strikingly better conserved, and extensive fatty change never occurred. The OWLD± H lambs had anisocytosis/-nucleosis, some few large vacuoles in hepatocytes in the periacyary (in 5) or centroacinar zone (in 1) and ceroid deposits in some cells. Only 1 (1983) had a moderate biliary hyperplasia. The findings indicated that the H lambs lacked the initial trigger which induced the sudden and extensive fatty change in the S lambs and led to a more serious liver damage.

In the H lambs there was lack of correlation between OWLD± changes and reduced lw gain. In 1985 and 1986, the H lambs classified as OWLD± were among the heaviest

within the group, and gained on average 7 kg more on pasture than the others. In 1983, however, the one H lamb with OWLD± was smallest and gained 15 kg less than the others. It showed no symptoms and was not blood sampled. This was the only H lamb with a moderate biliary hyperplasia.

Generally, there was good correlation between histopathology and clinical pathology (Ulvund 1990 a, b). In all S lambs, fatty change and hepatocyte necrosis were reflected in elevated serum GLDH (> 15 U/l), and fulminant OWLD was associated with increased plasma MMA (> 15 µmol/l) and lowered plasma B<sub>12</sub> (< 100 pmol/l, Table 2).

A certain correlation between suspect histopathology and blood chemistry was also seen in the H lambs. In 1985, the 2 OWLD± H lambs had plasma B<sub>12</sub> below 150 pmol/l throughout grazing, and plasma MMA between 1 and 11 µmol/l. From late July, serum GLDH was also highest in these 2 (> 15 U/l). In 1986, 2 of the 3 OWLD± lambs were blood sampled, and had the lowest plasma B<sub>12</sub> (< 100 pmol/l) and the highest plasma MMA (> 15 µmol/l) throughout Aug.-Sept., as well as the highest serum GLDH (> 70 U/l). The fact that these 5 lambs on average gained 20 kg more on pasture than the S lambs strengthens the hypothesis that they were not exposed to the initial OWLD »trigger mechanism«.

On the OWLD pastures, low amount of grass Co seemed to be correlated with seriousness of disease, as the higher content in 1984 (Ulvund & Pestalozzi 1990a) resulted in better growth and milder hepatic changes seen that year. The fact that H grass contained less Co than S grass in 1986 (0.08 mg/kg DM versus 0.12), further strengthens the suspicion that cofactors were present on the S pastures.

As judged from liver Cu, our OWLD lambs were Cu deficient (Ulvund 1990d), but sensi-

tive to Cu dosing (Ulvund 1990c), a fact which indicates that Co deficiency may predispose lambs to Cu toxicity. In hepatic damage due to pyrrolizidine alkaloids (Seaman 1987) or lupinosis (Gardiner 1966), similar mechanisms operate. Certain structural similarities exist at the cellular level as well. Features like anisocytosis/-nucleosis, fatty change, hepatocyte necrosis, Councilman bodies and bile duct proliferation occur in varying degree in alkaloid poisoning (Bull 1955, Hooper et al. 1974), lupinosis (Warmelo et al. 1970), annual ryegrass toxicity (ARGT), (Berry et al. 1982) and in the prehaemolytic phase in Cu poisoning of sheep. In Cu poisoning, the changes are thought to be due to loss of intracellular homeostasis (King & Bremner 1979). Loss of cell homeostasis could in fact be a common denominator for all these conditions.

Although Se dosing had no significant effect on lw (Ulvund 1990c), it increased liver Co (Ulvund 1990a), and the liver damage was less prominent than in the unsupplemented lambs (Table 4). The number of lambs was, however, too small to draw any conclusion.

#### *Changes in other organs*

Increased splenic haemosiderosis was a feature of our OWLD lambs, and has also been common abroad in lambs with OWLD (Sutherland et al. 1979) or Co deficiency (Filmer 1933).

In this area of Norway, we have had cases of polyvasculitis in lambs (Ulvund et al. 1984). We do not know whether these lambs were B<sub>12</sub> deficient, and further investigation of this may seem worthwhile. Polyvasculitis of the heart of B<sub>12</sub> deficient sheep was lately described by Mohammed & Lamand (1986). In experimental Co deficiency, Mac Pherson et al. (1976) reported vascular lesions in the brains of some sheep, and concluded that the relevance to Co deficiency had to be explored.

We found cerebrocortical necroses (CCN) in some earlier OWLD cases, but not in this material. In experimental Co deficiency, *Mac Pherson et al.* (1976) found CCN in some sheep, as did *Sutherland et al.* (1979) in OWLD lambs that had been blind. *Richards & Harrison* (1981) found status spongiosus and neuron necroses in basal cerebrum/cerebellum in 44% of the OWLD cases, while *Mitchell* (1979) found necrotic neurons in the cerebral cortex in some. Status spongiosus was also described in a goat with OWLD (*Black et al.* 1988).

CNS changes similar to the ones reported in our OWLD lambs were also found by *Fell* (1981) and *Fell et al.* (1985) in Co deficiency. Spongy change and development of Alzheimer type II cells have been typical for hepatocerebral disease in man, and in Wilsons disease due to abnormal Cu metabolism, and such changes in sheep may be an analogue (*Fell* 1981). In sheep injected with alkaloids, vacuolization of the brain stem showed a positive correlation with amounts of blood ammonia (*Hooper et al.* 1974). Brain vacuolization has also been found in sheep with lupinosis (*Allan & Nottle* 1979), Cu poisoning (*Doherty et al.* 1969), or ARG (Berry *et al.* 1980).

OWLD grass contained more Al than H grass (*Ulvund & Pestalozzi* 1990b). Al dosing in rabbits have resulted in fatty change and hepatocyte necrosis, pigment deposits in the spleen, and lymphocyte depletion and fibrosis of the splenic pulp (*Seibert & Wells* 1929). Al dosing of B<sub>12</sub> deficient lambs might be performed, to explore whether Al could have some significance in the OWLD condition.

The changes seen in PP and LN have not been described by others in OWLD. It is difficult to tell whether these are due to lack of B<sub>12</sub>, or to starvation. The changes may be associated with the decreased resistance to-

wards parasites and possibly other agents (*Ulvund & Pestalozzi* 1990a).

The present investigation supports the view that OWLD is a manifestation of B<sub>12</sub> deficiency, but that cofactors act at the cellular level triggering early hepatic fatty change leading to a sequelae of changes resulting in loss of cell homeostasis.

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**Sammendrag**

*Kvitleversjuke (kobolt/vitamin B<sub>12</sub> mangel) hos lam. Patologi.*

Mikroskopiske leverforandringer ble tidligst funnet etter 1 måned på sjukdomsbeitet, og omfattet utbredt fettinfiltrasjon i form av store vakuoler i levercellene, anisocytose/-nucleose og dannelse av Councilman-legemer. Senere kom ceroidavleiring, gallegangshyperplasi, Kupffercellehypertrofi, og infiltrasjon av betennelseceller.

Totalt 83% av alle undersøkte lam som gikk på sjukdomsbeite hadde typiske eller suspekterte leverforandringer. Økt hemosiderinavleiring i milten var vanlig. Hos alvorlig affiserte dyr var det en viss lymfocytuttømmning og fibrose i de germinative sentra i

peyerplettene i tarmen og i tarmlymfeknutene, samt neuronal atrofi og mikroaviteringer i hjernestammen. Fire hadde polyvasculitt.

Kobolt/vitamin B<sub>12</sub> doserte lam viste ingen spesifikke forandringer.

Lam som gikk på andre beiter (H lam), og som noen år var subklinisk Co/B<sub>12</sub> deficitte, viste ingen fulminante OWLD forandringer. Et år hadde 3 av 7 lam moderate forandringer suspekterte for OWLD, men uten særlig fettinfiltrasjon. Resultatene viser at OWLD er en vitamin B<sub>12</sub> mangel intensivert av samvirkende faktorer som forårsaker fettavleiring i levercellene og større leverskade, som resulterer i at den intracellulære homeostasen ødelegges og levercellene blir mer sårbare for f.eks. kopper.

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