

THE HISTOPATHOLOGY OF WESSELSBRON DISEASE IN SHEEP

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INTRODUCTION

In 1956, Weiss, Haig & Alexander described a virus associated with abortion in sheep and called it "Wesselsbron" virus (WB), strain van Tonder. In sheep infection with the virus caused a febrile reaction, the mortality rate not being high. Pregnant ewes aborted during the febrile reaction. The mortality amongst foetuses carried to full term and new-born lambs was very high — practically 100 per cent. A new-born lamb infected experimentally with this virus showed a severe febrile reaction and died about 72 hours after injection. Histopathological examination of the liver showed congestion and diffuse necrobiosis of the liver cells; in addition there was infiltration with lymphocytes and neutrophils showing karyorrhexis. The liver cells also showed bile pigmentation and the bile capillaries appeared prominent and in parts were distended with bile.

In 1958, Belonje published a report on the field observations he had made on Wesselsbron disease during a severe outbreak which occurred in the autumn of 1956/57 in the Middelburg District. He described various forms of the disease ranging from peracute to chronic and mild or abortive cases. Post mortem examination usually revealed jaundice to a greater or lesser degree, disseminated haemorrhages in the subcutis and intermuscular connective tissue and subserous and submucosal haemorrhages in the heart and intestines. The serous cavities almost invariably contained a blood-stained or yellow tinged fluid. In the peracute, acute and subacute forms of the disease the liver always presented a constant and typical picture — it was golden yellow in colour and of a firm consistency with the enlarged black coloured gall-bladder standing out in sharp contrast.

The author has had an opportunity of doing a histopathological examination on specimens which were collected during that outbreak. Specimens of the liver, spleen, myocard, kidney, intestine, gall-bladder and lungs were submitted in 10 per cent formalin without any details as to whether a particular set of specimens was collected from a peracute, acute or subacute case. Since the changes noticed histologically were of such a variable nature, an attempt at classification on that basis was not made. Specimens of the endocrine glands and brain were not submitted and the cases of encephalitis diagnosed clinically could not be confirmed histologically.

HISTOLOGICAL FINDINGS

As already stated, the histological changes presented a variable and inconstant picture and difficulty may be experienced in diagnosing isolated cases of this disease without the help of a biological test. The most striking histological changes were mainly confined to the liver. In what could be regarded as typical cases, this organ showed congestion and a diffuse fatty infiltration of the liver cells. The cells were markedly swollen and the typical cell cord pattern was disrupted. The nuclei appeared to be enlarged and some showed margination of the nuclear chromatin. In some cells the nucleoli were ill-defined, lost their basophilic properties and seemed to enlarge and coalesce to form irregular structures staining eosinophilically. These "inclusions" were actually only noticed in three cases (Fig. 1).

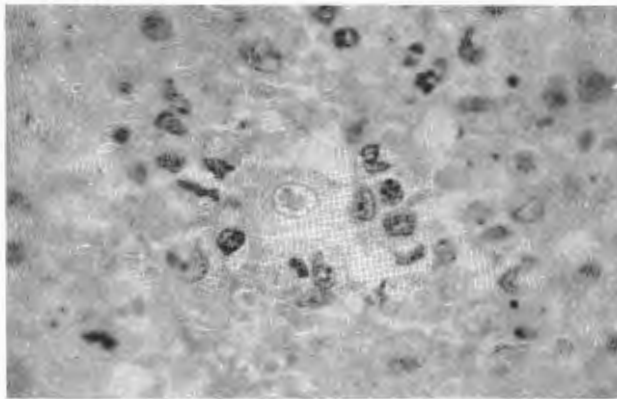


FIG. 1.—Specimen 53661. Liver. Liver cell showing intranuclear inclusion. Note the necrobiotic changes in the surrounding cells. HE.

It was interesting to note that isolated liver cells, diffusely scattered throughout the lobuli, had undergone necrobiotic changes. The cytoplasm showed condensation, hyalinisation and shrinkage; the nucleus faded or showed karyorrhexis and eventually the cell broke loose and became phagocytosed as a globular mass of debris by the reticulo-endothelial cells. Except in one or two cases, there was never any evidence of a well-defined centrilobular or focal intralobular necrosis (Fig. 2). In many cases only a few cells undergoing necrobiosis could be demonstrated in a section, whereas in other cases it varied from a large number of cells affected to almost a generalised necrobiosis affecting more or less all cells.

Pigmentation of the liver cells was a constant finding. The yellow discoloration noticed macroscopically was caused by fatty infiltration, bile stasis and bile pigmentation. In many cases the bile capillaries were prominent, dilated and distended with bile. In HE-stained sections yellowish-brown pigment granules could be demonstrated in the liver cells and Kupfer cells. The pigmentation did not seem to affect the cells according to any fixed pattern or to be accumulated in any particular part of the liver lobule. In many cells or groups of cells the cytoplasm was almost totally displaced by pigment granules (Fig. 3).



FIG. 2.—Specimen 53657. Liver. Necros.s. Note the cellular infiltration. HE.

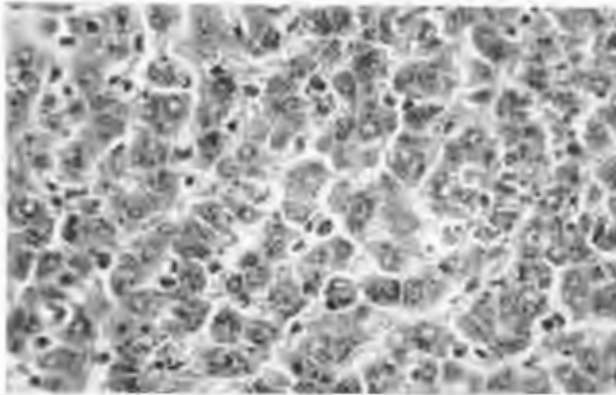


FIG. 3.—Specimen 53656. Liver. Extensive pigmentation of the liver cells. PAS.

In a few cases Giemsa-stained sections revealed the presence of cells containing pigment granules which stained a deep blue colour in addition to the cells containing pigment which reacted in the usual manner. This peculiar reaction of the pigment to Giemsa-staining was described by de Kock in 1928 in his studies on enzootic icterus. This condition must, therefore, be seriously considered in the differential diagnosis of Wesselsbron disease.

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Usually there was a mild cellular infiltration around the central veins, portal vessels and in the sinusoidal spaces (Fig. 4). The cells consisted mainly of fairly large mononuclear elements of the lymphocytic series and neutrophils -- the latter were often clumped together and showed karyorrhexis of the nuclei. The reticulo-endothelial cells appeared to be activated; they were enlarged and showed active phagocytosis of pigment granules, red blood cells, fragments of neutrophils and cell debris probably mainly derived from necrobiotic liver cells.

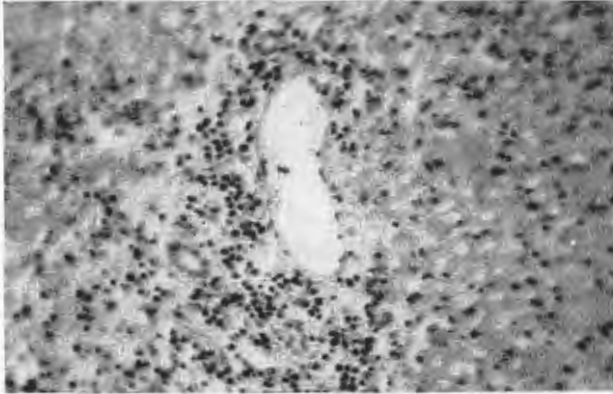


FIG. 4.—Specimen 53648. Liver. Lymphocytic perivascular infiltration. Note the fatty changes in the liver cells. HE.

No striking abnormal changes could be demonstrated in the other organs. In the majority of cases the kidneys showed congestion and fatty infiltration of the convoluted tubules. The myocardium invariably showed congestion and disseminated haemorrhages throughout the muscular tissue. Hyperaemia, emphysema and a slight lymphocytic infiltration of the septal walls were usually noticed in the lungs. Diminution of the cellular content of the malpighian bodies of the spleen took place in addition to the congestion and haemorrhage which were frequently present in this organ. The wall of the gall-bladder was often thickened as a result of oedema and the submucosa showed extensive haemorrhages. The intestine was similarly affected by haemorrhages.

Atypical cases

Whereas in the typical cases the diagnosis was based mainly on the combined presence of fatty changes, cellular infiltration, pigmentation and necrobiosis of scattered isolated liver cells, the atypical cases presented a variable picture which was rather confusing and difficult to interpret. In some cases there were extensive fatty infiltration and moderate pigmentation, in others these changes were reversed — there being almost no evidence of necrobiosis. Extensive diffuse necrobiosis and pigmentation without any fatty or cellular infiltration could be demonstrated in a few cases. Cases were noticed in which there was diffuse pigmentation of the liver cells only. Difficulty was experienced in differentiating some of these cases from enzootic icterus. Unless opportunities arise by which more experience can be gained of this disease, it will be difficult to give a reliable histological diagnosis in such atypical cases without the help of a biological test. However, it did seem as if these cases were mainly confined to adult sheep — the lambs tended to present a fairly constant and typical picture.

Experimental cases

Of three sheep which died after experimental infection the organs of two were available for histopathological examination, the third being too decomposed for examination. In a lamb, two-months old, a moderate, diffuse, fatty infiltration of the liver cells was present and a few of the cells showed condensation and hyalinisation of the cytoplasm with karyolysis of the nuclei. The sinusoidal spaces contained some neutrophils in the process of disintegration. The spleen was congested with a diminution of the lymphoid cells. The other organs showed congestion. In a five-months old lamb there was diffuse fatty infiltration of the liver and the reticulo-endothelial cells were prominent and swollen and contained phagocytosed pigment granules in the cytoplasm. There was no evidence of necrobiosis. The other organs were markedly congested. In general it could be stated that the changes corresponded to those described in the natural cases except that they tended to be very much less pronounced. Very little cellular infiltration, if any, could be demonstrated.

In experimentally-infected mice, the livers showed a moderate degree of fatty infiltration. Otherwise no striking abnormal lesions were present in the organs.

Lesions produced in foetuses after immunisation of pregnant ewes

It was found that pregnant ewes, after immunisation against Wesselsbron disease or combined immunisation against this disease and Rift Valley fever, gave birth to still-born lambs. Liver and brain specimens of seven foetuses were submitted for histological examination. The liver showed mainly congestion. The brain showed an extensive meningo-encephalitis. The blood vessels were congested and showed perivascular infiltration of lymphocytes and some neutrophils. These cells were undergoing karyorrhexis. The brain substance proper also showed focal accumulations of lymphocytes. In these areas there was evidence of demyelination and some of the ganglion cells showed degenerative and necrobiotic changes. Cellular infiltration was also noticed around the pial blood vessels.

DIFFERENTIAL DIAGNOSIS

During the initial stages of the outbreak, geeldikkop (Tribulosis) and even Rift Valley fever were suspected to be the cause of the mortality. The typical lesions produced by Rift Valley fever in the liver, adrenal, spleen and lymph nodes described by Schulz in 1951, could easily be eliminated. Geeldikkop had to be considered because the liver lesions in this condition showed a marked resemblance to some of the atypical cases of Wesselsbron disease. In 1918, Theiler described the histopathology of geeldikkop. The liver was affected by centrolobular necrosis, interstitial hepatitis with proliferation of the bile ducts and bile pigmentation. In a series of cases of this disease, Brown & Le Roux (1958) found the liver to be affected mainly by moderate fatty infiltration and extensive bile pigmentation. The kidneys showed degenerative changes, bile pigmentation and fatty infiltration. In such cases it is, therefore, also necessary to consider the history and other symptoms or lesions — in geeldikkop the eating of Tribulus plants and skin manifestations caused by photosensitisation.



FIG. 5.—Specimen 42043. Liver. Enzootic icterus. Note the accumulation of pigment-containing cells in the portal area.

As already pointed out, the staining reaction of the pigment in some of the cases of Wesselsbron disease was identical with that described by de Kock (1928) in enzootic icterus. This finding needs serious consideration in spite of the fact that the lesions and distribution of the pigment in enzootic icterus (Fig. 5) are different from those observed in Wesselsbron fever. Necrobiosis of the liver cells has not been observed in enzootic icterus, but marked degenerative changes have been observed especially during the acute phases of the condition. It may, therefore, be quite possible that the clinical and pathological picture of an animal becoming infected with the Wesselsbron virus during the initial phase of enzootic icterus, will be much more aggravated than will be the case in the uncomplicated condition. In view of the fact that the lesions in experimentally infected cases of Wesselsbron disease are much less pronounced, and the variable nature of the lesions observed in natural cases of the disease, the author feels inclined to favour the idea that the severity of the 1957 outbreak of Wesselsbron disease must, at least in part, be ascribed to the presence of subclinical enzootic icterus at the onset of infection with the Wesselsbron virus. Only a comparative study of the haematology and chemical pathology of these diseases can elucidate this question.

SUMMARY

1. The histopathology of Wesselsbron disease is described. The lesions are mainly confined to the liver. This organ shows fatty infiltration, bile pigmentation, necrobiosis of the liver cells and infiltration of lymphocytes and neutrophils. The histological picture tends to be variable and the lesions in experimentally infected cases are of a much milder degree compared to those observed in natural cases.
2. Immunisation of pregnant ewes produces meningo-encephalitis in the foetuses.
3. The differential diagnosis is discussed and it is suggested that enzootic icterus probably played a role during the 1957 outbreak of Wesselsbron disease.

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