CAROTENOSIS OF BOVINE LIVERS ASSOCIATED WITH PARENCHYMATOUS DEGENERATION

By John S. Buckley, Chief, Pathological Division; E. C. Joss, Assistant Chief, Meat Inspection Division; G. T. Creech, Associate Veterinarian, Pathological Division; and James F. Couch, Chemist, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

In the course of post-mortem inspection at slaughtering establishments operating under Federal meat inspection, various pathological conditions in bovine livers are observed by veterinary inspectors. Most of these conditions are of common occurrence, are recognized quite readily, and disposition in such cases can usually be made on the macroscopic appearance of the liver lesions. It occasionally happens, however, that a condition is observed which is immediately recognized as something out of the ordinary but can not be definitely diagnosed by gross examination of the affected liver.

During the last year the attention of the Meat Inspection Division was directed to an unusual condition in the livers of cattle, the outstanding gross characteristic of which was an intense yellow or reddish-yellow coloration of the liver tissue, while all other organs and tissues in the carcass, except the associated hepatic lymph gland which showed a yellowish mottled appearance, were normal in color and general appearance.

In cooperation with the Meat Inspection Division, the Pathological Division undertook an investigation of the liver condition in order to determine, if possible, the cause of the coloration. A request was sent to a number of inspectors in charge of slaughtering establishments for specimens of the yellow livers.

On receipt of the first lot of specimens, one of the writers (Creech) recalled that several livers similar in appearance had been received at the pathological laboratory a number of years previously. The laboratory records showed that the lot of yellow livers referred to had been sent to the laboratory in 1920 by J. S. Jenison, inspector in charge, National Stock Yards, Ill., for a determination of the cause of the yellow color.

The histological findings in the specimens from Jenison were "well-marked parenchymatous degeneration with round-cell infiltration and limited fibrous proliferation." Chemical studies of this lot of livers were made at the same time by W. N. Berg, formerly of the Bureau of Animal Industry. He found the pigmentation, or yellow coloration, to be due to "a substance identical with carotin." When these findings were made the condition was considered as being somewhat rare, and no further studies of yellow livers were made at that time.

Specimens of the yellow livers studied during the present investigations were received from five cattle-slaughtering centers, viz,
GROSS APPEARANCE OF AFFECTED LIVERS

The typical yellow livers have a characteristic appearance and should not be confused with livers showing other off-color conditions seen more frequently, such as bile discoloration in icteric conditions or yellowish, fatty livers. The affected livers are usually slightly enlarged, with well-rounded borders. In the early stages of the conditions the one outstanding characteristic, and in some cases practically the only deviation from the normal that can be detected in the gross specimen, is the intense yellow or reddish-yellow color of the liver tissue.

When fresh specimens of these livers are sectioned and handled, the knife and fingers are stained a deep yellow, which is distinctly different from the greenish-yellow tinge in bile-pigment staining.

As the pathological changes become more advanced the livers undergo fibrous changes. It has been observed that these fibrous proliferations may vary in the different liver specimens from a sprinkling of slight, fibrous areas barely visible in the gross specimen to advanced cirrhosis involving a large portion, possibly from one-third to one-half of the organ. The extreme cirrhotic changes are usually accompanied by more or less calcification.

In all cases examined in which the associated hepatic lymph glands accompanied the liver specimen, these glands exhibited a peculiar, yellowish, mottled appearance. The unusual deep-yellow color, with fibrous changes in some cases, as already indicated, and the lack of definite knowledge as to the cause of the condition, have led to the application of various terms, more or less descriptive, such as icteric liver, lutein liver, alkali liver, and Pictou disease, in designating this type of liver.

It appears that the condition is not confined to any particular age or sex. Some of the livers examined were from steers 2 to 3 years of age while others were from cows possibly 5 years old or older. The condition has been observed also in livers of aged range bulls.

It is of particular interest to note that no other abnormal changes are seen in any other organs or tissue of carcasses in which the yellow livers are found, with the exception, as previously indicated, of the hepatic gland. The fat and other tissue do not show any yellowish pigmentation, thus indicating that in all probability the condition is confined to the liver and associated lymph gland.

The history obtained thus far regarding the origin of cattle with yellow livers indicates that the condition is found most frequently, if not altogether, in cattle from the southwestern part of the United States.

CAUSE OF THE YELLOW PIGMENTATION

The close similarity between the livers examined in the present study and the specimens of yellow livers observed in 1920, in which the intense yellow color was found to be due to a substance identical with carotene, led to the belief that this was the same liver condition. It was decided, therefore, to make a chemical analysis of a number of specimens to identify definitely the coloring matter present.
CHEMICAL STUDIES

It was considered necessary to reinvestigate the coloring matter present in these livers because Berg had not published the evidence on which he based his conclusion that the substance was carotene; moreover, it was not certain that the more recent condition was the same as that studied by him. The coloring matter, therefore, was isolated in quantity and identified by chemical methods.

![Spectrophotometric measurements of a 0.05 per cent solution of carotene in carbon disulphide](image)

A composite sample of livers 1, 2, and 3, amounting to 4,110 gm., was finely minced in a meat chopper, placed in a 12-liter flask, and 4 liters of 5 per cent alcoholic potash were added. The whole was boiled four hours under reflux, when nearly all the liver tissue was liquefied. The product was strained through cheesecloth and the alcohol removed by distillation. When cold the aqueous residue was extracted with successive portions of petroleum ether until that solvent was no longer deeply colored. The combined petroleum-ether solutions were washed with 85 per cent alcohol to remove xanthophyll, if present, and other possible impurities, and then the petroleum ether
was removed by distillation. The residue was a blood-red, thick, unctuous mass. This was purified by boiling with 5 per cent alcoholic potash, removing the alcohol, extracting with petroleum ether, washing with 85 per cent alcohol, and distilling off the petroleum ether. The residue was dissolved in carbon disulphide and the solution poured into 5 volumes of alcohol. The precipitated carotene was collected, washed with alcohol, and warmed on a water bath to remove volatile impurities.

The substance so obtained was identified as carotene as follows: It responded to the ferric chloride and sulphuric acid color tests; it showed the solubility relationships characteristic of carotene; the triiodide prepared according to the method of Arnaud$^2$ melted at $138^\circ-139^\circ$ without decomposition. Palmer$^3$ gives the melting point $136^\circ-137^\circ$, which is sufficiently close for identification. Spectrophotometric measurements of a 0.05 per cent solution of the substance, made at the Bureau of Standards, United States Department of Commerce, are shown in Figure 1, through the courtesy of K. S. Gibson, of that bureau.

A number of determinations of the quantity of carotene present in yellow livers submitted for study, and in normal livers obtained from a local packing plant, were made. In these determinations the excellent method of Connor$^4$ was used. The results are given in Table 1.

### Table 1.—Carotene content and condition of affected and normal livers

#### CATTLE LIVERS

<table>
<thead>
<tr>
<th>Date</th>
<th>Animal</th>
<th>Carotene content</th>
<th>Condition of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sept. 24</td>
<td>Steer 1</td>
<td>30.4</td>
<td>Yellow.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 2</td>
<td>45.1</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 3</td>
<td>29.6</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 4</td>
<td>1.3</td>
<td>Normal.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 5</td>
<td>1.01</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 6</td>
<td>0.99</td>
<td>Do.</td>
</tr>
<tr>
<td>Nov. 5</td>
<td>Steer 4</td>
<td>40.8</td>
<td>Yellow.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 5</td>
<td>21.48</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 6</td>
<td>7.2</td>
<td>Cirrhotic.</td>
</tr>
<tr>
<td>Do</td>
<td>Steer 7</td>
<td>6.43</td>
<td>Yellow.</td>
</tr>
<tr>
<td>Dec. 22</td>
<td>Cow 1</td>
<td>18.9</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Cow 2</td>
<td>43.4</td>
<td>Do.</td>
</tr>
<tr>
<td>Feb. 14</td>
<td>Steer 8</td>
<td>16.6</td>
<td>Do.</td>
</tr>
<tr>
<td>Apr. 19</td>
<td>Steer 9</td>
<td>4.0</td>
<td>Do.</td>
</tr>
</tbody>
</table>

#### LIVERS OF RATS AFTER FEEDING ON TYPICAL YELLOW LIVERS

<table>
<thead>
<tr>
<th>Date</th>
<th>Animal</th>
<th>Carotene content</th>
<th>Condition of liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. 31</td>
<td>Rat 1</td>
<td>0.48</td>
<td>Pale, with necrotic areas.</td>
</tr>
<tr>
<td>Do</td>
<td>Rat 2</td>
<td>0.8</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Rat 3</td>
<td>(*)</td>
<td>Do.</td>
</tr>
<tr>
<td>Do</td>
<td>Rat (control)</td>
<td>(*)</td>
<td>Normal.</td>
</tr>
</tbody>
</table>

$^a$ Trace.

In all cases search was made for other lipochromes, particularly xanthophyll, but none were found. Since carotene itself is not a toxic substance, further study is needed to determine what deleterious compound is present in these livers. The investigation is being continued in the Bureau of Animal Industry.

**HISTOPATHOLOGY**

In the earlier stages of carotenosis of the liver the outstanding pathological change is that of a parenchymatous degeneration. The degenerative changes may vary in different parts of the same liver from a slight cloudiness, or granular appearance of the cell protoplasm, to complete degeneration and disintegration of groups of liver cells. In the well-advanced cases these areas of degeneration and necrosis are more extensive, including whole lobules or groups of lobules.

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The degenerative changes appear to have their beginning most frequently in the central portion of the lobules. There is a very noticeable vacuolation of the liver cells in the degenerated areas, and frequently the cells, particularly toward the periphery of the affected lobules, contain numerous fat droplets. (Figs. 2 and 3.)

In a few of the cases examined practically no normal liver cells could be found in any of the sections made from various parts of the specimens. (Fig. 4.) Some specimens show an engorgement of the central veins and capillaries with small hemorrhages here and there. Areas of round-cell infiltration are frequently seen. These are the first indication of the fibrous changes which occur in the later stages of the disease. The fibrous changes are first seen in those areas showing advanced degeneration and in the region of the portal canals. The capsule of the liver may be very much thickened. In those cases showing advanced fibrosis, extensive proliferations of bile ducts were noted. (Fig. 5.) In two cases examined there was complete cirrhosis of considerable portions of the liver. (Fig. 6.) Large cal-

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**Figure 3.** Photomicrograph of section of liver of steer No. 1, showing extensive degeneration of the cells. $\times 620$
Specific centers were noted in those areas showing the more advanced cirrhotic changes. Few of the livers examined histologically showed an excessive quantity of bile pigment.

The associated hepatic lymph glands exhibited very unusual and rather extensive degeneration of the gland cells. Few of the normal germinal centers could be recognized. A very large proportion of the gland cells had a peculiar, swollen appearance, and in a few places there were fusions of these cells and collections of nuclei simulating giant-cell formation. Toward the central portion of the affected glands large areas of cells had been transformed into necrotic, homogeneous masses. (Fig. 7.)

The yellow coloring matter in the livers and lymph glands had evidently been largely removed by the alcohols in the preparation of the tissues for sectioning. Slight traces of the carotene deposits were noted in some of the degenerated and fatty cells of the hepatic lymph glands, but none of the coloring matter could be seen in the liver sections.
RAT-FEEDING EXPERIMENTS

Being unable to understand this rather peculiar proclivity of the bovine liver to store up an excessive amount of carotene while other tissues in the body remained unaffected, the writers decided to conduct some experimental rat feeding with typical yellow livers in order to determine whether these animals would show the same tendency to carotenosis of the liver.

In addition to the liver ration all of the experimental rats received daily a small quantity of corn meal and crushed oats mixed.

As a preliminary experiment only three rats were fed. These were mature white rats and received 10 gm. daily of typical yellow liver from steer No. 2 during a period of approximately five weeks.

On post-mortem examination the livers of all the rats in this group were found to be very pale or light in color, but there was no distinct yellowish coloration of the liver tissue. Each liver, however, showed a number of small, whitish areas, evidently necrotic foci. On chemical analysis the three livers failed to show an excessive quantity of carotene, as may be observed in Table 1.
Histologically the livers showed an extensive degeneration of the liver cells. These degenerative changes were more in evidence immediately surrounding many of the central veins and larger blood vessels. (Fig. 8.) In places the complete degeneration and disintegration of the cells had resulted in small, necrotic centers which were seen in sections from all three of the livers. Groups of round cells were observed within and around some of these necrotic centers. (Fig. 9.)

While the preliminary feeding experiment gave negative results for carotene deposits, the very interesting findings with respect to the pathological changes in the livers of this small group of rats made it desirable to repeat the experiment in order to ascertain whether this same condition could be produced in a second or third lot of rats. Accordingly arrangements were made to feed two more lots of rats with yellow livers, and at the same time to feed a group of rats a similar quantity of normal bovine liver daily as controls in the experiment.
The rats used in the second feeding experiment were obtained from the Biochemie Division of the Bureau of Animal Industry, and more was known of their previous history and rationing than had been known in the case of the small group used in the preliminary experiment.

Ten rats were used in the second experiment. They were divided into two groups of three animals each, which were fed the yellow livers, and a third group of four rats, which were fed normal bovine liver. These rats were quite young at the beginning of the experiment, averaging in weight about 40 gm. each.

Lots 1 and 2 were fed liver specimens from steers Nos. 4 and 5, respectively, which were typical cases of carotenosis. Since the rats were rather small they were fed only 5 gm. daily of the liver at first, but during the last half of the feeding period the quantity was increased to 10 gm. daily. Like quantities of normal bovine liver were fed to the four control rats for the same period.

The rats in lots 1 and 2, fed the carotene livers, averaged only 141 gm. in weight at the end of the feeding experiment, an increase of
101 gm., while the rats fed normal liver averaged nearly 154 gm. each, an increase of almost 114 gm.

The post-mortem findings in the livers of rats in lots 1 and 2 were substantially the same as those in the livers of the three rats used in the preliminary feeding experiment; that is, they consisted chiefly of extreme paleness and evidence of necrosis as indicated by numerous small, whitish areas scattered through the livers. The lesions were somewhat more pronounced in the two lots of the second feeding than in the group of three animals in the first feeding.

The livers of all the rats in lots 1 and 2 were similarly affected, the only difference being the degree of involvement in the different animals. All other tissues of the rats in these two groups appeared to be normal.

The livers of all four of the rats in lot 3, fed normal bovine liver, were found on post-mortem examination to be normal in appearance, and the histological examination of these livers also showed them to be practically normal in structure and appearance. (Fig. 10.)
Histologically the livers of the rats in the two groups fed on carotene livers 4 and 5, in the second feeding experiment, showed more advanced degeneration and necrosis than the livers of the three rats in the preliminary feeding experiment. (Figs. 11 and 12.) The degenerative changes were found to be more extensive and the necrotic foci more numerous. In fact, in some of the specimens there was very little remaining normal liver structure.

There was no decided yellowish coloration of any of the livers in either of the two groups of rats fed on yellow livers in the second experiment, and a chemical analysis of these livers did not show the presence of excessive quantities of carotene in any of the livers of the experimental rats.

**ETIOLOGY**

The pathological changes found in the different specimens of bovine liver showing carotenosis indicate that the destructive processes in these cases are the result of some form of acute irritation. However, the studies made thus far, which include bacteriological, histological,
and chemical examinations, have failed to reveal the nature of the causative agent.

That the liver changes are due to some specific, toxic substance and that this substance is stored up in the bovine liver tissue in appreciable quantities is shown by the fact that similar destructive changes can be induced in the livers of rats which have been fed for a time on affected cattle livers. The similarity of the degenerative changes in the various cases examined also indicates that the causative element is in all probability of a specific nature.

![Figure 10](image)

The fact that the lesions are confined to the liver in cattle and also in the experimental rats suggests that the causative toxic substance has a peculiar affinity for the liver cells; at least it would appear from these observations that for some unknown reason the toxic substance does not pass beyond the liver after reaching that organ.

**SUMMARY AND CONCLUSIONS**

The results thus far obtained in studies of bovine livers showing carotenosis indicate that the destructive changes found in these livers are, in all probability, due to the presence of a toxic substance, the
nature of which has not yet been determined. That this substance is of a specific nature is indicated by the similarity of the lesions in the different cases studied.

In view of the evidence at hand and the limited available history of the cattle affected, the writers are of the opinion that the causative agent, or toxic substance, is a constituent of some plant indigenous to the region or locality where the affected cattle had their origin.

The yellow coloring matter, or pigment, found in the affected livers has been definitely identified as carotene, and it is thought that the carotenosis in these cases is simply an associated condition in which, for some reason difficult to explain, the excess carotene is stored up in the liver, while other tissues of the body remain unaffected.
For the purpose of securing further information relative to this peculiar tendency of the bovine liver to store up carotene in large quantities, feeding experiments were carried out with rats. The rats were fed 5 or 10 gm. daily of typical yellow liver for some weeks. On post-mortem examination the livers of these rats were found to be very pale or light in color, though there was no distinct evidence of yellowing; each of the livers also showed a number of small, whitish areas, which were subsequently found to be necrotic foci. On chemical analysis, however, none of the livers of the experimental rats showed an abnormal quantity of carotene. Control animals fed on normal bovine liver grew more rapidly than animals fed yellow livers of high carotene content.