STUDIES OF HISTOPATHOLOGY IN CATTLE PRODUCED BY FASCIOLA HEPATICA

by

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PREFACE

Histopathological investigations of any disease entity are valuable from the standpoints of:

1. Obtaining definite information as to the manner in which the disease progresses and the way in which it brings about injurious alterations within the host.

2. Acquiring information as to weak points in the course and transmission of the disease wherein prophylactic measures may be instituted.

3. Learning the most opportune conditions under which therapeutic measures may be instituted to advantage.

4. Determining, in the instance of animals consumed as food, whether the pathological alterations produced are of such a nature as to render the dressed carcass injurious or unsuitable for human consumption.

Successful investigations in any one or several of these points frequently result in a complete control or eradication of the disease or, in the least, forge a link in the chain of information which eventually accomplishes the same result.

In undertaking such studies, the pathologist must realize that the changes observed in the microscopic section represent, not the end-result, but only one stage in the long chain of processes which eventually lead to death or recovery. Allegorically, the microsection represents a single frame in the movie film of disease; and to complete such a cinema, not one or several, but many such histological pictures are essential.

Finally, underlying the entire picture, is the physiological activity of the organs or tissues involved. Pathogenic organisms, parasites, chemicals, extreme temperatures, and mechanical factors are of importance only so far as they disturb the normal function of the animal organism. With this in mind, the investigator necessarily must correlate the shifting tissue pattern with the fundamental physiology of the organ and therefrom view the entire moving process and its relationship to treatment, control, or eradication of the disease.

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Introduction

The economic importance of liver fluke in cattle is found to vary considerably with its geographic distribution. Though cosmopolitan in occurrence, the more severe infestations appear in those low-lying, marshy countries and districts which favor transmission to and development in its essential intermediate host, the fresh-water snail. Under such conditions, there are notable differences in both the number of animals parasitized and intensity of the parasitism within the individual.

In the United States, fascioliasis appears to be frequent along both coasts and the Gulf of Mexico, especially in the states of Oregon, Washington, California, Texas, Arkansas, Louisiana, Alabama, and Florida.(3) It is also known to be present in the North Central States(2) though is probably of little economic importance in this area. In Oregon, where these studies were made, liver flukes have been reported in all counties except Sherman, Wasco, Jefferson, Deschutes, Crook, and Wheeler, with heaviest infestations occurring in Klamath, the coastal, and Willamette Valley counties.(8)

Reports concerning the actual monetary loss from the condemnation of flukey livers are few. In the United States, Krull(5) reported the following in regard to animals

slaughtered at the Logan, Utah, abattoir:

> "A total of 2,364 salable carcasses were inspected, representing 835 cows, 1,241 heifers, 189 steers and 99 bulls. Because of liver fluke infestation the livers of 420 or 50% of the cows, 374 or 30% of the heifers, 46 or 24% of the steers, and 27 or 27% of the bulls were condemned. This represented a loss of 867 livers (37%) weighing 9,537 pounds. Valuing the livers at 12¢ per pound, the direct loss from liver condemnations alone was $1,144.44, an amount almost sufficient to cover the cost of slaughtering the animals."

It is probable that these figures are somewhat representative of the losses suffered in other endemic fluke areas. From limited observations of the author at an Albany, Oregon, packing plant, 20 - 33% of the slaughtered cattle over 2½ years of age show evidence of this parasite. From the viewpoint of the beef herdsman and dairyman, the question also arises as to losses incurred in the production of milk and flesh, and here again, more information is needed. Shaw(8) is of the opinion that in Oregon such setbacks in the total cattle population probably assume a greater figure than those resulting from the more severe and frequently fatal form seen in the sheep of this state.

One of the direct reasons for assuming this study was to determine the extent of the pathology produced in livers by this trematode and to learn, as far as possible, the activity of the parasite within this organ and the resulting tissue responses. While similar work has been carried out in sheep, only one reference(4) was available to work done

with cattle. Inasmuch as many such livers are at present being condemned as unfit for human consumption, it was hoped that such an investigation might throw more light on the situation and perhaps suggest a less costly disposal of livers which are now condemned with little consideration. Since facilities permitted, limited blood studies were also carried out, hoping to obtain information which would serve as an index of the general health of animals infested with this parasite.

As with any project involving large animals, there arose the problem of supplying cattle suitable for artificial infestation, since only under such controlled experiments can the true course and pathogenicity of such a parasitism be observed. Because funds for this project were limited, it was decided to use four animals of the departmental herd and to supplement these studies with material from abattoirs. While data concerning the source of such material, the time and length of exposure to infested pastures, age of the animals, and similar information could not be readily obtained, these livers would nevertheless broaden the picture of the histopathology involved in bovine distomatosis and at the same time give some indication of the extent of damage found in beef livers actually condemned under state inspection.

General Procedure and Methods

On June 18, 1941, the selected cattle were weighed and two of them, subsequently referred to as cases XI and XII, were each given about 1000 encysted flukes orally. These metacercariae were collected by Dr. Shaw on lettuce leaves and came from snails (*Galba bulimoides* Lea) kept and artificially infested in the departmental laboratory. To make certain the animals received all of the flukes, they were placed in one-ounce gelatin capsules and administered with a bolling gun.

At the time of infestation and weekly for the duration of the experiment, citrated blood samples were collected from all four animals. Routine determinations made on these samples included: hemoglobin (Newcomer method), erythrocyte and white blood cell estimates, and differential leucocytic counts. The Newcomer method was modified slightly to use larger quantities of blood, since it was observed that, in so doing, weekly variations were cut down. In making the initial determinations and when very irregular results were obtained, examinations were frequently repeated to keep errors in technic at a minimum.

The course of the infestation was further followed by fecal examinations for fluke ova, weights, and observations for external effects of the parasitism. Just prior to and following further infestation of case XII with 48

metacercariae on November 13, weekly estimates of ova were made by washing the eggs from 5.0 Gm. of feces and counting those present in one-fifth of the washings or the entire quantity when few were found.

The parasitized animals and one control (case C) were slaughtered on March 3, 1942. In this study, the control not slaughtered will be referred to as case D.

For the most part, liver tissues utilized here were fixed as soon as gross pathological changes were recorded and the desired photographs taken. Representative blocks (.5 x 1.5 x 2 cm.) were generally subjected to the following treatment:

1. Zenker's fluid - 24 hours
2. Running water - 24 hours
3. 80% ethyl alcohol - 24 hours
4. 95% ethyl alcohol - 24 hours
5. 100% ethyl alcohol - 12 hours
6. Cedarwood oil - 24-36 hours
7. Paraffin (56° C.) (two changes) - 24 hours

These paraffin-embedded blocks were then sectioned with a standard microtome and the tissue slices (5-7 microns in thickness) fixed to glass slides with an egg albumen-glycerin adhesive. When staining procedures required the use of absolute alcohol or 10% formalin as a fixative, steps 1 and 2 in the above outline were omitted.

Routinely microsections were stained with hematoxylin-eosin and permanent mounts made in Canada balsam. For further study, a limited number were also stained with

alum hematoxylin-basic fuchsin and Mallory's aniline blue technics, and several frozen sections were stained with Sudan IV for purposes of detecting extensive fatty changes. In all instances, the technics employed, with slight modifications, were adapted from Mallory.(6)

Preliminary Observations

External in vivo evidences of fluke infestation in cases XI and XII were very few. It was noted that both animals showed a limited degree of gauntness and mild depletion of subcutaneous fat, but actual weights (Table I) were inconclusive. No irregularities of appetite or other habits were observed.

Table I. Body Weight in Pounds

Date	Case C	Case D	Case XI	Case XII
6-18-41	420	505	550	595
8-19-41	495	465	580	590
10-23-41	420	515	645	680
1-28-42	550	640	740	670
3- 3-42	554*	-	752*	688*

*Estimated as 200% of the dressed carcass weight.

Ova were first found in the feces at eleven weeks and persisted until the animals were slaughtered. The effect of the superimposed infestation in case XII on egg estimates is well shown in the following table:

Table II. Estimated Number of Fluke Ova per Gram of Feces

Date	Case XI	Case XII
11-21-41	24	21
1-15-42	22	13
1-29-42	3	160
2- 5-42	1	41
2-12-42	.6	71
2-19-42	1	46

Table III. General Information Concerning the Origin of Livers Studied

Case	Date	Breed	Sex*	Age**	Weight***	Source of Animal
A	8-29-41	Grade Hereford	F	2	740	Linn Co., Oregon
B	8-29-41	Jersey	F	aged	852	Linn Co., Oregon
C	3-2-42	Grade Jersey	N	2	554	Benton Co., Oregon (Dept.V.M.)
I	7-24-41	Grade Hereford	F	2	736	Linn Co., Oregon
II	7-24-41	Grade Hereford	F	2	770	Linn Co., Oregon
III	9-4-41	Grade Hereford	F	2½	774	Portland stockyards
IV	9-4-41	Grade Hereford	F	3	960	Portland stockyards
V	9-4-41	Grade Hereford	F	2½	798	Portland stockyards
VI	9-4-41	Grade Hereford	F	3	966	Portland stockyards
VII	9-4-41	Jersey	F	aged	822	Linn Co., Oregon
VIII	9-4-41	Jersey	F	aged	856	Linn Co., Oregon
IX	9-4-41	Grade Shorthorn	N	3	898	Portland stockyards
X	9-4-41	Grade Hereford	F	3	922	Portland stockyards
XI	3-3-42	Grade Holstein	F	2½	752	Benton Co., Oregon (Dept.V.M.)
XII	3-3-42	Grade Shorthorn	N	2½	688	Benton Co., Oregon (Dept.V.M.)

* F - female, N - steer
** Estimated from carcass and buccal cavity
*** 200% carcass weight in pounds

Table IV. Summary of Bile Examinations*

Case	ml. of Bile in Cholecyst	pH	Specific Gravity	Fluke Ova Estimates in Cholecystic Bile	Flukes Present
A	60	7.22	1.025	negative	0
B	136	7.10	1.028	negative	0
C	122	7.20	1.022	negative	0
I	167	7.26	1.025	334	4
II	134	6.45	1.024	9,110	8
III	180	6.66	1.025	negative	5
IV	238	6.57	1.031	negative	0
V	200	6.90	1.025	negative	0
VI	215	6.47	1.026	13,760	7
VII	650	7.20	1.022	negative	0
VIII	638	6.98	1.025	negative	0
IX	220	6.84	1.024	75,240	13
X	45	6.36	1.031	69,750	2
XI	550	6.85	1.020	11,000	48
XII	145	6.78	1.021	3,700	554

*pH determinations were made with the use of a Beckman pH meter, and specific gravity was taken with a standard urinometer. Ova estimates were derived by actual counts of five 1-cc. quantities of bile, an average taken, and a product of this figure and the total volume of bile reached. Where huge numbers of eggs were present, the bile was diluted 1:9 with tap water before counting.

The following outline of pathological anatomy and related observations is given to correlate with microscopic findings and assist in their correct interpretation:

Case A.

Normal liver; weight - 4.4 Kg.

The shape of this organ was somewhat more round than is ordinarily seen in cattle. Two branches of the portal vein were visible for about 7.5 cm. on the omasal surface, but all extrahepatic bile ducts were hidden beneath portal fat. When these were exposed and transected, they were found to be thin-walled and collapsed with the release of bile from within.

Case B.

Normal liver; weight - 4.2 Kg.

This liver was of the usual greyish-brown color and firm consistency, and presented no irregularities in shape or other visible features. The main biliary channels were collapsible, with no evidence of fibrosis. One principal efferent duct from the ventral lobe measured 8 mm. (longest diameter when collapsed).

Case C.

Normal liver; weight - 3.6 Kg.

No irregularities were presented, and the liver might be described as "very clean." The bile ducts were unusually

small in caliber and thin-walled, with a maximum recorded diameter of 4 mm.

Case I.

Condemned liver; weight - 5.0 Kg.

Aside from a small, greyish area 3 mm. in diameter just below the falciform ligament, no lesions were observed on the surface or within the parenchyma. The main choleic duct was 10 mm. in diameter, non-collapsing, and thick-walled; and its larger tributaries showed similar changes. Two mature flukes were removed from these and two smaller ones from more distal branches.

Case II.

Condemned liver; weight - 5.6 Kg.

On the lower border of the visceral surface of the organ were numerous yellowish-white adhesions and scars covering an area 5 x 15 cm. The convex surface presented scattered focal lesions, greyish in color, under 4 mm. in diameter, and appearing very shallow. Two intraparenchymal branches of the main bile duct were visible on the omasal surface, and all other chief branches showed alterations resembling those of the previous case. The main trunk, measuring 12 mm., contained a brown, viscid bile and flakes of a hard, gritty material. Eight flukes were taken from these enlarged canals.

Case III.

Condemned liver; weight - 4.8 Kg.

In irregularities, this liver bore numerous grey foci about 1 mm. in size grouped together over an area of 4 sq. cm. on the dorsal border of the caudate lobe. Similar lesions were noted in the tissues of this area to a depth of 2 cm. The ventral tributaries of the main bile duct were visible in the visceral surface though appeared only moderately thickened. Upon opening the canals, three areas of calcification were found, each occurring at a duct bifurcation. Four mature flukes were enclosed in one of these and a single fluke in another, and aside from gross thickening in a few lesser ducts, no other abnormalities were seen.

Case IV.

Condemned liver; weight - 5.1 Kg.

The caudate lobe bore three brownish-yellow lesions 1 cm. in diameter and 1.5 cm. deep, and some enlargement and moderate thickening was obvious in the four branching bile ducts to the ventral lobe. While the organ was condemned as "flukey," no parasites or inorganic deposits were observed.

Case V.

Condemned liver; weight - 5.2 Kg.

Nine adhesions (3 - 6 cm. in diameter) on the

diaphragmatic surface were white, fibrous, and considerably cicatrized and took deep root in the parenchyma. Five similar smaller ones were present on the visceral surface. In this case only the smaller duct system showed scattered thickenings; and while definite evidence of fluke infestation is lacking, it is included in this study because of its possibilities and a history of coming from a lot of animals in which flukes were found.

Case VI.

Condemned liver; weight - 5.0 Kg.

Before removal of the gall-bladder and periportal fat, this organ appeared normal in all respects. Upon closer examination of the main ducts to the dorsal lobe, however, they showed marked thickening and were found to contain seven mature flukes. Other principal collecting channels showed lesser changes (enlargement and thickening) and contained a few loose, gritty flakes and thick, brown bile.

Case VII.

Condemned liver; weight - 9.0 Kg.

The organ appeared quite uniformly triangular in shape and possessed usual firm consistency except for the lateral border of the ventral lobe which was hard and sclerosed to a depth of 4 cm. from its edge. On the

diaphragmatic surface, four scattered foci under 1 cm. in width marked the surface but did not penetrate deeply. Three large biliary tributaries extending ventrolaterally appeared very tortuous and lay partially extraparenchymally. All of the other major canals showed typical changes, and three "cisterns" containing a few calcareous-like flakes were found.

Case VIII.

Condemned liver; weight - 8.4 Kg.

Numerous brown or white foci 1 - 2 mm. in size were seen on both sides of the ventral lobe, but in all other respects the parenchyma appeared normal. Its duct system showed enlargement and thickening throughout, with two principal branches 11 mm. in diameter and visible on the visceral aspect. Though no flukes or deposits were found, changes in the choleic ducts led to its condemnation and use in this study.

Case IX.

Condemned liver; weight - 4.4 Kg.

No surface lesions were noted, but chief branches of the biliary system coming from the ventral lobe were 8 - 10 mm. in diameter with markedly thickened walls. At several points in these, distentions 2 - 4 cm. long and 12 mm. in diameter were found to contain pipe-like concretions. In

most instances they were firmly adherent to the mucosa and occurred at the junction of two or more ducts. Thirteen flukes were present.

Case X.

Condemned liver; weight - 4.3 Kg.

As in case IX, gross pathology was confined to the choleic ducts. These were enlarged and thickened throughout, with those to the dorsal lobe showing more extensive changes. Two mature flukes and considerable brownish bile were present.

Case XI.

Experimental liver; weight - 6.3 Kg.

The ventral lobe of this organ showed considerable atrophy and sclerosis in its lower border. Two evaginations from the tissue proper (1/2 x 1 x 1 cm.) suggested proliferative processes and were especially firm and cicatrized. The dorsal lobe of the organ was very large in proportion to the ventral one and in this respect might be considered hyperplastic. The entire excretory system appeared enlarged with several ducts visible on the visceral surface to within 5 cm. of its lower border. Throughout these enlarged canals were the characteristic concretions which, in many instances, could be removed intact. The bile varied in color from yellow to brown and was intermittantly blood-

tinged. Forty-eight flukes were found, 25 of which averaged 17.2 mm. in length.

Case XII.

Experimental liver; weight - 6.8 Kg.

This liver presented a very similar picture to that of case XI, with more extensive involvement of the choleic system and a less tendency toward the formation of concretions. The ventral lobe, in its lower sclerosed border, showed four well-fibrosed abscesses containing small quantities of dry necrotic tissue. Scattered throughout the entire surface were various irregular lesions, some white and others concentrically white, red, and light brown; these were particularly numerous along the lateral border of the ventral lobe. As in the previous case, the dorsal lobe showed evidences of hyperplasia; and, in addition, its medial border was irregularly sclerosed. Transection of the liver at various levels gave a mottled appearance due to an increase in interlobular connective tissue. The common bile duct measured 30 mm. and presented a very small lumen. Incision of other passages revealed a thick, dark-brown secretion intermingled with flukes and flaky concretions. Five hundred and fifty-four flukes were washed from the organ and 25 of these were found to average 18.6 mm. in length. An apparent enlargement of the portal lymph node was also observed.

Histopathological Findings

Blood Determinations

Estimations made on the blood of the experimental animals are presented in Tables V to VIII inclusive. In the instance of the differential leucocytic counts, only a summary of the 37 weeks' findings is given because of the volume of material. Since the principal changes were noted in the eosinophilic ratio, however, this picture is more completely shown in Graph I.

A consistent decline in the erythrocytic counts of the parasitized animals was observed until 18 weeks following infestation. This was followed by an unsettled count for a period of six to eight weeks and then a slow, unsteady rise. It is noted that the onset of relative anemia varied in the two animals, beginning at four weeks following the ingestation of cysts in case XI and 13 weeks in case XII. In contrast, estimates of the controls remained fairly constant or showed a slight increase.

General agreement was seen between the hemoglobin determinations and the first-mentioned estimations. Though weekly findings did not compare in every instance, results were, for the most part, correlative.

Total white blood cell counts apparently did not help appreciably in tracing the course of the infestation, yet

differential leucocytic counts showed a marked rise in the percentage of eosinophiles within two or three weeks after infestation and an irregular fluctuation for the duration of the experiment. However, it was also observed that the control animals showed relatively high ratios during the months of December and January.

Table V. Red Blood Cell Estimates
(million per cmm. of blood)

Date	Case C	Case D	Case XI	Case XII
6-18-41	8.05	5.45	8.24	7.08
6-25-41	8.04	5.98	8.56	6.76
7- 2-41	8.17	6.43	8.60	6.85
7- 9-41	7.55	6.18	8.59	6.84
7-16-41	8.67	6.75	7.52	5.97
7-23-41	8.14	6.42	7.62	6.91
7-30-41	8.42	5.56	7.85	6.51
8- 6-41	7.33	5.94	6.89	5.77
8-13-41	7.58	5.82	7.26	5.94
8-20-41	8.57	5.64	7.55	6.89
8-27-41	7.71	5.53	6.85	6.85
9- 3-41	7.72	5.86	7.92	7.27
9-10-41	7.61	5.41	6.54	6.43
9-18-41	7.34	6.11	6.89	5.75
9-24-41	6.88	5.93	6.68	5.26
10- 1-41	7.62	6.38	6.03	5.93
10- 8-41	7.85	6.00	5.99	5.28
10-16-41	7.52	6.27	5.69	4.74
10-23-41	7.88	6.94	5.24	3.84
10-30-41	6.98	6.04	5.90	4.46
11- 6-41	7.13	5.83	5.13	4.73
11-13-41	7.20	6.23	5.26	3.87
11-20-41	7.70	6.76	5.87	4.08
11-27-41	8.02	5.87	5.74	3.85
12- 4-41	8.96	7.28	5.63	4.67
12-11-41	8.01	6.64	6.42	4.10
12-18-41	8.24	6.82	6.01	4.75
12-26-41	7.57	5.95	6.33	5.50
1- 2-42	8.41	6.50	6.66	4.80
1- 9-42	7.60	6.55	5.85	4.54
1-15-42	7.54	6.76	6.16	5.14
1-22-42	8.72	6.42	6.86	4.95
1-29-42	8.14	6.41	6.66	5.57
2- 5-42	7.40	5.91	6.41	5.62
2-12-42	7.15	5.90	6.22	5.35
2-19-42	7.79	6.20	6.03	5.07
2-26-42	7.52	6.60	6.34	6.37
Average	7.59	6.00	6.53	5.43

Table VI. Hemoglobin Determinations
(Gm. per hundred cc. blood)

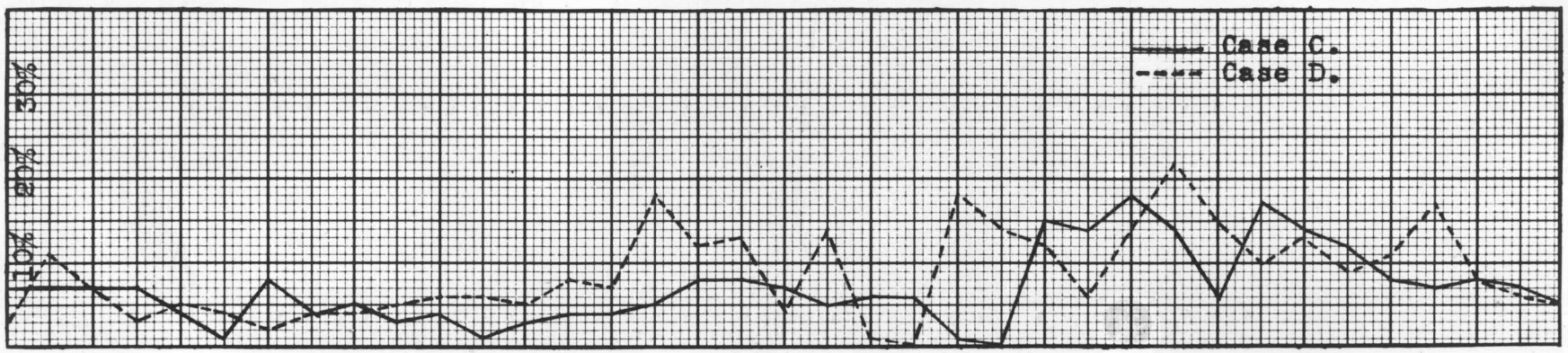
Date	Case C	Case D	Case XI	Case XII
6-18-41	10.14	8.42	10.38	9.93
6-25-41	10.43	9.59	11.05	11.40
7- 2-41	9.33	9.62	11.88	10.14
7- 9-41	9.32	9.69	9.50	13.20
7-16-41	10.91	10.75	9.56	9.44
7-23-41	8.71	9.79	9.74	10.43
7-30-41	12.32	9.24	9.86	12.93
8- 6-41	8.45	8.21	7.80	8.88
8-13-41	9.64	10.11	9.23	10.52
8-20-41	10.28	10.03	9.79	10.81
8-27-41	9.59	9.24	8.75	10.95
9- 3-41	7.94	9.02	8.10	10.52
9-10-41	9.95	9.50	7.95	11.29
9-18-41	10.00	9.09	8.50	9.97
9-24-41	8.78	8.82	8.17	9.33
10- 1-41	9.66	9.86	8.88	9.52
10- 8-41	9.79	10.14	9.21	9.93
10-16-41	9.00	9.45	7.84	8.45
10-23-41	9.36	11.19	8.37	8.66
10-30-41	9.95	10.44	8.37	8.37
11- 6-41	9.36	10.90	7.35	8.05
11-13-41	9.79	10.28	7.05	8.05
11-20-41	11.19	10.52	7.14	7.94
11-27-41	9.30	9.23	7.57	7.26
12- 4-41	9.50	10.11	7.18	7.61
12-11-41	9.30	10.40	7.67	8.21
12-18-41	10.03	10.28	8.26	8.84
12-26-41	8.60	9.30	8.37	8.42
1- 2-42	9.71	9.57	8.66	8.90
1- 9-42	9.43	9.02	7.84	8.37
1-15-42	11.38	10.81	9.36	9.30
1-22-42	10.52	9.95	8.10	8.60
1-29-42	9.87	9.71	8.66	9.95
2- 5-42	10.03	9.57	8.90	9.36
2-12-42	10.71	9.43	8.26	9.87
2-19-42	10.91	10.11	8.42	9.23
2-26-42	9.95	9.36	8.78	10.11
Average	9.81	9.75	8.61	9.53

Table VII. White Blood Cell Estimates
(thousands per cmm. of blood)

Date	Case C	Case D	Case XI	Case XII
6-18-41	11.3	10.5	12.0	11.2
6-25-41	10.1	9.1	10.5	7.2
7- 2-41	8.2	8.0	11.4	9.5
7- 9-41	8.1	7.0	12.0	9.8
7-16-41	8.3	7.9	13.9	10.1
7-23-41	8.3	8.2	10.0	9.8
7-30-41	7.6	8.1	15.2	9.5
8- 6-41	9.4	8.9	10.8	9.2
8-13-41	9.7	9.4	11.6	9.2
8-20-41	10.3	10.5	12.2	8.2
8-27-41	9.8	7.7	12.2	10.9
9- 3-41	7.2	7.2	10.2	9.6
9-10-41	8.3	7.6	11.5	9.2
9-18-41	8.4	8.8	12.2	11.8
9-24-41	7.7	9.8	11.1	8.5
10- 1-41	7.4	9.5	12.8	9.4
10- 8-41	11.3	7.9	10.5	8.2
10-16-41	8.6	9.5	10.3	8.3
10-23-41	8.0	10.07	10.9	9.3
10-30-41	8.4	10.9	12.4	10.5
11- 6-41	8.6	8.9	8.7	6.9
11-13-41	9.4	9.0	9.2	7.2
11-20-41	8.4	9.4	10.6	7.6
11-27-41	8.6	9.5	11.5	8.8
12- 4-41	11.3	10.8	11.1	9.1
12-11-41	9.7	8.0	9.7	8.1
12-18-41	10.4	10.1	11.9	9.2
12-26-41	10.4	11.9	12.8	11.9
1- 2-42	8.6	10.3	14.6	8.8
1- 9-42	8.3	8.9	12.8	11.2
1-15-42	9.7	10.5	13.0	9.8
1-22-42	11.1	9.2	13.5	11.0
1-29-42	8.2	9.5	10.0	9.1
2- 5-42	10.0	9.8	12.0	8.2
2-12-42	9.6	9.8	12.3	9.7
2-19-42	10.0	9.6	12.7	8.4
2-26-42	9.9	10.8	13.5	9.8
Average	9.0	9.1	11.4	9.1

Graph I. Weekly Eosinophile Estimations

A. Normal Animals



B. Infested Animals

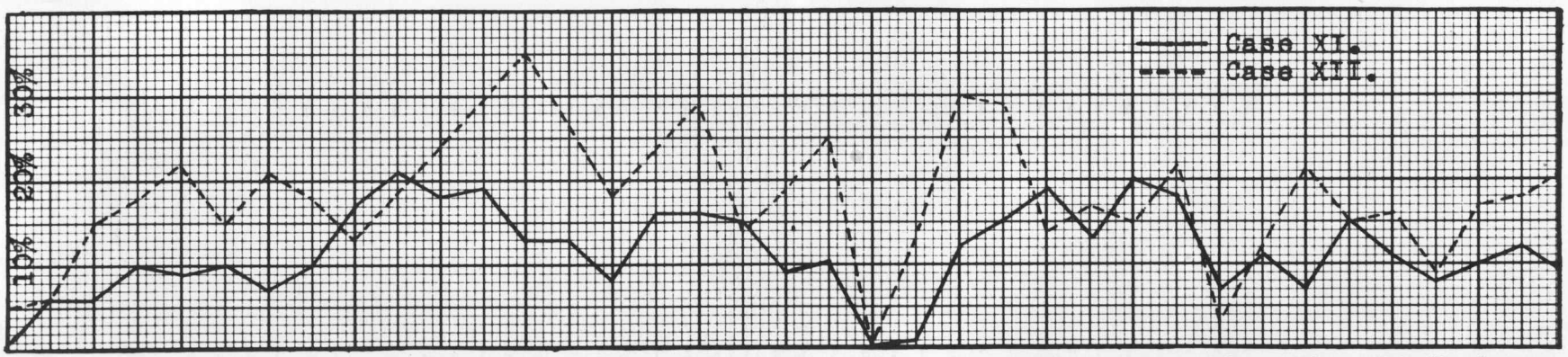


Table VIII. Averages of the Differential Leucocytic Counts
(number of cells per 100 leucocytes)

Type of Leucocyte	Case C	Case D	Case XI	Case XII
Eosinophiles	7.03	8.70	11.41	18.05
Basophiles	.11	.19	.05	.00
Myelocytes	.00	.03	.03	.00
Juvenile Neutrophiles	.43	.35	.64	.35
"Stab" Neutrophiles	1.13	1.81	1.75	.97
Segmented Neutrophiles	17.16	18.86	18.11	17.43
Lymphocytes	73.27	69.00	66.84	62.46
Monocytes	.86	1.05	1.16	.73

Liver Histopathology

In this part of the study, over 100 microscopic sections were made and examined, in addition to duplicates used in making collagen and mucin stains.

Alterations in the biliary canals were preeminate as might be suggested from gross observations. Depending upon the severity of the infestation, parenchyma, blood vessels, Glisson's capsule, and connective tissue were also involved. For convenience and coherency, the changes seen in all twelve cases are grouped and discussed under these and related headings.

Bile Ducts:

The epithelium of parasitized channels varied considerably in its appearance. In small ducts harboring large flukes, it was frequently completely destroyed, along with the lamina propria, and the trematode found lying

directly against the muscular coat. Occasionally, where no contact with a fluke existed, the epithelium retained its normal pseudostratified or simple columnar structure. Probably the most frequently observed response was that of proliferation, especially in the larger collecting ducts. This apparently proceded in both directions. Toward the lumen, the cells proliferated and threw up tall folds, so numerous at times that their basement membranes would approximate each other. In the opposite direction, the epithelium invaginated into the lamina, branching as it progressed. The end result was the formation of a compound tubular gland-like structure. In areas where irritation had persisted for some time, fibroblastic activity frequently laid down a framework of collagenous fibers which gradually separated the "tubules." As with other chronic proliferative processes, there was an extensive multiplication of capillaries to supply the newly-formed tissues. When changes in the individual epithelial cell are considered, it was observed that the nucleus lay closer to the basement membrane and its chromatin appeared heavier and usually clumped about the nuclear membrane. The cytoplasm protruded well into the lumen of the duct and frequently bulged, giving the cell the features of Goblet cells found in the intestinal mucosa. Similarly, many of them apparently ruptured and discharged their contents into the bile stream.

This mucin-like substance and the cytoplasm of unruptured cells stained only lightly with aniline blue and basic fuchsin dyes. The activity of biliary epithelium was further shown in formation of new ductules, noted particularly in the walls of large, hyperplastic ducts, in sclerosed portions of the liver proper, and in the spaces of Kiernan.

In most instances the lamina propria lost its identity, either through replacement by proliferating epithelium as previously mentioned or through the deposition of dense white connective tissue simultaneously being laid down in the adjacent muscular layer.

The muscle in the duct wall apparently reacts passively. In many sections observed, the newly-formed collagenous fibers were seen intramuscularly, separating the bundles and permitting the infiltration of more capillaries, lymphocytes, and similar types of cells seen in inflammatory response. Occasionally, growing epithelium further interrupted the muscular continuity by extending clear into this part of the duct wall.

Perimuscular connective tissue of infested canals, in practically every case, showed extensive cicatrization. The collagenous bundles were laid down in a circular manner, reinforcing the wall and giving it a laminated appearance. These were separated only by blood vessels,

small proliferating bile ducts and cellular infiltrations. Actual thickening was obviously more pronounced in this part of the wall than in any other.

In the proliferating mucosa, particularly where epithelial growth was rapid, necrosis was observed. Not infrequently, the fibrosed underlying tissues were the site of the inorganic deposition seen grossly. These were observed histologically as homogeneously-staining, purple areas approximating the spines of the fluke and supporting the deeper tissues.

For the sake of comparison, a limited number of both normal and pathological bile ducts were measured. As a standard, the internal diameter of the accompanying branch of the hepatic artery was also taken and a ratio set up. That of the normal ducts was found to be 1 : 1.3 : 2.7 (internal diameter of the artery : internal diameter of the duct : external diameter of the duct), and the hyperplastic ducts, 1 : 1.8 : 8.5.

Hepatic Parenchyma:

Changes noted in the hepatic tissue proper were of two types: those resulting from direct injury incurred in the migration of flukes, and those secondary alterations related to inflammatory reactions in the bile ducts and interlobular connective tissue.

There was unavoidably a certain amount of mechanical destruction of hepatic cells, sinusoids, biliary canaliculi and related tissues associated with parasitic activity. Adjacent structures also showed the effects of coagulation necrosis, subsequent phagocytosis, and their replacement with connective tissue. In sections taken from the sclerotic ventral lobes of the artificially-infested animals, an extreme condition was noted in which only a few isolated hepatic cells remained, and these undergoing pressure atrophy. However, the majority of the abattoir specimens did not show this extensive involvement except in random sections taken from the sclerotic margins of these mildly-infested organs.

Frequently the hepatic cells immediately adjacent to enlarging choleic ducts were pyknotic and possessed a deeply-staining cytoplasm - both conditions characteristic, together with their compressed condition, of a progressive pressure atrophy. In most observations only a single row of cells was involved, probably indicating that previously atrophied ones had been removed by phagocytosis. Occasionally proximal lobules showed swelling and vacuolization, but this was not common in most instances.

For the most part, microsections taken from normal-appearing portions of infested livers also failed to demonstrate definite pathology as evidenced by the

appearance of the liver cells proper. In cases I, VIII, IX, and X some swelling was observed, but no indications of extensive degeneration were presented.

Permanent section of one subcapsular lesion of case VII showed pronounced vacuolization and swelling, observations generally taken to indicate fatty degeneration. No tissue of this case was formalin-fixed, however, so a fat stain could not be employed to check this observation. Where Sudan IV was used (cases XI, XII, C), no fat could be demonstrated histologically.

Blood Vessels:

In areas where considerable inflammation and necrosis were involved, a phlebitis was frequently seen. The endothelium usually underwent hypertrophy and was pushed lumenward in irregular folds by a subintimal infiltration of cellular elements from the blood stream, particularly polymorphonuclear leucocytes and lymphocytes. These were eventually replaced by fibrous connective tissue, leaving polypoid masses or longitudinal folds protruding from the intimal wall.

As is usually seen in chronic inflammatory responses, the sclerotic ducts, hyperplastic interlobular connective tissue, and replaced parenchyma each showed an increase in its capillary and arteriolar beds.

Interlobular Connective Tissue:

The increase in connective tissue of the spaces of Kiernan was probably, next to the enlarged choleic ducts, the most common change noted in mildly infested livers. This alteration appeared greatest and most common in areas adjacent to infested ducts.

Collagenous fibers were apparently laid down by residual fibroblasts and completely veiled the normal, loose reticular fibers in many cases. Commonly, bundles of these newly-formed fibers were separated by small, proliferating bile ducts and cellular infiltrations. Their encroaching effect on the surrounding parenchyma has already been mentioned.

It must be stated that in a majority of the livers studied, no trouble was experienced in locating areas wherein histological changes in the interlobular connective tissue were not detectable.

Glisson's Capsule:

In three normal cases, the average thickness of the liver capsules was 46 microns. Of six infested ones, none were thinner than 49 and one measured 97; an average taken was about 74 microns. As might be expected, the capsule covering burrow lesions and sclerosed margins was thicker than that over normal parenchyma. Except for an occasional infiltration of leucocytes and an increase in collagen

bundles, the histological pattern remained much the same.

It was noted in a single lesion of case III that the serosa covering the capsule directly over a focus of inflammation was a low columnar in type in contrast to the simple squamous mesothelium normally seen.

Cellular Infiltration:

The predominating cellular reaction seen in these studies was the migration of eosinophilic leucocytes to the region of invading or harbored flukes. These were seen in massive numbers about the parasitized ducts, in their mucosa and wall, and in the adjacent spaces of Kiernan. Many of these cells also invaded the parenchyma about and in the path of the parasites. Heaviest accumulations were noted immediately around bile ducts and ductules, and smaller blood vessels, particularly those in the walls of hyperplastic canals.

Frequently, foci of lymphocytes were present in the proliferating mucosa and in extensively sclerosed areas. In more severe reactions such as those involving necrosis of a duct mucosa or inflammation in the wake of fluke activity, neutrophiles played an important part in addition to the eosinophiles and lymphocytes already mentioned.

Discussion

By using data obtained in weekly blood examinations and external observations, the detrimental effect of *Fasciola hepatica* upon its bovine host must be considered small. The anemia and eosinophilia noted do indicate some damage, but failure of the parasite to produce appreciable effects on general condition would suggest the ability of cattle to more than maintain their part of this host-parasite relationship.

Since the number of flukes recovered in either of the two experimental animals far exceeded those found in the abattoir specimens, it is expected that the pathology noted in these cases might be considered relatively severe when compared with an average natural infestation. It is true that the former were not subjected to repeated infestations and their associated inflammatory reactions to young migrating flukes, yet the number of cysts fed was perhaps as large as a pastured animal might ingest in several seasons.

Considering this heavy infestation, the relative amount of normal functional parenchyma remaining is remarkable. It is known that the liver possesses unusual powers of regeneration and in cases XI and XII the huge size of the dorsal lobe, the liver weight, and the position of the falciform ligament with reference to the atrophying ventral lobe might be taken as evidences of a compensatory

hyperplasia. Histologically, the formation of new parenchyma was not detectable, but this does not nullify the possibility since, according to Maximow,[7] regenerated liver resembles the normal gland six to eight weeks following hepatectomy.

The detrimental effect of Fasciola hepatica on liver parenchyma, based on these observations, is relatively small in the average naturally-infested animal. It appears to involve only those tissues in the path of migrating flukes and those immediately adjacent to inhabited choleic ducts. There is considerable probability that a temporary strain is placed upon the hepatic cells from the time the young fluke reaches the liver until it makes its final home in a bile duct, because of their duty in detoxifying excretory products of the fluke and toxic principles released with tissue destruction. Histologically, such effects could not be demonstrated in this study because of the limited number of animals and the necessity for slaughtering and examining recently-infested cases.

Depending upon its proximity to a fluke, the mucosa of biliary channels responds by erosion and proliferation. Mechanical pressure of a fluke in the smaller ducts and the trauma produced by its cuticular spines frequently produces extensive damage to both mucosa and lamina propria. On the other hand, it is probable that the irritation produced by

toxic products of fluke metabolism acts as a stimulus to the epithelial cells and results in the general hyperplasia seen when not impaired by pressure of an inhabiting parasite. Blanchard refers to this epithelial proliferation as an adenoma (Kouri[4]); however, Boyd[1] says:

> An adenoma is an innocent epithelial tumor of glandular structure which closely approximates that of the gland from which it arises. Unfortunately, the matter is not quite so simple as it sounds. Many so-called adenomas are not true tumors, but merely examples of localized compensatory hyperplasia.

On this basis, it is felt that the reaction should not be referred to as an adenoma, but rather a reactionary hyperplasia.

Differences in the extent of calcification between cases equally infested suggest several points for speculation and further consideration. A possibility of direct relationship to host resistance is presented. The relatively few flukes present, near-normal eosinophilic picture, and the extensive calcification seen in case XI when compared with case XII may indicate the deposition of inorganic salts in the duct mucosa to be an important defensive reaction on the part of the host. Further contributing evidence is a difference noted in average size of flukes from these cases. Comparative studies made by the author on two yearling wethers, using metacercariae from the same source, revealed no inorganic deposits and an

average length of 26.0 mm. in recovered parasites. It is probable that the calcification seen in cattle interferes with feeding habits of the fluke and these effects are noted in its size.

The question of a possible relationship of blood calcium and phosphorus to this deposition is also raised. Further studies should be undertaken with this in mind and a decision reached as to the merits or contraindication of these elements in the therapy of human fascioliasis.

Some controversy remains as to the manner in which the liver fluke reaches the choleic ducts in cattle. Krull(5) says:

> There is no evidence to indicate that in cattle the young flukes enter the bile ducts by puncturing the capsule and migrating through the liver tissue as has been reported in the case of sheep.

On the other hand, it does not seem likely that the same parasite producing similar pathology in the two species would be selective in its mode of entrance. Several observations made in this experiment may have a bearing. In some microsections, lesions were noticed directly beneath the thickened Glisson's capsule. In one instance, the serosa covering the lesion was columnar in type, indicating that there had been some irritation to the normally squamous endothelium. While this may have resulted from subcapsular inflammation, the absence of this metaplastic

change in other sections presenting similar lesions suggests the possibility of an attack from the peritoneal cavity. Another bit of evidence comes from the fact that frequent involvement of the ventral lobe may result from penetration of the intestinal wall and gravitation of the young flukes to the floor of the peritoneal cavity before reaching the liver. As an explanation for the occasional infestation of the dorsal and caudate lobes, it is noted that a part of the duodenum lies in proximity to them, from which escaping flukes might readily attack. At the present time, further experiments are contemplated which are expected to settle this controversy.

It seems feasible, on the basis of the observations made in this study, that a considerable part of many of the livers now totally condemned might be salvaged under proper supervision. The practical limitation of pathology to the larger choleic ducts would lend itself to practical trimming, and the relative amount of normal parenchyma remaining could be utilized to a very great advantage in hepatic extracts, liver sausages, and other products which permit incision and examination of all parts of the organ in addition to special processing. While the aesthetic viewpoint must be considered, it is felt that the economic aspect and unusual value of liver tissue both as a food and therapeutic agent warrants this special consideration.

Summary

1. General observations as to the effects of *Fasciola hepatica* on its bovine host indicate them to be limited to a mild anemia and eosinophilia, and slight, if any, lowering of general body condition.
2. The most marked alterations produced by this parasite occur in the bile ducts. They consist in the sclerosis of lamina propria, muscle wall, and perimuscular connective tissues, a reactionary hyperplasia of the epithelium with an elaboration of a mucin-like substance; the deposition of inorganic salts in the mucosa; and an erosion and necrosis produced by parasites inhabiting them.
3. In naturally-infested animals, damage incurred in the hepatic parenchyma is relatively slight. When more severe injury is produced by a heavy infestation, it may be offset by compensatory hyperplasia in other parts of the organ.
4. An increase in interlobular connective tissue was frequently observed, especially in proximity to infested ducts. However, many microscopic sections were studied in which no change could be detected.
5. The thickening of Glisson's capsule, presence of focal subcapsular lesions, and the metaplasia seen in overlying peritoneum are submitted as suggestive histological

evidence that the young fluke probably reaches the choleic ducts by migration from the peritoneal cavity through Glisson's capsule and the hepatic parenchyma.

6. Considering the value of liver tissue as a food and therapeutic agent, the observations made herein seem to indicate that special effort should be made, under adequate supervision, to utilize mildly-infested organs which are now discarded without consideration.

PHOTOGRAPHIC PLATES

Figure 1. Fasciola hepatica (about natural size). The three upper flukes are those recovered from yearling wethers and the lower ones were obtained from case XI.

Figure 2. Two pipe-like concretions removed from a main bile duct of case XI.

Figure 3. Cross-section of the ventral lobe of case C. Only a single bile duct is perceptible, directly over "3".

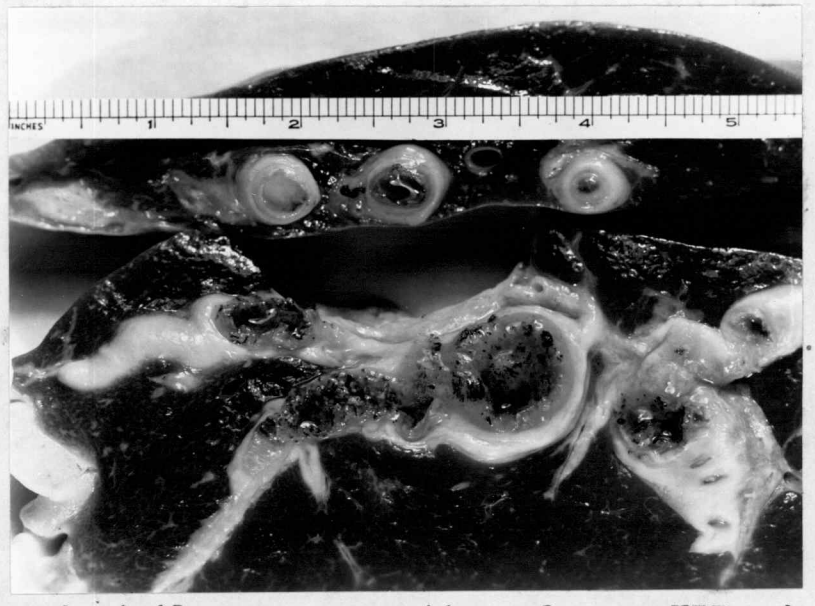


Figure 4. A similar cross-section of case XII, showing an increase in connective tissue, extensively fibrosed biliary canals, and flaky calcareous deposits.

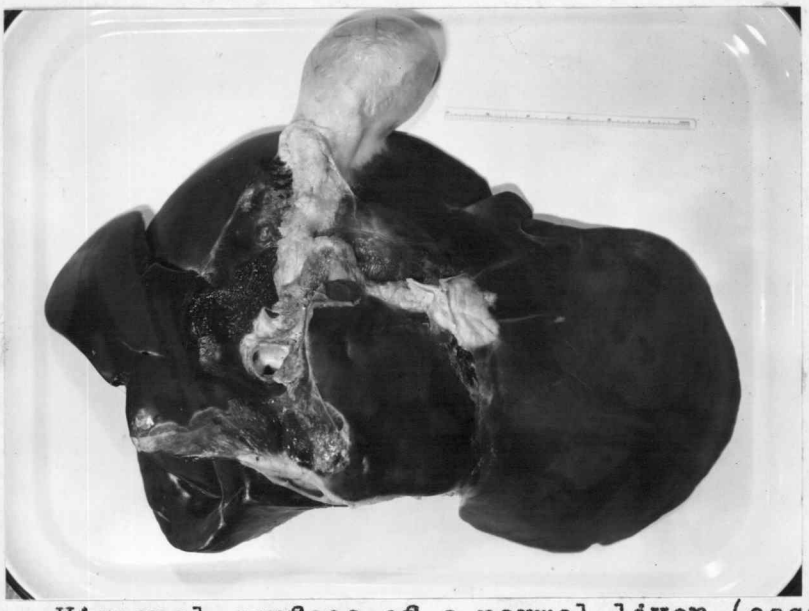


Figure 5. Visceral surface of a normal liver (case C).

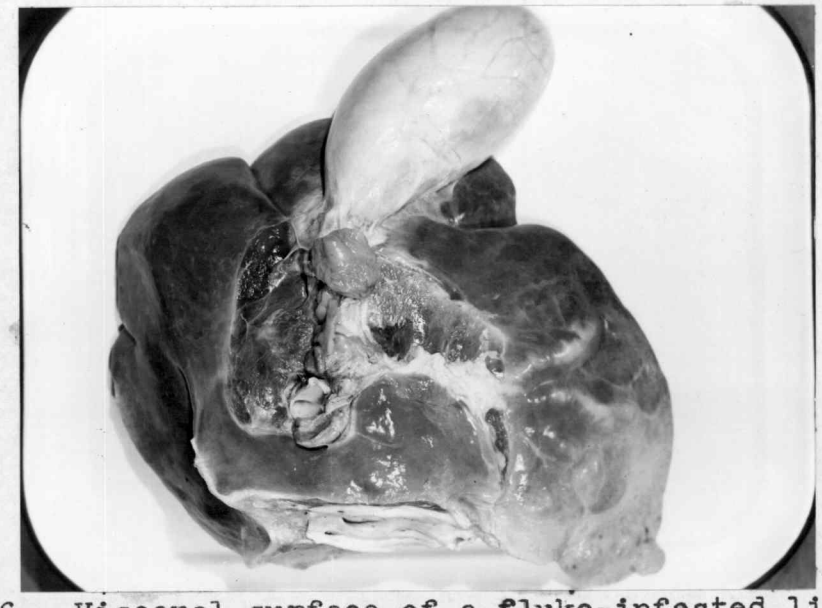


Figure 6. Visceral surface of a fluke-infested liver (case XI). Note the extensive fibrosis of ventral lobe, the enlarged and visible bile ducts, and the general difference in shape when compared with Figure 5.

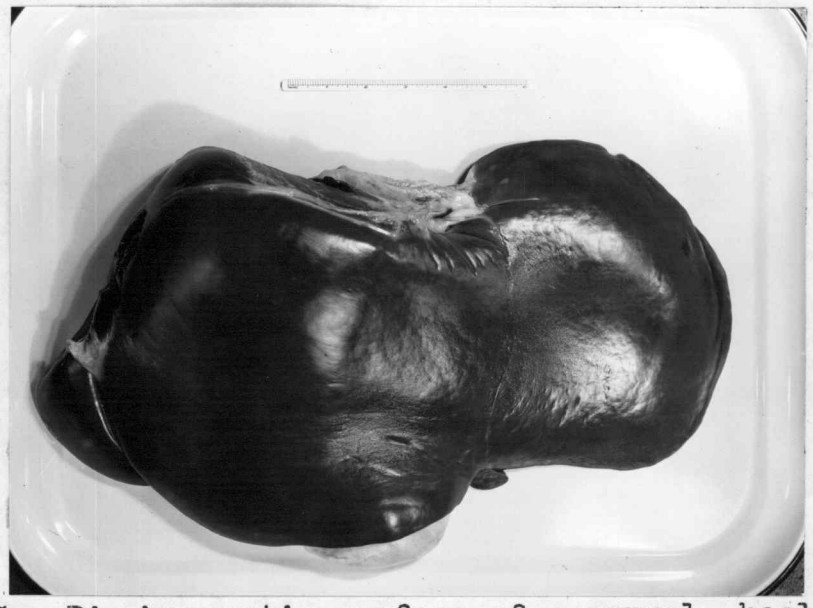


Figure 7. Diaphragmatic surface of a normal, healthy liver (case C).

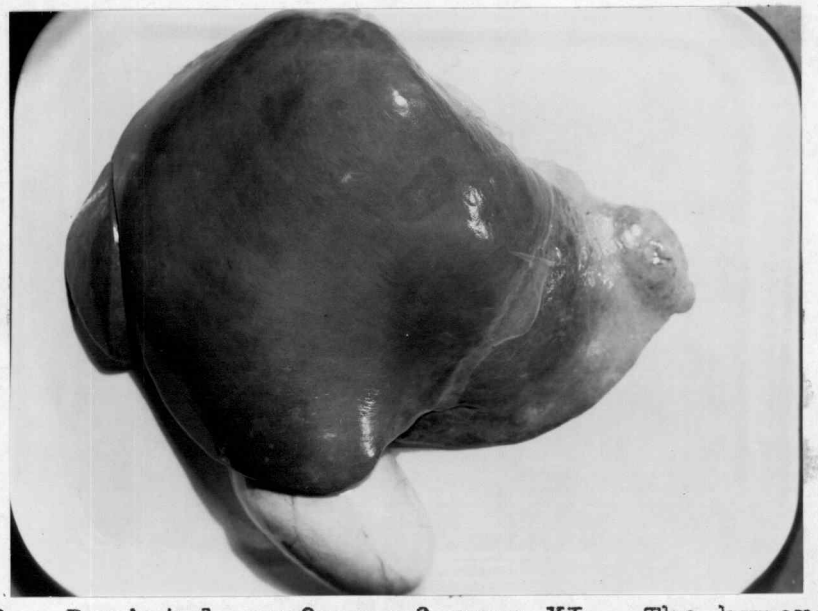


Figure 8. Parietal surface of case XI. The hyperplasia of the dorsal lobe and shrinkage of the ventral one is illustrated by general appearance and the position of the falciform ligament.

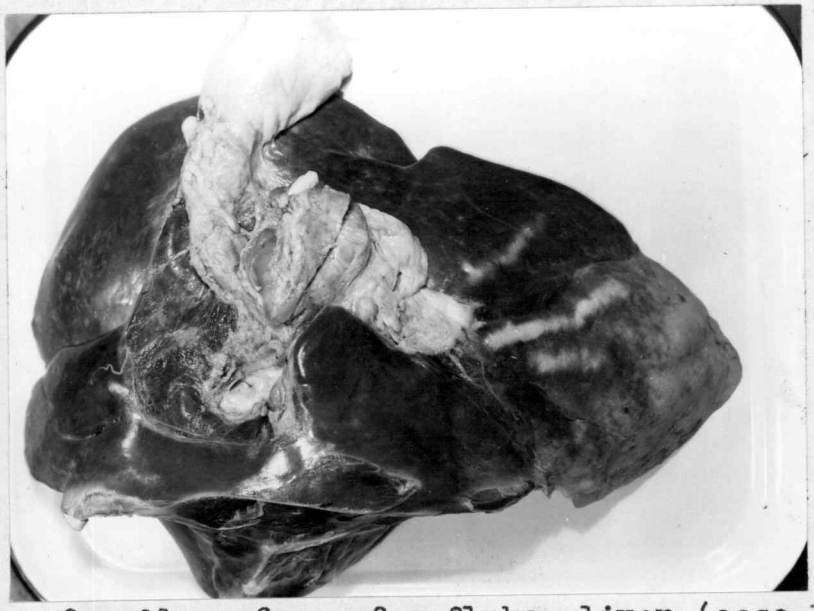


Figure 9. Omasal surface of a flukey liver (case XII) showing numerous small, greyish lesions on the dorsal lobe and lateral border of the ventral one. Three hyperplastic bile ducts can also be seen spreading into the much-fibrosed lower lobe.

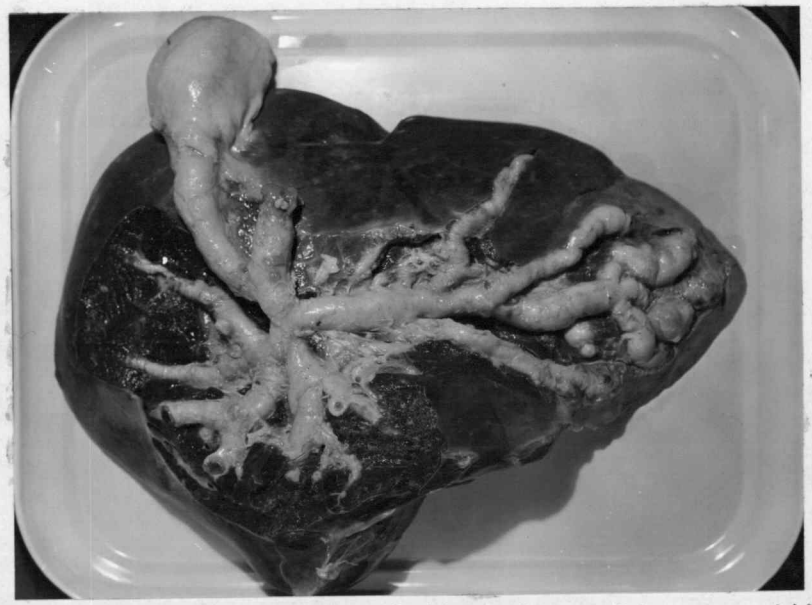


Figure 10. A similar view of the above liver, with the caudate lobe removed and small portions of hepatic parenchyma dissected away to reveal an extensively-involved choleic system.

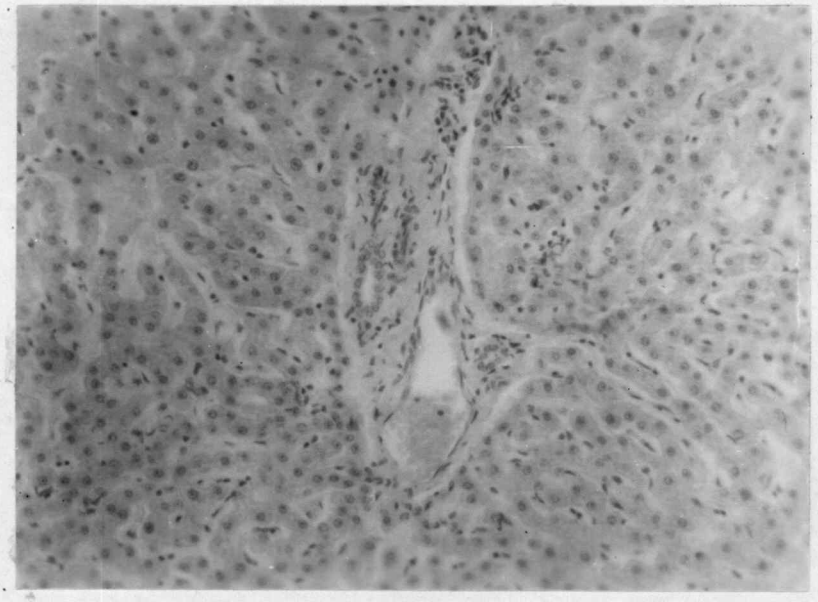


Figure 11. Parenchyma and interlobular connective tissue of a normal organ (case C). 200x. H-E stain.

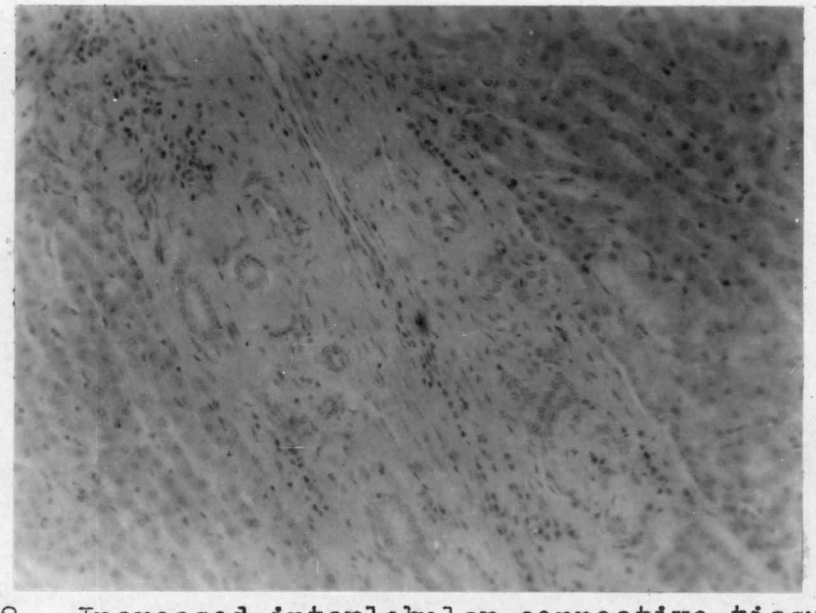


Figure 12. Increased interlobular connective tissue in a fluke-infested liver (case XII). 200x. H-E stain.

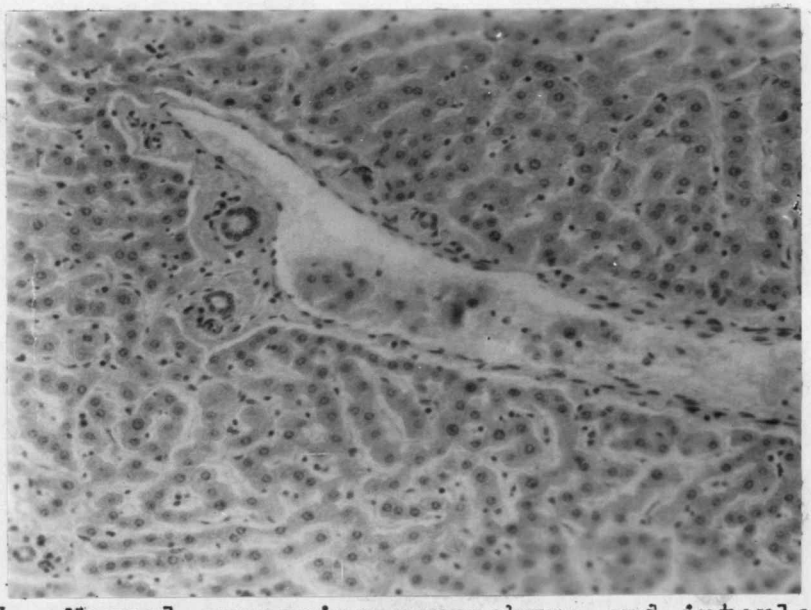


Figure 13. Normal-appearing parenchyma and interlobular structures from the liver of case I. 200x. H-E stain.

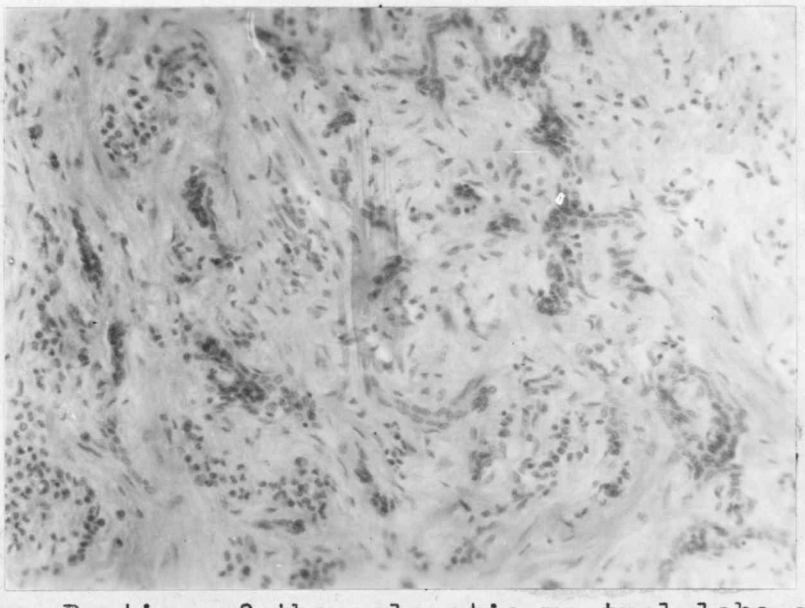


Figure 14. Portion of the sclerotic ventral lobe of case XI. Complete absence of parenchyma, extensive fibrosis, and the formation of numerous ductules probably originating from residual biliary epithelium are noted. 200x. H-E stain.

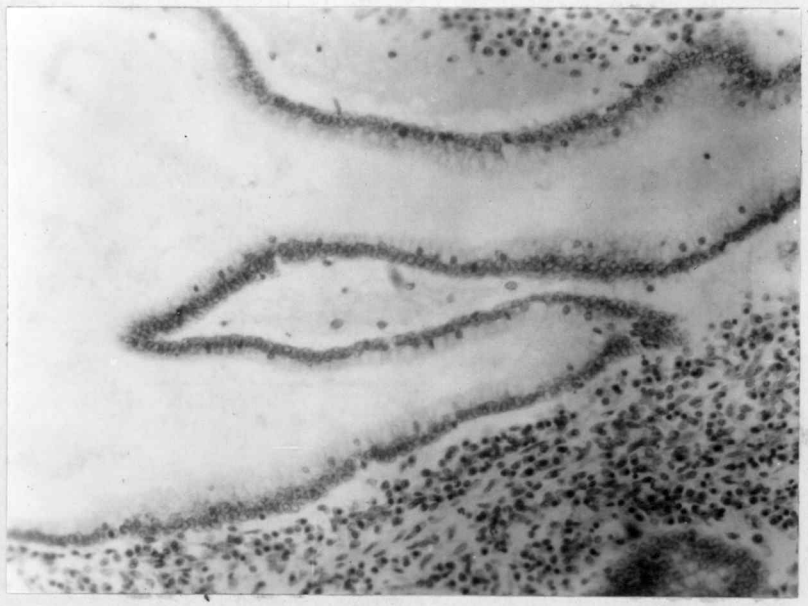


Figure 15. Extensive hyperplasia of the epithelium and main bile duct. Note the relative absence of stroma and crowding epithelial cells. In the lower right-hand portion considerable cellular infiltration is also seen. Case II. 200x. H-E stain.

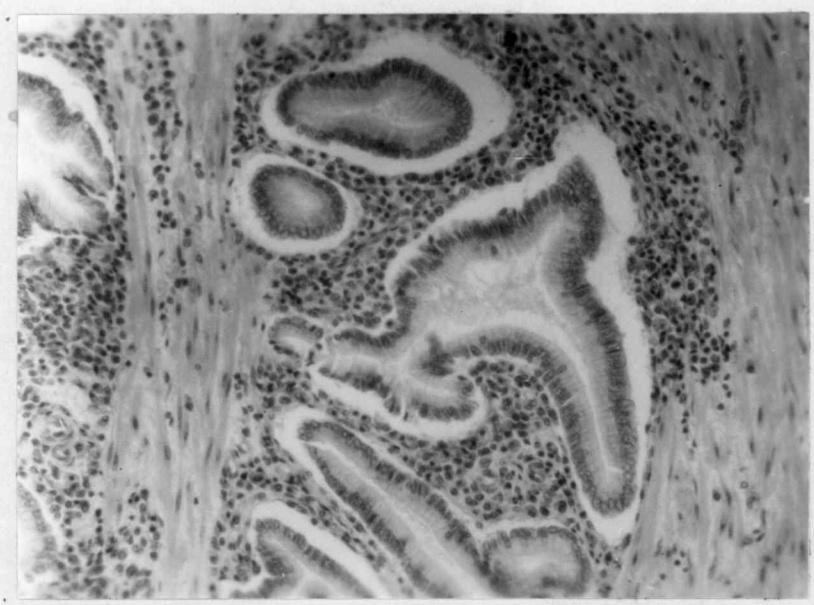


Figure 16. Gland-like extension of epithelium into the muscular layer of the duct wall and accompanying cellular infiltration (case IX). 200x. H-E stain.

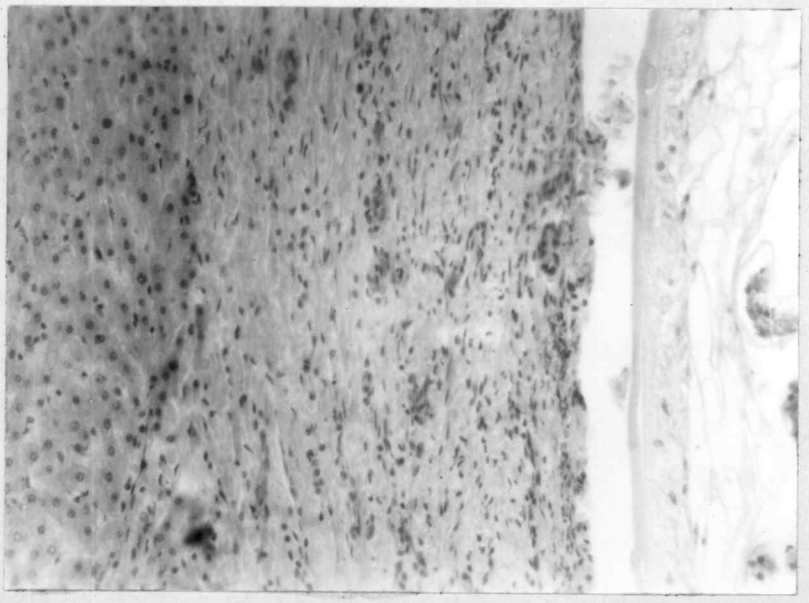


Figure 17. A liver fluke approximating the eroded and thin wall of a duct from case III. 200x. H-E stain.

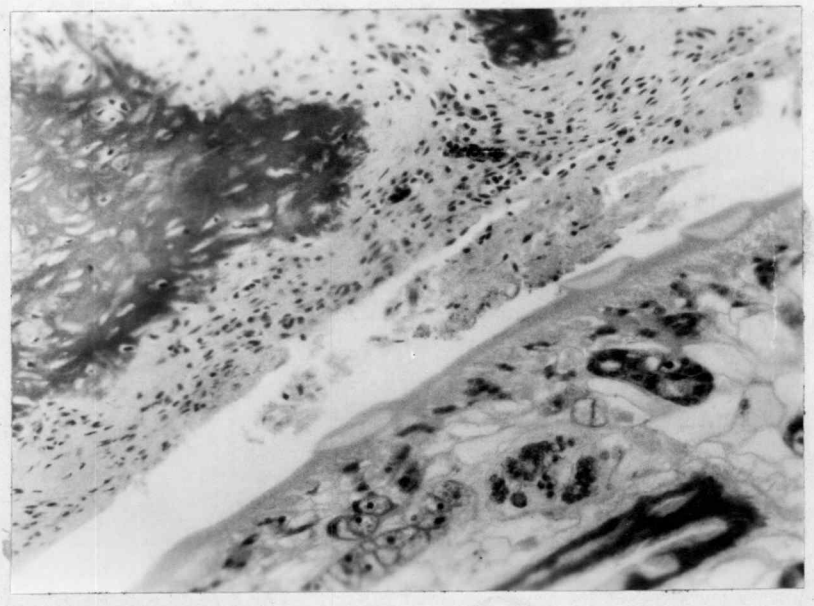


Figure 18. A similar picture in case IX, showing in addition the calcium deposits seen grossly. 200x. H-E stain.

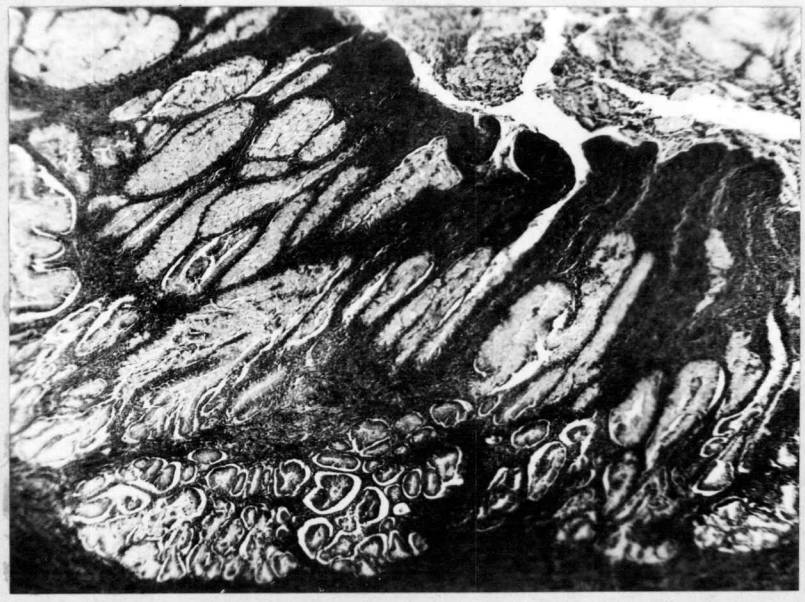


Figure 19. A general perspective of the proliferating epithelium and pseudo-mucin production (case II). 40x. Mallory's aniline blue stain.

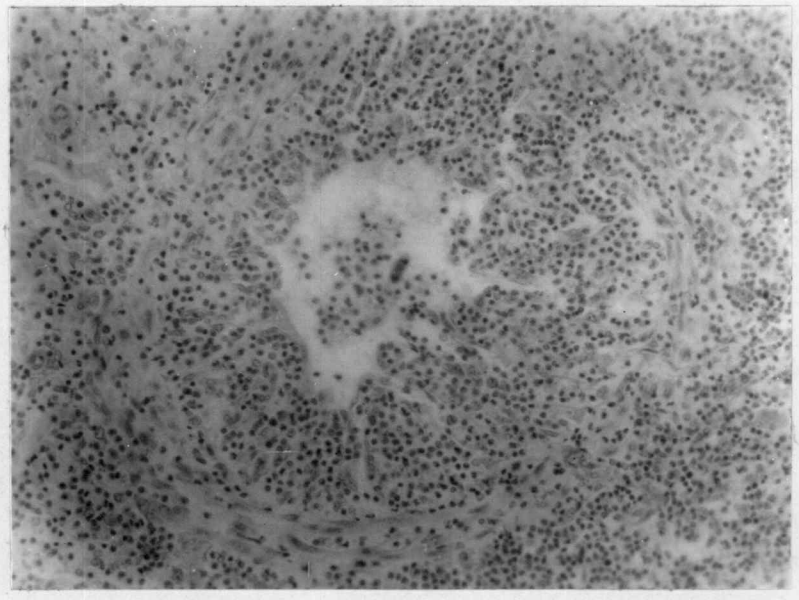


Figure 20. Inflammatory reaction in an interlobular vein, showing the subintimal infiltration and proliferating endothelium. Extensive perivascular accumulation of leucocytes (principally eosinophilic) is also noted. Case III. 200x. H-E stain.

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