THE ROLE OF INTESTINAL BACTERIA IN THE DEVELOPMENT OF DIETARY CIRRHOSIS IN RATS*

By ALEXANDER M. RUTENBURG, M.D., EDMUND SONNENBLICK, IRVING KOVEN, M.D., H. ARTO APRAHAMIAN, M.D., LEOPOLD REINER, M.D., AND JACOB FINE, M.D.

(From the Departments of Surgery, Harvard Medical School, and Beth Israel Hospital, Boston)

PLATES 1 TO 3

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Antibiotics delay the development of dietary hepatic necrosis and fibrosis in rats (1, 2). This protective effect is lost after the intestinal flora develop resistance to the antibiotic. Furthermore, rats fed a necrogenic diet, but maintained in a germ-free environment, do not develop liver necrosis (2). Clearly, then, bacteria are involved in the development of necrosis or fibrosis. But the way in which bacteria are involved is obscure. Gyorgy (1) believes that the metabolic abnormalities which lead to cirrhosis are enhanced by bacterial activity. At the same time he suggests that antibiotics delay the development of cirrhosis by an undefined protective effect upon the metabolic mechanisms.

Experiments were done in this laboratory to explore more precisely the role of the intraintestinal bacteria in the development of cirrhosis in rats. For this objective it was considered necessary to use non-absorbable[1] as well as absorbable antibiotics, in order to distinguish between a systemic effect of antibiotics and their effect upon the intraintestinal flora. In this communication we report the results of the effect of orally administered antibiotics upon rate of growth, survival time, and the pathological changes in the liver of rats on a choline-deficient diet.

Method

Male rats of the Wistar strain, each weighing 95 to 100 gm., were housed in an air-conditioned laboratory in wire cages with raised screen bottoms. Their diet consisted of choline-

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free peanut meal and casein[2] (3, 4), supplemented with lard and appropriate vitamins and minerals, as first described by Copeland and Salmon (3). The constituents of this diet were thoroughly mixed mechanically and stored at 4°C. The diet was fed *ad libitum* once daily, and water was allowed *ad libitum*.

The rats were divided into seven groups, as indicated in Table I. To prevent death from acute choline deficiency (5) all rats except those in group II received 10 to 15 mg. of choline in the daily diet for the first 10 days. The choline was decreased stepwise to zero during the next 2 weeks. The rats in group II received a daily supplement of 25 mg. of choline for the duration of the experiment. Groups I and II received no antibiotics; groups III to VII inclusive received antibiotics as indicated in Table I. The antibiotic, freshly prepared in solution, was added to the diet daily for the duration of the experiment. Group I served as a control for the effect of the antibiotics; group II served as a control for the effect of the choline deficiency.

The rats were examined daily and weighed weekly for the first 4 months of the experiment. Thereafter, they were examined daily, but weighed only once a month. Any rat found dead was immediately removed from its cage and autopsied. Sick rats were isolated from the rest until they improved or died.

Stool cultures were obtained from rats in each group at frequent intervals during the experiment. After 136 days one rat from each of groups I to V was killed by exsanguination. Liver, spleen, kidney, blood, and gastrointestinal tract were cultured. All bacteria isolated were tested for sensitivity *in vitro* to the antibiotic administered by a modified tube dilution technic (6).

The liver was weighed and examined at death in every case. Specimens of liver, spleen, and other organs showing gross abnormality were fixed in formalin, dehydrated, and embedded in paraffin for microscopic examination. Routine sections were stained with hematoxylin and eosin. Silver impregnation was used for reticulum in liver, and trichrome stains for fibrous tissue in liver.

Similar pathological and bacteriological studies were made on rats fed a stock diet.

The duration of the experiments is shown in Tables II to VII.

RESULTS

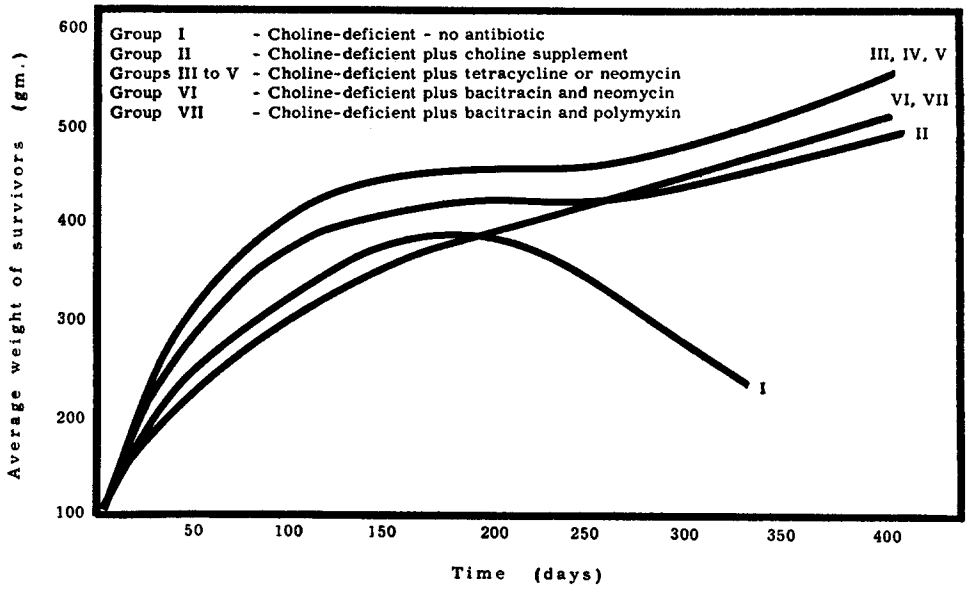
I. Rate of Growth, General Appearance and Survival Time

Initially all the rats were active, healthy, and showed a lusty appetite with an average daily food consumption of 10 to 15 gm. per rat.

Text-fig. 1 depicts the average weight curves of the various groups. The rats in control group I (choline-deficient—no antibiotic) were the slowest to gain weight. By the 175th day they showed a profound weight loss, which continued in spite of ascites until death. The rats in control group II gained weight steadily and maintained their maximum weight until killed. The rats receiving tetracycline or neomycin (groups III to V) gained weight most rapidly, in accord with the reported effect of antibiotics on the growth of domestic animals (7, 8). But the rate of gain in excess of that of the rats in

[2] Commercial peanut meal is a by-product containing 45 per cent protein. Choline was extracted from this product and from commercial casein with 95 per cent methanol and 95 per cent ethanol respectively by reflux on a steam bath four 2-hour periods.

group II began to decline after the 250th day. The average weight gain in rats receiving 25 mg. of tetracycline daily (group III) did not keep pace with that of rats receiving 50 mg. of tetracycline (group IV) or neomycin (group V). Rats receiving bacitracin and neomycin (group VI), or bacitracin and polymyxin (group VII) showed a less rapid average gain in weight than the other groups receiving antibiotics during the first 200 experimental days. Their weight curves were much like those of the rats in control group I (choline-deficient—no antibiotic) during this period. Thereafter, all but two of



TEXT-FIG. 1

the rats in groups VI and VII continued to gain weight at about the same rate as did the rats in control group II. Eventually all rats in group II, and all those receiving antibiotics except those of group III, reached about the same maximum weight. In doing so they acquired large amounts of abdominal wall fat. Weight was generally maintained, but in several cases in which cirrhosis developed, a preterminal severe weight loss occurred.

The rats in control group I (choline-deficient—no antibiotic) showed a progressive decline in activity after the 30th experimental day. They consumed less food and appeared sickly and listless. By the 175th experimental day several of these rats showed yellowish discoloration of the paws and nose, suggestive of jaundice. At this time they were lethargic, consumed very little food and displayed prominent ascites. One of this group was killed on the

136th day, and ten others died by the 313th experimental day (Table I). The remainder (19) were killed in a preterminal state between the 305th and 316th days, after being subjected to special studies.[3]

During the same period, all rats in control group II (choline supplement —no antibiotic) appeared healthy and active. There was no evidence of jaundice or ascites during the entire experimental period. None of this group died spontaneously. Three were killed after 135, 316, and 400 days respectively,

TABLE I

Effect of Antibiotics on the Spontaneous Mortality of Rats, on a Choline-Deficient Diet

Group	No. of animals	Antibiotic supplement per rat per day		Mortality*	
				Number	Per cent
I‡	30	None	None	10	100
II§	10	"	"	0	0
III	10	Tetracycline	25 mg.	0	0
IV	10	"	50 "	0	0
V	10	Neomycin sulfate	15 "	2	20
VI	10	Bacitracin Polymyxin B	1,000 units 30,000 "	0 0	0
VII	10	Bacitracin Neomycin sulfate	1,000 " 15 mg.	0	0

* Exclusive of animals killed for interim observations.

‡ Rats fed unsupplemented choline-deficient diet.

§ Control animals fed a choline-deficient diet supplemented by choline (25 mg. daily).

and 4 others about 70 days later. The remaining 3 continued well, and were killed after about 500 days.

The rats receiving 25 mg. of tetracycline (group III) appeared well until killed at intervals up to 400 days, but four showed yellowing of the nose and paws after 300 days. Of the rats receiving 50 mg. of tetracycline daily (group IV) two were killed after 30 and 136 days respectively. Four appeared well when killed after some 300 days. The remaining four looked sick and had matted coats by the 400th day, but there was no ascites or weight loss.

Of the rats receiving neomycin alone (group V) one died on the 240th and

[3] These rats were compared to normal rats for their ability to dispose of *Escherichia coli* labelled with I^{131}, introduced into the portal circulation. The results will be reported in a separate communication.

one on the 243rd experimental day. Before death both of these rats appeared ill and lost weight rapidly. At autopsy the livers were large and yellow, but without cirrhosis. Both rats had pneumonia, which apparently was the cause

TABLE II

Necropsy Findings in Rats Receiving Choline-Deficient Diet (C.D.D.) (Group I)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
136	Killed	—	—	Small, smooth, pale	4+	0
234	Died	234	—	Nodular, pale	3+	3+
268	"	262	—	" "	4+	3+
273	"	—	—	" "	3+	2+
294	"	260	17.5	" "	3+	3+
297	"	248	12.0	" "	1–2+	1+
300	"	260	16.0	" "	1–2+	3+
303	"	235	11.5	" "	3–4+	0*
305	Killed	—	—	" "	3–4+	1+
305	"	—	—	Finely granular, pale	2+	0*
305	"	311	19.5	Nodular, pale	3–4+	3+
305	"	326	—	Finely granular, pale	2–3+	0*
305	"	327	—	Nodular, pale	3+	3+
305	Died	211	14.0	Coarsely granular, pale	3+	3+
306	Killed	—	—	Nodular, pale	4+	0*
306	"	305	18.5	Finely granular, pale	3+	0*
312	Died	311	19.0	Coarsely granular, pale	3–4+	2+
313	Killed	342	23.0	Nodular, pale	2+	3+
313	"	355	20.0	" "	3–4+	3+
313	"	194	11.5	" "	—	—
313	Died	276	14.0	" "	2–3+	3+
313	Killed	—	14.8	" "	—	—
313	"	390	20.0	Granular "	2–3+	3+
313	"	241	13.5	Nodular "	2–3+	3+
313	"	314	17.5	" "	2–3+	3+
313	"	370	22.3	Granular "	3+	1+
315	"	430	29.0	" "	3–4+	3+
316	"	320	22.5	Nodular "	3–4+	3+
316	"	387	25.0	" "	3+	3+
316	"	367	21.0	" "	2–3+	2+

* All 5 rats without cirrhosis had moderate fibrosis of the liver.

of death. The remaining eight rats continued well until killed at various intervals up to 500 days (Table V).

There were no spontaneous deaths among the rats receiving bacitracin

and neomycin (group VI) or bacitracin and polymyxin (group VII). All of these animals appeared well up to the time they were killed, after periods up to 750 experimental days.

II. Bacteriological Findings

Nearly all cultures of liver, spleen, blood, and kidney were sterile. *E. coli* or *Staphylococcus aureus* was cultured from the lung abscesses found in 6 rats in group I. In two of these there were liver abscesses containing *E. coli*. *Staph. aureus* was cultured from the lungs of the two rats of group V (neomycin) which had died of pneumonia.

TABLE III

Necropsy Findings in Control Rats Receiving Choline-Deficient Diet (C.D.D.) Supplemented with Choline (Group II)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
136	Killed	242	9.4	Normal	0	0
316	"	426	12.5	"	0	0
400	"	600	16.0	"	0	0
469	"	450	12.5	"	0	0
473	"	480	16.5	"	0	0
473	"	495	19.5	"	0	0
473	"	560	17.5	"	0	0
480	"	600	18.4	"	0	0
480	"	610	17.6	"	0	0
500	"	605	18.2	"	0	0

The initial pattern of the intestinal flora remained unchanged in groups I and II. Stool cultures of rats receiving neomycin alone generally showed *Proteus vulgaris*, very few *E. coli* and many Gram-positive rods throughout the experiment. Stool cultures of rats receiving tetracycline were free of *E. coli* for the first 190 days. Thereafter, *E. coli* appeared in increasing abundance. *Proteus vulgaris*, *Streptococcus faecalis*, and other Gram-positive cocci were found in abundance throughout the experiment. Stool cultures of rats receiving bacitracin and neomycin showed a few *E. coli* and *Proteus vulgaris*, and almost no Gram-positive cocci, but many showed lactobacilli. Those rats receiving bacitracin and polymyxin showed only a few colonies of *Pseudomonas aeruginosa* and *Proteus vulgaris*. There were no *E. coli* or Gram-positive bacteria.

In vitro tests for sensitivity showed that in every instance the intestinal flora eventually developed resistance to the antibiotic administered.

TABLE IV

Necropsy Findings in Rats Receiving C.D.D. Supplemented by Tetracycline (25 mg./Day) (Group III)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
136	Killed	330	—	Smooth, pale	4+	0
145	"	370	21.4	" "	3-4+	0*
303	"	430	23.9	Nodular, "	3+	3+
400	"	446	21.4	" "	3+	0*
407	"	455	20.0	" "	3-4+	0*
407	"	356	27.8	Finely granular, pale	3+	0
407	"	325	27.8	Coarsely granular, pale	2-3+	3+
460	"	318	22.6	Coarsely granular, pale	3+	1+
516	"	358	27.3	Nodular, pale	1+	3+
516	"	340	28.4	" "	1+	3+

* Mild to moderate fibrosis.

TABLE V

Necropsy Findings in Rats Receiving Choline-Deficient Diet (C.D.D.) Supplemented by Tetracycline (50 mg./Day) (Group IV)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
30	Killed	—	—	Normal	2+	0
136	"	326	8.5	Normal, slightly pale	4+	0
307	"	400	20.0	Smooth, pale	2+	0
322	"	404	25.5	Nodular "	3+	3+
335	"	500	16.0	Smooth "	3+	0*
335	"	524	19.8	" "	3-4+	0
393	"	540	21.3	Granular "	3-4+	3+
393	"	540	22.0	" "	3-4+	2+
410	"	516	21.6	Nodular, ", firm	4+	3+
415	"	510	20.9	Granular "	3-4+	3+

* Fibrosis.

III. Pathology

Cirrhosis was considered present if there was distortion of the liver architecture by diffuse fibrosis and nodule formation. Localized proliferation of bile ducts was taken as further evidence of cirrhosis. Fibrosis without nodularity was classified as such.

The gross and microscopic findings are summarized in Tables II to VIII, in which the degree of nodularity formation is graded 1 to 3 plus.

(*a*) *Group I.*—(Control rats: Choline-deficient diet—no antibiotic). All rats

TABLE VI

Necropsy Findings in Rats Receiving Choline-Deficient Diet (C.D.D.) Supplemented by Neomycin (Group V)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
136	Killed	—	—	Normal	3+	0
240	Died	260	18.0	"	4+	0
243	"	258	17.4	"	1+	0*
261	Killed	346	18.1	Nodular, pale	3-4+	0
400	"	515	21.5	Normal	1-2+	0*
400	"	505	22.4	"	3+	0*
497	"	645	17.4	"	2+	0*
499	"	498	21.9	" pale	3+	2+
499	"	387	17.4	Finely granular	3+	3+
499	"	405	20.6	Normal, pale	3+	0*

* Fibrosis.

TABLE VII

Necropsy Findings in Rats Receiving Choline-Deficient Diet (C.D.D.) Supplemented by Bacitracin and Neomycin (Group VI)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
353	Killed	—	—	Smooth, pale	2+	0
450	"	423	18.1	Normal "	3+	0
464	"	447	18.6	" "	1+	0
464	"	455	19.1	Smooth, "	2-3+	0
465	"	485	19.0	Granular, yellow	1+	3+
470	"	337	17.4	Smooth, pale	4+	0
470	"	423	18.1	" yellow	2+	1+
485	"	510	20.2	" pale	3-4+	0
490	"	505	20.6	" "	4+	0
490	"	515	19.8	" "	1+	0

in this group showed substantial weight loss, which was in part masked by the ascites. Twenty-nine of 30 livers were abnormal in the gross. They were large, pale, and yellow. Both lobes showed gross nodularity (Fig. 1). The

left lobe did not appear more involved than the right, contrary to the findings of others (9). In many instances there were large, subcapsular fatty cysts which added to the gross nodularity. The portal system in most instances was engorged, with numerous collaterals present on the inferior surface of the diaphragm. Several rats had engorged spleens. Two livers had multiple abscesses. The other organs appeared normal in the gross except that in 6 rats there were multiple lung abscesses. The noses and paws of these rats were yellow, but there was no microscopic evidence of bile stasis in the livers.

TABLE VIII

Necropsy Findings in Rats Receiving Choline-Deficient Diet (C.D.D.) Supplemented by Bacitracin and Polymyxin (Group VII)

Experimental period	Mode of death	Body weight	Liver			
			Gross		Microscopic	
			Weight	Appearance	Fat	Cirrhosis
days		*gm.*	*gm.*			
310	Killed	419	20.3	Nodular, pale	4+	2+
400	"	430	21.4	Normal	1-2+	0
448	"	510	20.6	"	2+	0
470	"	514	22.4	"	2+	0
470	"	530	21.4	"	2+	0
650	"	536	19.5	Smooth, yellow brown	3+	0
750	"	465	30.0	Yellowish, nodular	3+	3+
750	"	599.2	22.0	Yellow, smooth	2+	0
750	"	504.3	18.7	" brown, smooth	2+	0
750	"	540	21.0	Brown, smooth	1+	0

All livers of this group showed fatty infiltration graded 1 to 4 plus (Table II). No fibrosis or cirrhosis was found in the liver of the rat killed on the 136th experimental day. Five livers showed varying degrees of fibrosis, but no cirrhosis. Twenty-two livers showed well established diffuse cirrhosis (Figs. 1 to 4). In eight of these there was extensive localized bile duct proliferation. This is in accord with the findings of others (4).

(*b*) *Group II*—(Control rats: choline-deficient diet plus choline—no antibiotic). Autopsy of all ten rats showed no gross abnormalities (Fig. 5 and Table III), except that, compared to rats on a stock diet, there was a marked increase in the amount of fat in normal depots. There was no engorgement of the portal vein nor of its tributaries. Microscopic examination showed normal liver architecture (Fig. 6).

(*c*) *Group III*—(Choline-deficient diet plus tetracycline 25 mg./day). Of the ten rats in this group (Table IV), one killed on the 136th day showed only fatty infiltration. A second rat killed after 145 days showed fatty infil-

tration and mild fibrosis. Of seven rats killed after 400 days, four showed cirrhosis. The livers of the remaining three showed increased fibrous tissue but no definite nodules. Fatty infiltration was present in all the rats in this group. The two rats killed after 500 days showed the most extensive cirrhosis, and the least fatty infiltration. All other organs were normal.

(*d*) *Group IV*—(Choline-deficient diet plus tetracycline 50 mg./day). The livers of two rats killed at 30 and 136 days revealed only fatty infiltration (Table V). The livers of all four rats killed at 307, 322, 335 and 335 days respectively showed fatty infiltration. One showed cirrhosis, and another some increase in fibrous tissue but no cirrhosis. The livers of all four rats killed at 393, 393, 410, and 415 days respectively were cirrhotic, with extensive fatty infiltration. All other organs were normal and there were no ascites.

(*e*) *Group V*—(Choline-deficient diet plus neomycin 15 mg./day). The livers of rats killed after 136 days showed fatty infiltration. A second killed after 261 days was nodular because of fatty cysts. The livers of six rats killed after 400 days showed fatty infiltration. Four of these livers showed some increase in fibrous tissue without architectural distortion or regeneration of liver cells. The livers of the remaining two had cirrhosis with extensive bile duct proliferation and marked fatty infiltration. The two rats which died at about 240 days showed *Staph. aureus* pneumonia but no cirrhosis.

(*f*) *Group VI*—(Choline-deficient diet plus bacitracin and neomycin)

Group VII—(Choline-deficient diet plus bacitracin and polymyxin). The pathologic findings in both groups were similar (Tables VII, VIII). Two livers from Group VI and two from Group VII were nodular or granular and showed cirrhosis. One of the latter two was killed after 750 days. The livers of 3 killed after 750 days had fatty infiltration. All other livers were smooth and pale (Figs. 7, 10) and without fibrosis (Figs. 8, 9, 11, 12). Fatty infiltration was present in all livers, but in general the amount of fat was less than in the livers of groups III to V.

COMMENT

Briefly summarized, the results are as follows: 80 per cent of rats (24 of 27[4]) developed cirrhosis after 300 days on a choline-deficient diet. By this time all of these rats were extremely ill, and indeed close to death. 73 per cent (8 of 11) of rats fed absorbable antibiotics (tetracycline) with the choline-deficient diet showed cirrhosis when killed after some 400 days or longer. Of 10 rats fed a poorly absorbable antibiotic (neomycin[5]) with the choline-

[4] There were 30 rats in this group. Three were killed at intervals prior to 300 days, and three others were free of cirrhosis after 300 days.

[5] Although not more than a few per cent of neomycin is absorbed, a detectable blood level is produced.

deficient diet, two showed cirrhosis. These were among the group of six killed after the 400th day.

Only 17 per cent (3 of 18) given non-absorbable antibiotics with the choline-deficient diet and killed at varying intervals between the 400th and 750th day of observation showed cirrhosis.

Since non-absorbable antibiotics were superior to absorbable antibiotics, one can exclude systemic activity of the antibiotics as having anything to do with the protective effect.[6] It is, therefore, proper to assert that cirrhosis in rats on a choline-deficient diet is caused by intestinal bacteria, and not by the choline deficiency.

DISCUSSION

Hartroft (12, 13) has put forward the view that fatty infiltration leads to cirrhosis by a collapse of fat "cysts" in the parencyhma, which allows the original reticulum framework to form the septa characteristic of cirrhosis. In our rats the fatty infiltration was not prevented by antibiotic therapy. The failure of livers with extensive fatty infiltration to develop cirrhosis does not determine whether fatty infiltration is a necessary precursor to cirrhosis. Whatever the role of fatty infiltration, a bacterial factor is required for the development of cirrhosis. Since the absorbable antibiotics are distinctly inferior to non-absorbable antibiotics (14–17),[7] it may be inferred that the intraintestinal flora are the primary offenders.

Our data do not disclose the way in which bacteria induce the fibrosis. According to Himsworth (9) fatty infiltration produces a relatively anoxic environment which may lead to fibrosis. As yet unpublished data (18) provide an observation which is more pertinent; *i.e.*, that the damage induced by the choline deficiency impairs the ability of the R.E. system to dispose of invading intestinal bacteria. From the available evidence, however, we cannot determine whether the prevention of cirrhosis by non-absorbable antibiotics is due to the elimination of bacterial activity in the liver or of the absorbable intraintestinal products of bacterial activity which may stimulate fibrous tissue proliferation in the liver.

Since the prevention of cirrhosis by antibiotics appears to be due only to the suppression of bacterial activity, one cannot conclude that choline-deficient rats if maintained for more than 750 days on non-absorbable antibiotics, would continue to remain free of cirrhosis. Sensitivity tests showed

[6] The possibility that the antibiotics protect by virtue of the ability of resistant strains to synthesize choline can be dismissed, because fatty infiltration, the mark of choline deficiency, is not prevented by antibiotics.

[7] The evidence that bacitracin and polymyxin B are non-absorbable is that no detectable amount of these antibiotics was found in serial blood and urine samples from animals and patients taken after oral administration of these antibiotics in therapeutic doses.

that resistance of the intestinal flora to the non-absorbable antibiotics developed much less rapidly than to the absorbable ones. Moreover, the non-absorbable antibiotics succeeded in keeping the bacterial population in the gut considerably reduced, even after the flora had become resistant. This reduced population may account in part for the more prolonged protective effect of the non-absorbable antibiotics.

SUMMARY AND CONCLUSION

Diffuse hepatic cirrhosis develops in rats on a choline-deficient diet within 300 days. Absorbable broad spectrum antibiotics added to the daily diet do not prevent the development of fatty infiltration, but they delay the development of cirrhosis for about 100 days more. Non-absorbable antibiotics added to the daily diet prevent the development of cirrhosis in most rats for as long as 750 days.

The superiority of non-absorbable antibiotics to absorbable antibiotics excludes a systemic effect of the antibiotics and demonstrates that intestinal bacteria are largely, if not wholly, responsible for the cirrhosis in rats on a choline-deficient diet.

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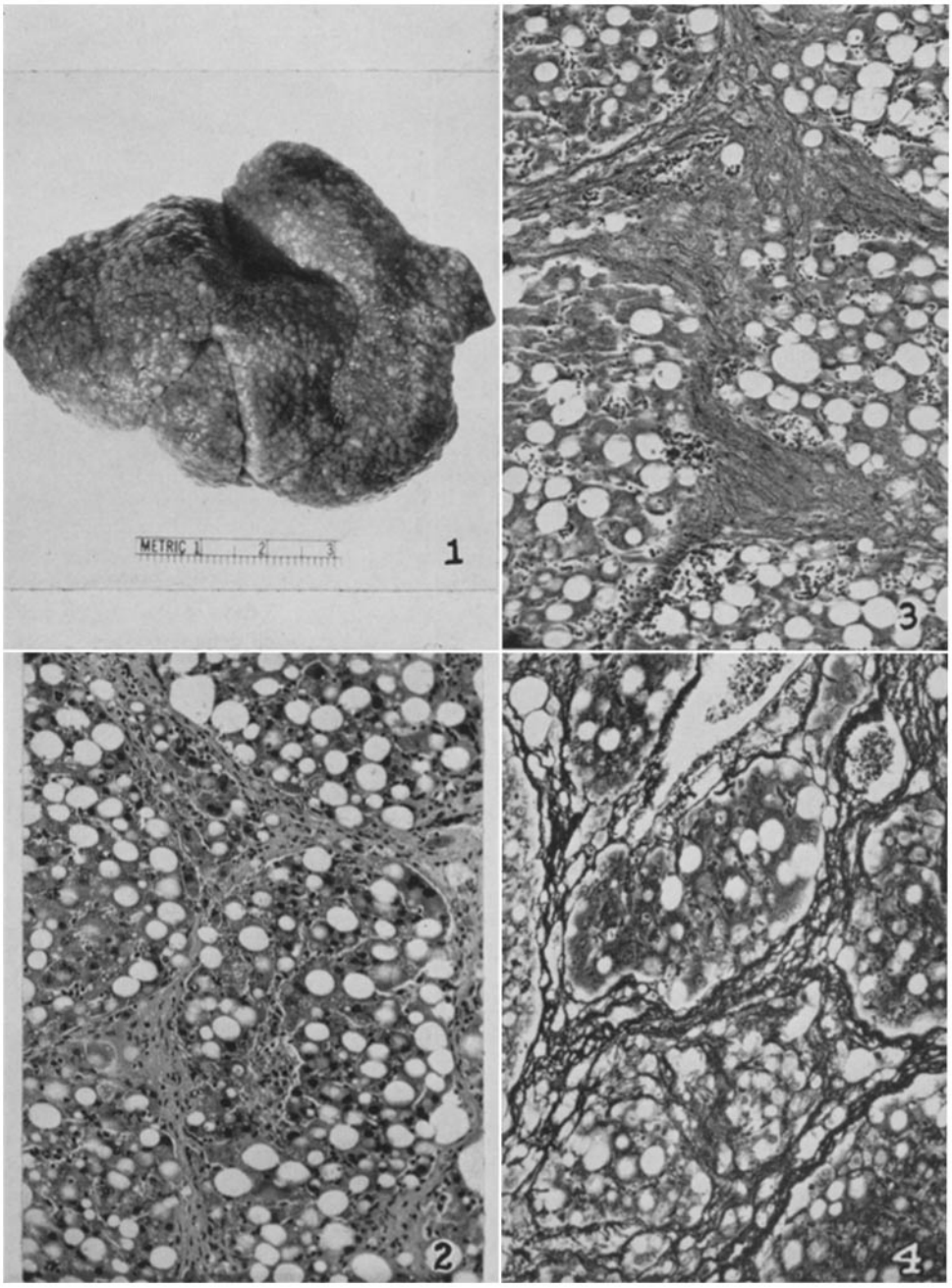
EXPLANATION OF PLATES

PLATE 1

FIG. 1. Group I animal. Liver after 304 days of choline-deficient diet. The organ is nodular in the gross. × 1.2.

FIG. 2. Group I animal. Liver after 305 days choline-deficient diet. Sections show fibrosis with formation of nodules and distortion of liver architecture. Hematoxylin and eosin. × 360.

FIGS. 3 & 4. Group I animals. Sections of liver after 305 days of choline-deficient diet. Sections show extensive fibrosis with formation of well defined nodules. Fatty changes 3+. Silver reticulum stain. × 360.



(Rutenburg *et al.*: Dietary cirrhosis)

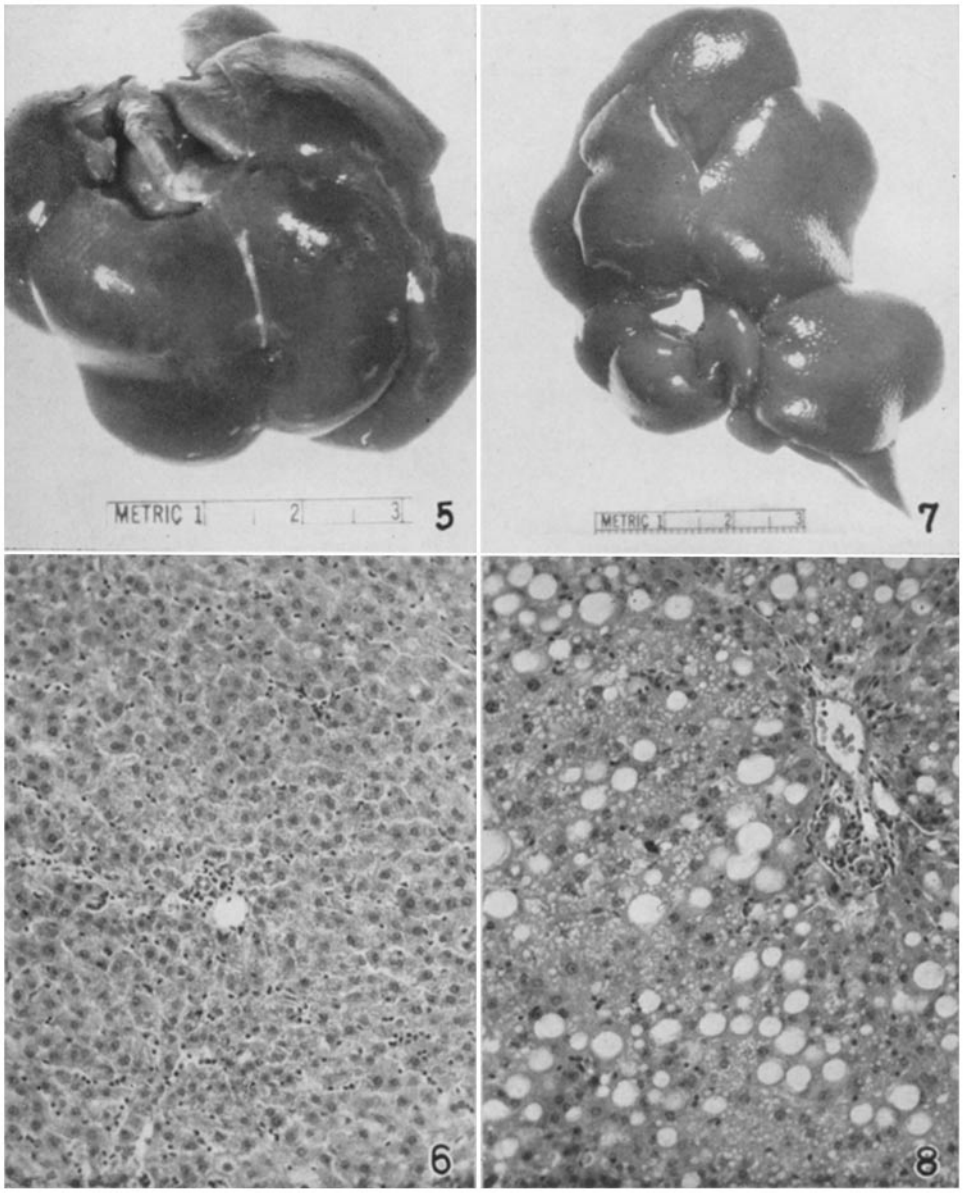
PLATE 2

FIG. 5. Group II animal. Liver of control rat fed choline-deficient diet supplemented by choline (25 mg./day) for 316 days. × 1.2.

FIG. 6. Group II animal. Section of liver after 316 days of choline-deficient diet supplemented by choline. Normal liver. Hematoxylin and eosin. × 360.

FIG. 7. Group VI animal. Liver after 750 days of choline-deficient diet supplemented by bacitracin and polymyxin (Group VI). × 1.2.

FIG. 8. Group VI animal. Section shows moderate fatty infiltration and minimal periportal fibrosis with maintenance of normal liver architecture. Hematoxylin and eosin. × 360.



(Rutenburg *et al.*: Dietary cirrhosis)

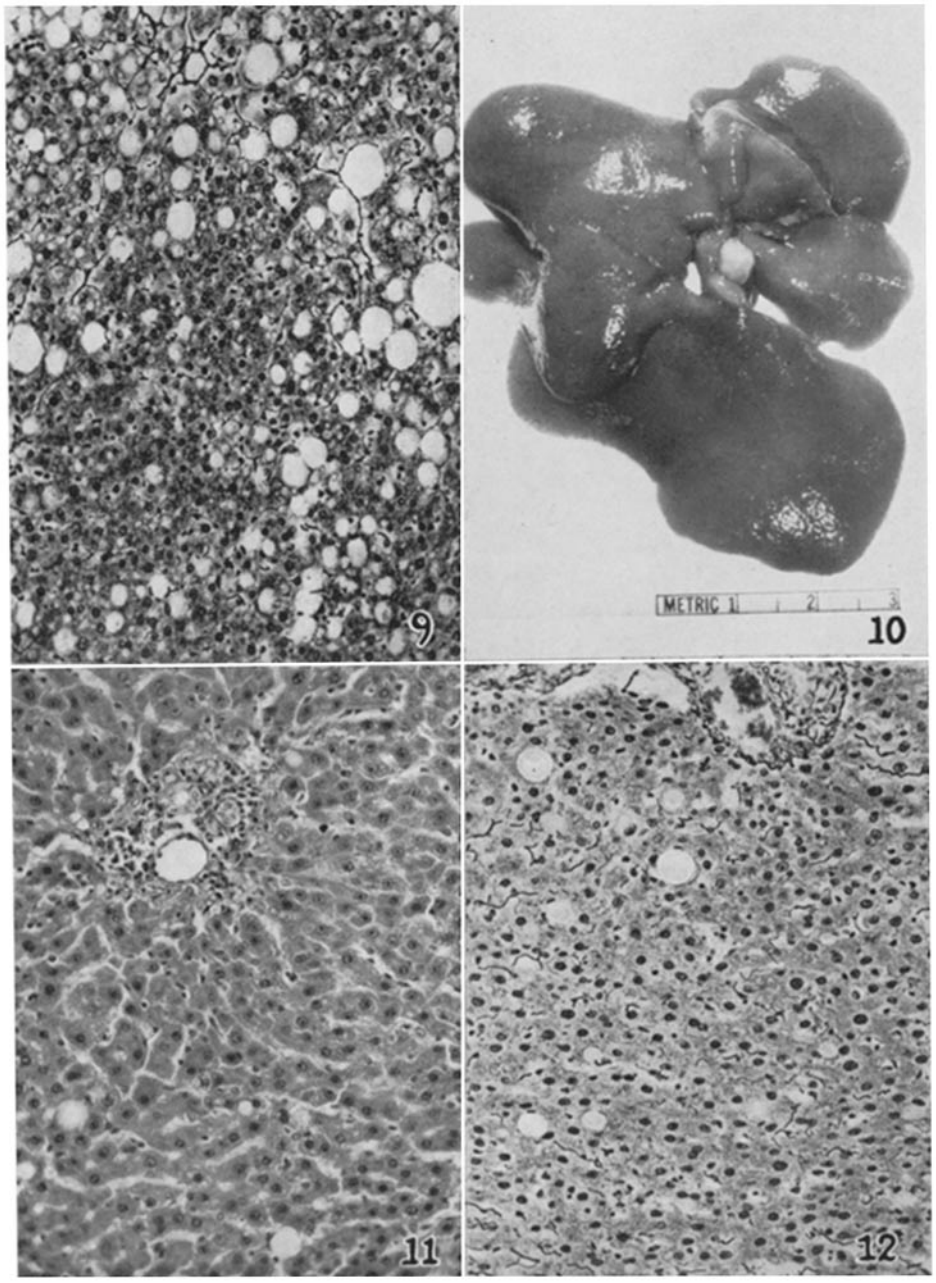
PLATE 3

FIG. 9. Group VI animal. From liver similar to that in Figure 11. Note minimal increase in reticulum. Silver reticulum stain. × 360.

FIG. 10. Group VII animal. Liver after 490 days of choline-deficient diet supplemented by bacitracin and neomycin. × 1.2.

FIG. 11. Group VII animal. Section shows normal liver. No fatty change. Hematoxylin and eosin. × 360.

FIG. 12. Group VII animal. No increase in fibrous tissue. Reticulum stain. × 360.



(Rutenburg *et al.*: Dietary cirrhosis)