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Experimental cirrhosis of the liver

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EXPERIMENTAL CIRRHOSIS OF THE LIVER

BY

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A THESIS

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DEPARTMENT OF PATHOLOGY AND BACTERIOLOGY, MEDICAL SCHOOL.

STATE UNIVERSITY OF IOWA.

1913.
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By the term cirrhosis of the liver we refer to the condition where there is a hyperplasia of connective tissue in this organ. This increase in the amount of connective tissue above the amount normally found in this location may be due to a number of etiological factors such as various toxins, infections, and other irritants. These irritants can reach the liver by the portal vein, the hepatic artery, the lymphatics, the bile passages, or by direct extension. This gives us several distinct possible groupings, such as portal cirrhosis, biliary cirrhosis, capsular cirrhosis, etc.

It is much more simple to classify the various forms of cirrhosis from pathological or histological findings than from clinical findings. Attempts have been made by various pathologists to correlate the views of both pathologists and clinicians in a classification that would be used as a working basis by both.

Adami uses the following:

I- Portal cirrhosis with enlargement.

II- Portal cirrhosis with contraction (Laennec's cirrhosis, hob-nailed liver, gin-drinker's liver).

III- True biliary cirrhosis (Hanot's form).

IV- Obstructive biliary cirrhosis with enlargement.

V- Obstructive biliary cirrhosis with contraction.

VI- Pericellular or diffuse cirrhosis.

VII- Capsular cirrhosis (perihepatitis with cirrhosis)
VIII- Senile atrophy and arteriosclerosis.
IX- Cirrhosis from passive congestion.

Briefly each may be described in the following:

I- Portal cirrhosis with enlargement - he believes to be in many cases merely an early stage of Laennec's form. However, he puts the form with moderate cirrhosis but with a great amount of fatty degeneration - a form often found in connection with pronounced alcoholism - in this class.

II- Portal cirrhosis with contraction - is the most common form met with and was formerly supposed to be directly due to alcohol. This part of the subject will be taken up more fully in the discussion to follow.

Grossly we find the liver very much diminished in size and covered with granulations or nodules varying in size. In consistency it is harder than normal and its color is apt to be greenish from bile stains. The left lobe is more often affected and on its anterior side. The capsule is usually thickened and there may be adhesions with other organs. Histologically we find an immense proliferation of fibrous connective tissue running more or less in bands, connected one with another. These bands tend to isolate lobules or groups of lobules. In younger portions of the lesions we find infiltrations with leucocytes and young fibroblasts, but the older portions have few nuclei and cellular material. We also get the so-called bile
capillaries about which opinions differ. Some consider that they are bands of liver tissue that have reverted in type, others that they are new growths of bile capillaries that have persisted in the process. New thin-walled blood vessels run into the cirrhotic portion from the hepatic artery. The portal vein and its branches are more or less compressed by the contracting connective tissue and to this is due many of the clinical manifestations of the disease.

III- True biliary cirrhosis is rare except in France. It is characterized by enlargement of liver and severe persistent jaundice. The liver is smooth and firm. It is also bile stained. Histologically we find increase in connective tissue with invasion by it of the lobules themselves. There is also the presence of the bile capillaries mentioned above. It is supposed to be infective in origin.

IV- Obstructive biliary cirrhosis is a form due to some severe obstruction to escape of bile. It is doubtful if secondary infection is not also needed to bring about this condition. Grossly the liver is enlarged, smooth, and bile stained with dilated bile ducts the walls of which also show bile staining. Histologically, we find central bile ducts dilated, surrounded by bile capillaries, before noted, and new-formed connective tissue both interlobular and intralobular.

V- The form known as obstructive biliary cirrhosis with contraction is probably a later stage of the last and had diffuse overgrowth of connective tissue and very much
VI- Pericellular cirrhosis differs from the above form in that the overgrowth of connective tissue is more diffuse and tends to surround cells alone or in small groups. This is the form more often resulting from syphilis and tuberculosis.

VII- Capsular cirrhosis is the form rarely seen where the capsule of the liver becomes enormously thickened and as a result of its contraction the liver becomes smaller in size. Histologically the liver shows brown atrophy and passive congestion with an increase of connective tissue growing into the parenchyma directly from the capsule.

VIII- Senile atrophy of the liver is a condition that gives increase in the amount of connective tissue but this increase may be only relative and due to disappearance of the liver cells. Nicholls considers this condition to be merely a replacement fibrosis.

IX- Cirrhosis from passive congestion. It is well described by its name. The fibrosis is apt to only occur after atrophy or degeneration of the cellular elements and can be considered like the last as a replacement fibrosis.

Councilman divides cirrhosis of the liver into two forms - atrophic (chronic interstitial hepatitis) and hypertrophic; while he speaks of biliary, syphilitic, cardiac, etc., as types, his descriptions of the various conditions
found do not vary from that of Adami to any important extent.

Delafield and Prudden use the same classification as Councilman. Both they and Councilman place the types of biliary and pericellular under these two headings. The former consider syphilitic hepatitis as a different disease.

Mallory considers that we have two forms of cirrhosis of the liver, the hypertrophid and the atrophic, but he considers that these terms mean but little. He considers that in all but one form - to be discussed later - that they are merely different stages of the same thing, namely, that the atrophic form is merely the result of repair. He prefers to consider the disease entirely from the point of view of etiology. In so doing he makes five types of cirrhosis, the toxic, the infectious, the pigment, the syphilitic, and the alcoholic cirrhoses.

He says that fatty infiltration has no significance except its presence will protect cells from necrosis, that chronic passive congestion does not bring about cirrhosis, but that the cells atrophy as a result of toxins liberated and the connective tissue trabeculae contract and give only a relative increase in connective tissue, that is merely the appearance without there being any increase at all; and that a general bile stasis will not by itself cause proliferation of connective tissue. In this he agrees with Adami.
As I have accepted this classification as a basis for whatever experiments I have performed, to be described later on, I will go into a description of these five types somewhat more minutely than I have the others.

I—Toxic cirrhosis. Mallory considers that central necrosis from toxins circulating in the hepatic vein is a common infection, also that mid-zonal necrosis is also fairly common. Either type of lesion is apt to be pretty evenly distributed throughout the liver. Generally in the milder types the polymuclear neutrophile and endothelial leucocytes quickly invade the necrosed part and dissolve the cells and carry them away. If the patient recovers the tissue regenerates. In this regeneration we get liver cells being produced from liver cells and bile capillaries from bile capillaries; sometimes if the necrosis is severe the bile ducts will be seen attempting to grow toward the central vein but not going further than two thirds of the way, and never do they produce liver cells.

From this it is seen that simple injury to the liver cells without injury to connective tissue or blood vessels will not cause increase of connective tissue. A rabbit chloroformed will show this necrosis of liver, but if allowed to live the cells all regenerate. The connective tissue in severe lesions that do not fully regenerate does contract, thicken and bring about an appearance of
increased amount. Although there is as said really no hyperplasia yet the term cirrhosis is applied to the resulting conditions. Microscopically the connective tissue is found irregularly distributed but the lobules are found in the contracted fibrous tissue still holding their original shape although considerably shrunken. It is not known yet whether the contraction will continue far enough to cause obstruction of bile ducts or bloodvessels. Of all the forms of cirrhosis this is the only one that is acute from an etiological standpoint.

II- Infectious cirrhosis - This form is due to bacteria that have invaded the tissues particularly by way of the bile ducts. This form is very often a secondary accompaniment of bile stasis from either an impacted gall-stone or a tumor pressing on the bile duct. The increase in connective tissue here is due to several factors any of which in any tissue of the body will produce the same hyperplasia of the same tissue, viz., fibrin, a stretching from leucocytic invasion, and direct injury from toxins. In some parts of the lesion besides the last mentioned we may often find that the connective tissue increase is merely preparative from fitting in a space lost from abscess formation.

Microscopically the exudative inflammation is around the bile ducts. The process evidently spreads evenly from
the portal vessels to the hepatic veins without disturbing the lobular arrangement. The dilatation and tortuosity of the bile ducts makes them appear in greater numbers than normally. The injury to the connective tissue brings about a great increase in fibroblasts which together with the great exudation gives a very markedly enlarged liver, which is smooth both on surface and on section. Recovery of course in time will give a liver that is atrophic from the connective tissue contraction.

III. Pigment cirrhosis occurs in hemochromatosis and seems to be entirely from mechanical causes. The blood pigment set loose is taken up by endothelial leukocytes that proliferate from the lymphatics and blood vessels. These migrating through the tissues and collecting in numbers stretch the connective tissue stroma and as a result of this mechanical injury it proliferates. Welch has already described a similar condition as being caused by carbon particles. This action is shown in a similar manner in the lung, in both brown induration and anthracotic pigmentation, as well as in many other organs.

In the acute stage of this type of cirrhosis we get a large smooth liver. Later on the connective tissue contracts and we get an atrophic liver. In any stage, pigmentation is an important characteristic. The connective tissue is evenly distributed and the liver surface is
always smooth.

IV- Syphilitic cirrhosis is a condition where the liver connective tissue reacts to direct injury from the treponema pallidum in exactly the same way that it reacts to this same injury in any other part of the body. Typically the congenital type is a diffuse infiltration whereas the acquired is a focal one but they may be combined in either case. It has been found that the treponemata are situated in the tissue directly between the fibrils in the interstitial supporting tissue, especially between the collagen fibrils. The reason that gummata form is due to the fact that the connective tissue in the walls of the blood vessels are injured in the same way and their proliferation shuts off the circulation to the part which naturally becomes necrotic.

V- Alcoholic cirrhosis - this is a chronic, progressive form which is characterized by a peculiar form of hyaline degeneration of the liver cells followed by cell necrosis; a coarse hyaline, irregular sort of net-work appears in the cell cytoplasm. This stains deeply with eosin. It may be found in single cells or in large groups and may be found in any part of the lobule but more often at the periphery. Both the cytoplasm and nucleus are swollen which gives the hypertrophic stage - the early stage of this type. Soon polynuclear or endothelial leucocytes invade the cell and
destroy it - the hyaline part remaining until the last. Degeneration takes place rapidly as seen by the mitotic figures, also the fibroblasts commence to proliferate. This stage (hypertrophic) gives a very large smooth liver which is increased in consistency.

This type of cirrhosis is very apt to be complicated with fatty infiltration which condition seems to have no effect on the rapid degeneration of the liver cells. The reason for the connective tissue proliferation is not known but is probably due to stretching of the reticulum by the swollen cells together with the leucocytic invasion. Later the connective tissue contracts and we get the atrophic form of Laennec with the resulting lesions from bile and venous stasis. Mallory believes that the so-called pseudo-bile ducts are sometimes new growths of bile capillaries as seen in necrosis mentioned above and sometimes rows of liver cells that have been squeezed out by the connective tissue as proved by the fat vacuoles and by the presence of this same hyaline degeneration which does not as far as we know appear in the cells of the bile capillaries.

He also believes that the words monolobular, multilobular and pericellular should be given up as meaningless as the types vary so in individual livers. He believes that they have reference to the wrong end of the process and that the etiological factors are the only ones in order.
HISTORICAL CONSIDERATIONS OF CIRRHOSIS.

Vasalius in 1514 first, as far as can be found from the literature, described a hardened condition of the liver. In 1819 Laennec gave the name, cirrhosis, to the disease but made the mistake of considering the yellow masses of parenchymatous cells as a malignant new growth. Johannes Müller in 1843 declared that cirrhosis of the liver was mostly a connective tissue hypertrophy which extended in between the lobules at the expense of the parenchymatous portion and was steadily a progressive lesion. In 1860 a great advance was made by Diehl who noted that sometimes a hypertrophy of the whole organ resulted as well as the atrophic condition noted by Laennec. In 1891 Ponfick came forward with the idea that the new formation of regenerated tissue had too small a functioning power to be of any value in replacing the tissue destroyed. This view was not held very long as Hanot and Gilbert in 1890, Fraenkel in the same year, Beale in 1889, and Dieulafoy in 1881 had all stated that, sometimes even after the recognition of definite clinical symptoms, cirrhosis might be recovered from completely, in so far as the health of the patient was concerned. Von Heykelom in 1894 gave as his opinion that cirrhosis of the liver depended on a necrotic process followed by a production of fibrous connective tissue. Kretz, in 1890 goes still further and added to this a regeneration of the liver.
cells themselves. He defined cirrhosis to be "a hard, white, localized, connective tissue, chronic degenerative process with progressive regeneration of the parenchyma". This view is the one now held by all pathologists, namely that we must first have some injury to the liver cells themselves which stimulate the connective tissue to proliferation as a repair process principally. The various viewpoints will be rapidly gone over in the following discussion:

Special researches in regard to the pathological aspects of cirrhosis of the liver.

Since the early work already mentioned above in discussing the historical aspect of cirrhosis there have been many and various researches made as to cirrhosis. These have been somewhat limited either to the anatomical considerations, the etiological factors, or the question of liver regeneration. It is impossible for one to give a detailed account of all this in a paper necessarily as limited as this must be; however, a rather brief review will be given and in general will take up each research by itself.

After Laennec had described the condition of atrophic cirrhosis, others became very much interested in the subject and ever since that time a great deal of work has been carried out on this subject. Cruveilhier in 1833 and Andral in 1834 first described liver regeneration. This idea did not
find general acceptance for a long time and was disputed by Albers and Weismann. Aschoff believed that the connective tissue regenerated but would not believe that the liver cells had much regenerative power. In 1862, Wagner described the so-called new bile ducts in cirrhosis of the liver and opened up one of the biggest questions that has ever been asked as to cirrhosis, a question which is not yet universally settled. He noted that these "ducts" often seemed to communicate with the columns of atrophied liver cells, but did not with the bile ducts running between the lobules. He considered that they must be ramifications of the hepatic artery. Later he found that he could not inject them through the artery so he changed his idea and admitted that they were either branches of the portal vein or atrophied liver cells. Liebermeister believed them to be derived from vessels or connective tissue that was present in the liver before the injury - in other words they resulted from the proliferation of these. Rokitansky believed that these "ducts" were found mostly from the portal vein branches. Eppinger published an article in 1875 in which he stated that they were probably found by proliferation of connective tissue since they were most numerous near the interlobular veins. Cohnheim(1876) believed that they were formed from the white blood corpuscles. Schmidt, 1880, considered
that they originated from the lymphatics. As there is such an intimate relationship between the bile ducts, the branches of the portal vein, and the lymphatics in the portal spaces, and as these investigations were made mostly by injection experiments, it is easily seen how such divergent views were obtained. The next view that was put forward was that there are always even in adult tissue certain undifferentiated cell constituents that when necessity arises can become differentiated and regenerate tissue. This idea was supported by Herring and Simpson, 1906, and Franklin Mill in the same year. Schaper and Cohen the year before expressed the same theory and believed that these cells differentiated into ducts but also into new liver cells.

These ducts have been described as originating from the interlobular bile ducts or from the liver cell columnus, either from sprouting or by atrophy. Klara, MacPhedron and MacCallum Kronig and several others believed that these ducts resulted simply from the collapse of the necrotic liver substance with merely a crowding together of the bile ducts. Zenker 1872, Lewitsky and Brodowsky 1877, Dinkler 1887, Manglesdorf, 1882, Hederius 1884, Hischberg, 1886, Orth 1887, Shafer 1889, Millar 1908 and others believed that these ducts were sprouts directly from the bile ducts while Thierfelder and many others believed both views - namely that they physically aggregated and
also partially new formed. Cornil, Friedlander 1877, 1,
2, and Ackerman 1880 believed that the liver cells atrophy
and the duct becoming embedded in a sort of embryonic
tissue gets stretched by the proliferation of this tissue
and becomes lined by epithelial cells that grow down from
the nearest bile ducts. In 1906, Hyani published an ar-
ticle in which he gave support to the last hypothesis
by claiming that these new sprouts grew down from the
bile ducts along the line of atrophied cells. A great
number of authors have confirmed these words, viz:-
MacCallum 88 observed the widening of the basil membrane
of the bile duct through which came the newly formed bud.
Kretz 1894, 1900, 1902, Hyani 1897, Marchand 1892,
Janowski 59 Charcot and Gombault 25 have observed these so-
called ducts connected with both liver cells and bile
ducts.

Those that believed these ducts to be simply
atrophied liver cells are Perls, Brieger, Heukelo 51,
Aschoff 9 Orn th l1, Posner, Klebs 72, Goodhardt, Janson 60
Rollston and others.

Those believing them to be formed by the proliferation
of the liver cells are Kelsch and Kiener, Schmidt Wanne-
broucq and Kelsch, Barbacci, Ribbert, and Melchior. Hanot
and Gaston thought where the excretion of bile was shut off
that owing to the loss of the antiseptic powers of the bile,
toxins formed in the intestine irrit ated the liver cells and
caused them to proliferate as ducts as an attempt on Nature's part to pick up lost connections. Hess while working on experimental rupture of the liver believed he found the liver cells at the edge of the wound becoming changed into bile ducts.

Many have believed that these ducts no matter how developed have the ability to form new liver cells. Waldeyer, Klöbs, Zenker, Hedernus, Schlichthoist, Shoebe, Rolliston, Dreshfeld, Hanot, Hirschberg, Orth, Mangelsdorf, Schaper and Melchor all adopted this view. Medel believed that for regenerating liver cells the bile ducts were much more important than the liver cells themselves. Steinhaus, Hyami and some others would never accept these opinions.

The more the subject of cirrhosis was studied the more it became evident that the proliferation of connective tissue was a secondary process and that the regeneration of liver tissue and these so-called "bile ducts" were not the primary process but rather one resulting from some injury to the liver - one that was only reparative in nature. The injury to the liver that was most often found was necrosis which was followed direct injury, the administration of poisons like phosphorous, alcohol, etc., infection, chronic passive congestion and anything that stopped the outflow of bile. The subject of necrosis has turned out to be an immense
subject and can be touched on here but lightly.

Kretz made profound studies of cirrhosis in man as found at autopsy and decided that the disease was essentially a repair process due to repeated focal injuries to the liver cells. He believed that the primary lesion was a destruction of the liver cells at the periphery of the lobule. Next the liver cells start to regenerate, are again injured and this repeated injury with the constant stimulation of repair forces a hyperplasia of the connective tissue. Weigert believed that the primary lesion was cell death and that alone stimulated the connective tissue to new growth. Kirikow believed that not only must we have cell death but also the continual injury from some toxic substance in order to stimulate the connective tissue to proliferate. W.G. MacCallum, Mac Phedran and A. B. MacCallum, Meder, Marchand, Stroebe, Barbacci, and W.G. MacCallum all agreed with the hypothesis advanced by Kretz. Podwyssozki, concluded that small injuries are repaired by hyperplasia of either the liver cells or else those of the bile ducts and that connective tissue repair only followed loss of tissue. Ponfick, came to the same conclusion. Salmon, Smith, and Kilborne found that the toxin of hog cholera would bring about necrosis of the liver cells which was sometimes followed by cirrhotic changes. Kelly described at length the reparative processes taking place in acute yellow atrophy of the liver and
showed that the cirrhotic condition here was particularly
a replacement fibrosis due to the complete necrosis or
loss of tissue. Baduel says that cirrhosis of the
liver in certain cases is due to a primary chronic tor-
pid peritonitis involving the liver by the lymphatic
route. He says that certain cases of pericarditis can
be explained in the same way, both that and cirrhosis
being of lymphatic origin from some primary source in
the peritoneum. The changes in the liver he thinks
are too extensive to be referred to congestion and his
experimental research has confirmed the assumption of
a lymphatic route between the peritoneum and the in-
terior of the liver. He injected a stain into the peri-
toneum and after eight or ten hours found it in both
liver and spleen. He injected a much larger amount and
found it again in the liver and could find no evidence of
it in either the kidneys, heart, lungs, or veins. He
described two autopsies, one in a girl of fourteen and
the other in a man of forty in neither of whom was there
an alcoholic history, which he thinks confirms this
theory. No other observers seem to have adopted this
view.

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Milne, believes that in ordinary cirrhosis the
condition is merely one of replacement fibrosis.
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W.G. MacCallum describes a liver he found at autopsy
which is normal except for the presence of irregular
fine lines of scar tissue throughout its substance and is
of a class that is called "incipient cirrhosis". He thinks
that all cases of such are produced by an attack, in
earlier life, of some infectious disease. In other words
the condition differs from ordinary cirrhosis in that
it is not progressive. Alessandro, describes two types
of cirrhosis of the liver both of which are due to al-
cohol and are similar to the type that follows typhoid
fever. In neither case does he think that a long interval
between the exciting cause and the lesion should tend to
rule out either of them as the cause. Kelly, thinks
that the changes found in cirrhosis of the liver are pri-
marily degenerative changes involving the parenchyma of
the organ. In addition to changes in the cells he says
they become arranged irregularly as regards each other
and the central vein, in some lobules the vein cannot be
recognized. This degenerative change is followed by
reparative processes on the part of the parenchyma as
well as by hyperplastic changes in the connective
tissue. MacCallum, concludes that in the ordinary type
of interlobular cirrhosis there is a primary destructive
process leading to the disappearance of portions of the
cell mantle surrounding the central vein of each lobule.
The framework of the lobule persists but usually collap-
ses and there is produced an irregular lobular mass in
which the central vein is in places left more thinly
covered by the radial strands of liver cells, or even completely exposed and surrounded only by the connective tissue framework. The remaining liver cells proliferate rapidly by mitosis and generally increase the size of the portion of the lobule that remains. In this process they sometimes assume temporarily the form of bile duct cells. The connective tissue bands which run through the liver and separate these irregular hypertrophied remains of lobules consist of preexisting connective tissue together with some newly formed. Numerous bile duct-like canals course through these bands, and it is thought that these are produced by proliferation from the preexisting bile ducts. The degeneration of bands of compressed liver cells which produce this appearance is not thought to be important. That the canals are composed of proliferating cells is shown by reference to a fresh case in which mitoses were frequently seen in the walls of these ducts and by the fact that they can be seen sprouting out, forming separated masses of new liver cells. The bile duct epithelium and the liver cells are shown throughout these processes to be equivalent so far as the regeneration of the liver cells are involved. The anatomical picture presented by a cirrhotic liver is one where usually the regenerative processes are the most striking while the destructive process may or may not be
present and may only be represented by scar tissue. He defines cirrhosis of the liver as "a chronic disease in which destruction processes, probably often repeated, result in a loss of the functional liver tissue immediately followed by the formation of a scar, the healing process, and later by an attempt at the restitution of the liver to normal by regenerative processes."

It seems as though this definition, just given, can be taken as the modern conception of the pathological processes in cirrhosis. I think that all agree with this except in one particular and that is in regard to the time of regenerative attempts. It appears to me that most observers have come to the final conclusion that regeneration immediately follows the primary injury to the liver cells by the causative exciting factor and that it keeps up throughout the following repetitions of that injury; and that the connective tissue hyperplasia is a later process and when once under way prohibits further regeneration, by its infiltration and subsequent contraction, in that particular locality.

As to what agents may be the prime excitors, I have not deemed it necessary to go into further than was done in the beginning. In discussing the classifications of Adami and Mallory this was pretty well taken up in
detail. It is only necessary in a broad way to repeat that any irritant to the liver cell which will bring about necrosis can excite cirrhosis. Exceptions to this may be found in the case of certain specific infectious agents as are found in syphilis, tuberculosis, etc., which work directly on the connective tissue itself and cause a primary hyperplasia of that, in which case the necrosis is supposed to be a secondary factor.

FORMER EXPERIMENTS.

We will now briefly endeavor to review the past attempts of many investigators who tried experimentally to produce cirrhosis of the liver so as to establish scientific proof of what had already been assumed to be the facts relative to exciting agents.

Wooldridge, injected into the jugular vein of a dog a complex proteid substance derived from the thymus and other glandular organs. Infarctions associated with thrombi in the branches of the portal vein were produced. In animals, that survived for fourteen days, he noticed scattered foci of repair which he thought resembled cirrhosis.

Ponfick, while doing experimental work on repair produced cirrhosis of the liver.

Flexner while studying the lesions produced in the organs of a rabbit after injection of dog serum,
observed a well marked cirrhosis due to repair about multiple necroses. He was unable to reproduce the same lesion on further experiments.

von Heukelom, in 1896 reviewed the literature on experimental cirrhosis and found nineteen different methods of experimentally producing cirrhosis.

Leyden, in 1866 made experiments on animals for the purpose of studying the proliferation of bile ducts in bile stasis.

Foa and Salvioli called attention to the fact, that the results of different experimentors varied so, was due to the diversity of methods and the peculiar reaction of individual animals.

Nothnagel inoculated animals with chloroform and obtained parenchymatous changes. This work was confirmed by Junkars, Ungars, Strassman, Ostentag, Fraenkel, Merthens, Ajello, Marthen, Heintz, Bandler, Muller, Wells, Whipple and Sperry, Aubertin, Howland and Richards, Muskens, Hildebrandt and others.

Adami while working on the so-called Pictou Cattle Disease obtained from the livers, which showed cirrhotic changes, a very minute organism which he thought was the cause of the disease. Later he examined cirrhotic livers from various parts of the continent and discovered in every case this same organism. Nothing is heard of
this at the present time so I presume it was proved to be some artefact but I could find nothing in the literature as to the later developments.

Harley and Barratt while carrying on a set of investigations on metabolism for several years succeeded in experimentally producing cirrhosis in that part of the liver that was affected by ligating the left hepatic duct. The animals used were cats and dogs and were kept alive for from five to sixteen months. As a result of the whole series of experiments they gave the following conclusions:-

"When a single bile duct is ligatured, the portion of the liver remaining outside the area of ligature remains unaffected, while the following changes occur in the ligated area:-

1- A development of interlobular fibroid tissue occurs. In this tissue small collections of multipartite nuclei are occasionally met with, but such collections form but a small fraction of the interlobular tissue.

2- The larger bile ducts become dilated, and there is a marked hyperplasia of the smaller bile ducts lying between the lobules, which become tortuous and appear considerably increased in number.

3- The hepatic lobules atrophy; the atrophy commencing at the periphery and proceeding from
without inwards.

4- The rapidity with which these changes develop, and their intensity vary considerably in different animals of the same species, though the operative procedure is the same in all cases.

5- The functions of the liver cells in the atrophied lobules still continue, as is shown, by the elimination of sodium sulphindigolate, by the presence of bile in the larger bile ducts, and by the unaltered aspect of the liver cells. All these facts are observable when extreme cirrhosis has occurred.

The mode of production of cirrhosis after ligature of a single duct appears to be as follows:-

1- The interlobular fibrosis is attributable to the continued slight irritation set up by bile which passes through the walls of the smaller bile ducts by osmosis, caused by the increased pressure of the bile resulting from the ligature. Rupture of the smaller bile ducts probably is not an effective factor in experimentally produced cirrhosis.

2- The dilatation of the large bile ducts and the marked increase in the smaller ones is, in part, at any
rate, directly due to the ligature, and is comparable to the extreme elongation and increase in size of the veins and venules, which is seen in considerable degrees of varix of the lower extremities and in varicocele. Our observations have so far failed to afford proof of the modes of formation of interlobular bile ducts.

3- The atrophy of the lobules is due chiefly to the irritant effect of bile which has passed out of the bile ducts and which acts principally, if not almost exclusively upon the peripheral portions of the lobule. It does not appear that pressure upon the lobules caused by the newly formed interlobular fibrous tissue is an effective factor in causing atrophy."

Weaver inoculated animals with a bacillus belonging to the colon group that was isolated from a guinea-pig dying spontaneously. In animals that died within a few days merely degeneration and necrotic changes were noted but in those that lived longer there was associated with this necrosis a proliferation of connective tissue.

Hektoen, inoculated animals with a bacillus of the so-called pseudo-diphtheria group which was isolated from a lesion of Blastomycetic dermatitis on the back of the hand. In guinea-pigs after subcutaneous injection besides local necrosis at point of injection necrosis and cirrhosis of the liver resulted. Intraperitoneal injections
were followed by the same results. The cultures were filtered through porcelain filters and the filtrate injected with the result that necrosis and death usually resulted but no fibrosis. The cultures were killed and injected and here also nothing resulted. Intravenous injection gave no results but that into the anterior chamber of the eye of a rabbit gave the characteristic lesions noted above. Cirrhosis was produced by subcutaneous injections in a dog but in a grey mouse and a white rat nothing resulted. Finally the cultures became attenuated and the series of experiments had to cease.

Marchwold says that with frequent injections of small amounts of antipyrin in frogs, rabbits, and other animals he induced cirrhosis of the liver as the reaction of the organism to the primary destruction of the liver cells, while the injections of large amounts caused acute destruction of the organ.

Joannovics did experimental work on repair and succeeded in producing cirrhosis of the liver mostly as a replacement fibrosis.

Adler says that in experimental cirrhosis of the liver he has noted that at a very early stage, proliferation is going on in the interior of the bile ducts in the affected areas. He has noticed this in the
larger bile ducts, the common smaller bile ducts, and down to the most minute ones where no liver cells could go. This proliferation of the epithelium is followed by the widening out and distortion of the basilar membrane and finally the sprouting into branches. He induced his cirrhosis experimentally by feeding his animals tobacco.

Bostroem worked on the question of chronic passive congestion of the liver and as a result he concludes that the congestion causes destruction of liver cells through nutritional and functional disturbance. These conditions he believes are associated with rupture of capillary walls and escape of blood into the tissues. He also finds that thrombosis is often a concomitant condition and has a close relationship to destruction of the liver cells. He says that repair starts in the portal spaces by fibrous proliferation growing toward the center of the lobule and that gradually cirrhosis results.

Symmers while working on the Bilharzia haematobia found that the ova would produce cirrhosis of the liver. He reports a case of human infection.

Silvestri says that certain Italian experimenters have proclaimed that some of the albumin ingested, such as that in meat, is liable to escape the assimilating
action of the gastro-intestinal mucosa and to make its way into the circulation where they induce the formation of antibodies. Silvestri argues that if this heterogeneous albumin enters the circulation in unusual amounts the organism is a prey to autointoxication of a much more complex nature than is generally supposed. The heterogeneous albumin reaches the liver practically unmodified with the other waste products in the blood, and in time exerts a seriously injurious action on it. The damaged liver cells in time launch new nucleoproteids into the circulation, the effects of which are superadded to those of the primal cause. The future, he thinks, will reveal the importance of the part played by these factors in inflammation of the liver, but experimental researches to date indicate that it is a very important one. He thinks that the injurious action of these nucleoproteids in the circulation is similar to that of intoxication from phosphorus, alcohol, and other substances which are capable of producing cirrhosis. This conception, he feels, explains certain cases of cirrhosis in which no etiological factor can be discovered. He does not go into the nature of his experiments.

Joannovics was able to induce lesions, similar to those in man, by administering to guinea pigs, by mouth, ammonium carbaminate and carbonate; by inhalation of alcohol,
by repeated subcutaneous injections of chloroform, and by the action of poisons like toluylandiamine which destroy red blood corpuscles and indirectly induce icterus and lesions in the liver tissue.

Podwysozki, did experimental work on liver repair using the rat, cat, rabbit, and guinea-pig. He finds in 24-48 hours after injury a new formation of liver cells. By the fifth to the seventh day these new cells are very abundant. Mitoses were usually seen in endothelial and connective tissue cells, and in Kupffer's cells. The newly formed liver cells were usually large, pale, and round or oval. The bile ducts, especially the interlobular, appeared to develop freely and from them new liver cells.

Jovanovics reviewed the literature on the subject and added several new methods of experimentally producing cirrhosis of the liver to the nineteen already reviewed by von Hekelom. He divides all experimental work on cirrhosis into two main classes: one in which the formation and the increase of interlobular connective tissue takes place and also where there is sclerosis of the liver, and the other where we get degeneration of the liver cells. To the first, he says, belong all the operative interferences whether working on the common bile duct, the portal vein, or the hepatic
artery. Also in this group he places the injection of irritating substances in the common bile duct or into the parenchyma of the liver itself, as well as the methods that mechanically irritate the surface of the liver. To the second group belong those poisons which bring about a degeneration of the liver cells, whether given by mouth, subcutaneously, intravenously, or by inhalation. These different methods have been gone into pretty thoroughly while reviewing the work of each individual experimenter and will not be touched on at this point.

Pearce intravenously injected dogs with an immune haemolytic serum and noticed that there resulted a primary necrosis which in animals that survived was repaired with hyperplasia of connective tissue resulting in due course of time to a true cirrhosis of the liver. No such condition as this has ever been seen in man, but these experimental lesions are of great scientific interest in that they gave good pictures of the early stages of hepatic cirrhosis. He used rabbit serum immunized against dog corpuscles and found that when the animals died right away the histological picture was that of a severe passive congestion of the liver added to which there was always a sort of hyaline degeneration of the red blood corpuscles so that they formed a sort of
fibrinous plug. After forty-eight hours a hyaline necrosis was seen throughout the whole of the liver tissue, leaving only small areas of normal tissue about the portal spaces. The destruction was complete throughout. Occasionally an infiltration of leucocytes was present. Pearce believes that this necrosis is in the nature of an infarction due to the fibrinous plugs. In all, forty-three animals were injected and of these fifteen survived long enough to show results. In a later article(113) he describes in great detail the various steps noted in the process of repair in these fifteen animals together with one other animal. The most of the animals died during the first week so he got good evidence of the processes of repair up to and including the seventh day. One animal lived thirty-six days and gave a late stage of the process but the time in between these periods was not represented. He found considerable variation in the time element of the various processes but in general the conditions were as follows:— the first evidence of repair was noted at the end of thirty-eight hours after injection of the serum. In this case the necrosis was very diffuse and there was a slight infiltration with leucocytes. In the affected area not only were the liver cells destroyed but also the endothelial cells as well. Between the necrosed
cells and the normal cells which were situated near the large portal spaces was a layer of cells which had their cell structure still preserved but which were very much vacuolated. He noted that regeneration came only from the normal cells and never were mitotic figures seen in these cells or in the necrotic ones.

The period from forty-eight to sixty hours was represented by fine animals. In these the repair was the same as in the others except that the regeneration had progressed further. Also the endothelial cells lining the capillaries in the necrotic mass had proliferated and were acting as phagocytes. By the third day connective tissue proliferation had begun. This together with the proliferation of the endothelial cells was rapidly replacing the spaces formerly occupied by the necrotic liver cells. Up to the end of the seventh day these processes steadily progressed. The intercellular fibrillae of the connective tissue began to become visible on the fourth day and gradually increased in amount. By the thirty-sixth day a condition which was essentially a cirrhosis had developed and had replaced the necrotic cells. This connective tissue appeared in bands and was very vascular. Pearce believes from his observations that new bile ducts are formed from pre-existing bile ducts and that their epithelial cells can
form new liver cells. Also that liver cells at times
have a tendency to arrange themselves in long linear
masses that occasionally may resemble bile capillaries.

Fiessinger reports extensive experimental research
which reconciles the interstitial and parenchymatous
theories of the development of cirrhosis. He says that
the anatomic evolution is complex; that the cicatricial,
biliary, vascular, and pericellular processes are com­
bined in various proportions, and both the inter­
stitial and parenchymatous tissues are involved; and
he thinks that previous contradictory findings were due
to preexisting lesions in the liver of the animals ex­
perimented on by the various investigators.

Fischler and Brandts carried on a series of ex­
periments and produced cirrhosis in their animals, the
results being about the same as those obtained by
former investigators.

Gougerot said that he had succeeded in reproducing
in guinea pigs all the various processes of human tu­
berculous hepatitis. Some of them were reproduced
to the finest details, especially the forms of cirr­
hosis with hypertrophy and atrophy, all the evidence
presented confirms the role of the tubercle bacillus
in the developments of such affections in man. The
tuberculous nature of certain forms of cirrhosis,
formerly attributed to alcohol, is fully established he thinks, by the research related in which he inoculated animals with pure cultures of the tubercle bacillus and cirrhosis of the liver developed in consequence - the series being too numerous to be ascribed to mere change. He claims that the experiments absolutely show that the tubercle bacillus alone, without the aid of alcohol, is able to induce typical cirrhosis of the liver.

Opie has shown by experiments that bacteria in association with toxic substances that have a special affinity for the liver, such as chloroform and phosphorus can produce changes which neither are able to do when acting alone. He thinks that in man some metabolic disturbance may produce similar changes in the liver as do these poisons as in the toxemia of pregnancy where we get persistent vomiting together with central necrosis of the liver similar to that caused by chloroform. He says his experiments show that one of these poisons may have its activity so intensified by bacterial infection that a quantity of the poison which alone would produce but slight change may in combination with the colon bacillus or the streptococcus cause destruction of perhaps the entire mass of liver parenchyma. Also some bacteria such as the colon which have but little pathogenicity for the normal animals are virulent
for the animals which have had their livers injured by chloroform or phosphorous.

It is possible, he thinks, that those instances of acute yellow atrophy which accompany infection with streptococcus are dependent on some disturbance of metabolism or other form of intoxication that has made the liver much more susceptible than normal. His experiments showed that bacterial infection could influence or even determine the development of cirrhosis of the liver. Degenerative changes that would ordinarily be repaired, when combined with bacterial infection will often go on into sclerosis. He found that chloroform alone may cause cirrhosis yet the bacteria bring the change much quicker. He says, "the poison in large quantity rapidly causes death, but a much smaller quantity in association with a relatively non-pathogenic micro-organism produces a lesion from which recovery is possible. Such results repeated at intervals produce the chronic changes of cirrhosis".

By administering colon bacilli together with chloroform he succeeded in producing advanced cirrhosis in twenty-three days. His experiments were more comparable to the conditions that occur in man for he succeeded in this way in producing a portal obstruction with its ordinary secondary results. He is of the opinion as a result of his experiments that it is explained why some
alcoholics escape cirrhosis whereas temperate people and even total abstainers do not always. He believes that the production of poisons in the body is an important factor in primarily causing the disease.

His experiments are given more in full as regards technique and seem to more nearly approach the conditions as we get them in man, from an etiological standpoint hence I will give them with considerable detail. The following is quoted nearly word for word.

1- A dog was given 1 c.c. per K. of chloroform on three successive days followed by a rest of three days, in turn followed by chloroform so that it has received in all 218 cc of chloroform. It became slightly jaundiced and the superficial abdominal veins have been considerably dilated. At autopsy the veins of the portal system are dilated, the tissue about the pancreas is oedematous and the peritoneal cavity contains a small quantity of fluid. The liver is small, yellow and resistant to the knife. Examination showed that about one-third of the area of the section consists of a very cellular, newly formed connective tissue situated about the central veins and in contact with the sublobular vessels. The liver cells are in columns which radiate from the portal spaces; they have the
appearance of regenerating hepatic parenchyma being in double columns with the nuclei on either margin. A few mitotic figures were seen. Near the center of the lobule in the new connective tissue were a few of the so-called bile ducts composed of cubical cells in well defined tubules.

2- This animal, a dog, received 2 cc of chloroform per K. on three successive days; a rest of six days has been followed by the chloroform repeated as before, the animal dying after sixty-days when it had received 424 cc of chloroform. The liver is large with superficial indentation corresponding to the lobulation, and was bright yellow in color. Thick bands of fibrous tissue transverse the organ. Much fatty degeneration was present. The lesion is like the one with fatty degeneration which is believed to occur as the result of excessive beer drinking.

3- A dog lived three months and received 32 doses of 1 cc per K of chloroform. There was jaundice and dilatation of superficial abdominal veins and fluid in the peritoneum. The liver is pale yellow. There is much irregularly placed connective tissue. This lesion seemed to follow as in the others, the areas of injury with the toxin.

4- A dog has been given 7.6 c.c. of chloroform
.5 c.c. per K. on four successive days. On the fifth day, .5 c.c. of a 24 hour bouillon culture of B. coli was injected into the jugular vein. On the eighth day chloroform was again administered. The animal was killed on the ninth. The liver was red and large, and the bile gave B. coli in pure culture. The central part of each lobule, occupying one-half the space from the central vein to the portal space, shows fatty degeneration and vacuolation, at the periphery of the lobule the liver cells are well preserved. In a circular zone inside this is evidence of hyaline degeneration.

5- Four rabbits received 1/50 gr. of phosphorous per oram daily skipping some days so that animals received 14/50 grains in 24 days. Two of these animals were repeatedly inoculated in the ear vein with Streptococcus pyogenes dying on the same day. The other two were killed and showed no particular change. The first two show profound fatty degeneration and the hyaline necrosis noted in the last experiment.

6- A dog received on four successive days 8.3 cc of chloroform (.5 c.c. per K); on the fifth day received .5 cc of a 24 hour bouillon culture of B. coli. Subsequently the dog received six more doses of
chloroform and died on the twenty-third day, twenty-four hours after a second injection of B. coli. There was well marked jaundice, the liver was smooth and B. coli was not recovered. Almost the whole liver had undergone disintegration and was represented by a narrow zone of intact cells encircling each portal space. There was some fat infiltration. In a few places the supporting frame work and endothelial cells persisted but in most places there was merely a mass of lymphoid, large mononuclear and polymuclear leucocytes and red blood corpuscles with fragments of necrotic liver cells. The same hyaline zone mentioned before was present, connective tissue was progressively proliferating especially about the portal spaces. Active proliferation of bile ducts was seen.

7- A dog received 10 cc of chloroform (1 cc per K) on three successive days; on the fourth day of the experiment it had lost about one-twelfth of its weight. On the eighth, ninth, thirteenth, and fifteenth days it received the same amount. On the twenty-first and second, half that amount. It was killed on the twenty-fourth day. Shows nothing but fatty degeneration.

8- A dog received 15, 2 cc of chloroform (2 cc
per K) and on the following day received usual dose of B. coli. Subsequently it received twelve doses of 7.1 cc of chloroform, and two more injections of B. coli. Death took place in thirty days. Liver was yellowish brown. The central one-third of liver lobule was replaced by loose cellular connective tissue containing many mononuclear cells. Also showed growth of bile ducts.

9- A dog received usual dose of B. coli, sixteen days later on three successive days got 5 cc of chloroform. On the following day .01 cc of colon. Later interrupted doses of chloroform for seventeen doses also interrupted doses of colon. Death occurred in forty-three days. Liver is pale reddish yellow and large. Parenchyma showed far advanced degeneration. There was no proliferation of connective tissue, but there were present numerous newly formed bile ducts.

Two of the animals were used and proved that this strain of B. coli in large doses would not stimulate connective tissue to proliferate but would only cause severe degeneration. Another series of experiments with Streptococcus pyogenes in place of B. coli gave essentially the same results as the foregoing.

Milne103 carried on a series of transplantation
experiments, transplanting portions of liver tissue into the subcutaneous tissue, onto the surface of the spleen, and in folds of the omentum. He used rats and rabbits as the animals experimented on. In all cases but two where the liver tissues persisted without change he got simply necrosis as a result. In another series of experiments by tying the portal vein he obtained cirrhosis. After two days he found that necrosis was present with a beginning proliferation of fibroblasts in the periphery of the lobule.

Summing up his results from both autopsy, (human) and animal experimental work he says: "Cirrhosis of the liver depends on a primary necrosis of tracts of liver cells and on these areas being replaced by fibrous tissue which is developed from adjacent portal spaces. When a definite ring of liver cells is destroyed at the periphery of the lobule a condition of monolobular cirrhosis is induced.

Ordinary atrophic cirrhosis is the result of very repeated damage to the liver parenchyma with a corresponding development of fibrous tissue in place of necrotic areas. There occurs also a compensatory liver cell hyperplasia which accounts for the extremely varied histological and macroscopical picture of this disease. In practically all destructive conditions in
the liver where fibrous tissue is being laid down, numerous "bile-duct-like" structures can be observed ramifying. They are often in connection with either the old interlobular bile-duct or a liver cell trabeculae. In some pathological conditions these ducts are lined by a fine somewhat flattened epithelium, and in others they are lined by a very definite cubical type of cell. Experimentally, they become conspicuously evident about the fourth or fifth day as tubes lined by an attenuated epithelium, by the tenth day they are definitely cubical in type, and from two months onwards they atrophy, and although their lining cells sometimes multiply locally, they never reproduce any cells with any close resemblance to liver cells. These 'ducts' then, seem to be formed from a becoming evident of the delicate bile-conducting channels which extend between the liver cells and the interlobular bile ducts. When these structures, which are apparently more resistant to destructive agents than the liver cells become exposed in granulation tissue, their lining cells swell up in the same way as do the lining cells of the air alveoli in the lung in interstitial pneumonia. By these means the cubically lined ducts are formed which so commonly are observed in pathological developments of fibrous tissue in the liver".
Lancereaux conducted experimental researches for years on cirrhosis of the liver particularly the type that is seen in France. As a result he concludes that alcoholic cirrhosis of the liver is the result of the action of substances used in wine, beer, etc., for their preservation, especially potassium bisulphate. He was able to produce the typical lesions of gin-liver by feeding animals with this salt. The other salts of potassium did not seem to cause disturbances of this kind.

Joannovics carried on a series of experimental researches in bile stasis and got very slight results as his animals lived but a short time. He reported that the connective tissue proliferation that he obtained and observed growing in from the interlobular spaces toward the necrotic center of the lobule, never reached a high grade of development.

Sabourin obtained cirrhosis by ligating the hepatic duct and announced that the connective tissue proliferation was "bivenous" coming from the sheaths of both the hepatic and the portal veins.

Nasse believed that better results followed when only one branch of the hepatic duct was ligated in that there would be less danger of killing the animal. He observed that the part of the liver from which this
led underwent atrophy and he also claimed that this lobule became surrounded by a broad hand of connective tissue but the general opinion of others that have repeated this experiment is that this is only a relative increase and not due to proliferation. He also noted that the other lobules not affected hypertrophied. He carried on experiments by tying the hepatic artery and reported a similar but not so high-grade an atrophy and that connective tissue rings developed about the lobules. That the chains of liver cells became smaller and the cells lost their characteristic granulation and finally fused into a homogeneous mass and that owing to the cell atrophy the intratrabecular blood capillaries could be easily seen. He thinks that the connective tissue increase is due entirely to the metabolic changes due to cell atrophy.

Malphi, Glisson, Kohnheim, Litten and others carried on the same experiments and found no change in the liver tissue or else it was due to suppuration from the operation.

Janson in the same sort of experiments noted progressive necrosis, cyst formation and increase in the connective tissue.

Pilliet injected silver nitrate and Josselin de Jong carbolic acid, directly into the liver substance causing a necrosis which became repaired with
connective tissue. The latter placed tinfoil between the liver and the diaphragm and got reparative changes. This sort of experiment was not long in vogue due to the fact that the conditions were not comparable to any found in human beings.

Wegner\textsuperscript{142} gave small doses of phosphorous to a rabbit and found that a connective tissue proliferation and bile duct extension resulted from an incipient high grade fatty degeneration. This was marked enough to correspond with certain types seen in human cirrhosis.

No other observer except Joannovics has been able to repeat this experiment and obtain a similar result although numerous attempts were made. Joannovics thinks that this is due to the fact that the dosage and the time interval was not given by Wegner.

Zeigler and Obolonsky\textsuperscript{150} tried similar experiments with arsenic and got no other effect than fatty degeneration. Lead in the hands of other observers gave the same results.

Mertens\textsuperscript{102} by the prolonged absorption of chloroform mixed with paraffin oil, succeeded in producing a typical picture of cirrhosis similar to that seen in human atrophic cirrhosis. He also obtained similar results by allowing his animals to inhale alcohol and by the subcutaneous injection of chloroform in paraffin oil.
Joañnovics repeated the chloroform experiments and confirmed the results of Mertens.

Boix on the ground of clinical evidence and pathological research came to the conclusion that human cirrhosis was due to some derangement of the stomach, which was enlarged in these cases, due to the abuse of alcohol. He believed that the stagnant stomach contents gave rise to the lower fatty acids and that the absorption of these caused cirrhosis. He fed animals lower and higher fatty acids with the food and came to the conclusion that the higher acids and ketones could produce no change but that the lower ones, and butyric and acetic acids also could produce changes in rabbits similar to human cirrhosis.

Josselin de Jong, tried the same experiments but could not repeat the same results. Others have had the same results. Boix meets this with the statement that the conditions were not right. However, his theory has been practically abandoned. Joannovics tried the same experiments with great carefulness but got no particular results.

Rovighie and Portiole obtained cirrhosis of the liver by feeding animals on carbonate of ammonium. These results as mentioned before are confirmed by Joannovics. He also got the same results with ammonium.
carbonate and hence believes that the ammonium and not the salt is the exciting agent. His same results with toliuylendianin has already been mentioned.

Lindemann with "Oleum Pyglegii" and Ehrlich with cocain produced vacuolar parenchymatous degeneration of the liver cells and a condition similar to human cirrhosis due to chronic poisoning.

Iwanoff experimented on frogs with antipyrin and obtained congestion of the liver with degeneration of the cells.

Joannovics tried to confirm the results of Iwanoff and Marchwold but was unable to do so.

Wells attempted to obtain by intravenous and subcutaneous injection of peptone dissolved in water, the increase of interstitial connective tissue and vacular degeneration of the liver cells in a rabbit but with indifferent results.

Many attempts, and with success, have been made by various experiments in guinea pigs with intraperitoneal injection of tubercle bacilli to reproduce the cirrhotic change found sometimes in the human liver affected with tuberculosis.

Joannovics and Christian both say that occasionally one sees a cirrhotic condition of the liver in rabbits and guinea pigs that have not been used for
experiments and were supposed to be healthy animals. The latter in a study of experimental myocarditis by spartein sulphate and adrenalin chloride found that in some rabbits a hepatic change resulted comparable to chronic passive congestion, which changes were in harmony with the view expressed by Mallory, that the changes resulting in the liver cells around the central vein are due to toxins liberated by stasis rather than by pressure. The same views are held by Ogata, Christian also obtained cirrhosis of the liver in one case but ruled it out for the reasons given above.

Richardson carried on some experiments by ligating the common bile duct in rabbits. Changes took place after twenty-four hours in the liver. First a necrosis which seemed to begin at the edge of the portal spaces and extend toward the central vein. Occasionally the portal space connective tissue was involved in this. The necrosis seemed to tend toward fatty degeneration. In all stages there was great dilatation of the bile ducts. These areas increased in size and at the end of two weeks had entirely disappeared. Mitotic figures appeared in the bile duct epithelium at the end of forty-eight hours together with bile duct sprouts. At the same time there could be seen an increase of fibroblasts. By the end of four weeks the bile ducts were
extremely dilated and tortuous. The removal of necrotic areas have made the liver lobule smaller in size, the loss of tissue being somewhat made up by the increased size of the frontal spaces. The cells in the center of the lobule appeared degenerated - some showed fat vacuoles and some showed merely a loosely granular appearance. The normal cells at the periphery of the lobule seemed to be continuous with the new formed bile ducts. He believes that the increased bile pressure causes the proliferation of the bile ducts and that the injury to the parenchyma is the cause of the connective tissue proliferation. He believes that the bile escapes at the junction of the bile ducts with the bile capillaries and either acts directly on the cells or else on the capillaries in which case the secondary anaemia causes the cell necrosis. He says that Bunting and Brown found by experimentation that bile even applied externally to rabbit liver tissue is very toxic to that tissue.

Bauer tied the common bile duct in guinea-pigs and rabbits and obtained a connective tissue increase which started in the portal space and extended into the lobule. In dogs under the same conditions he only got areas of necrosis with slight increase of interlobular connective tissue.

Belousson performed the same experiments in
the same animals and found in guinea pigs only an interlobular increase of connective tissue, in rabbits, he found this intralobular as well, and in dogs he found typical necrotic areas and cirrhotic changes. He believed that the proliferation of connective tissue was only a process of repair.

Burker experimented with rabbits with the same technique found both inter- and intra-lobular increase of connective tissue. Canalis by the same method in guinea-pigs and dogs obtained typical necrotic and cirrhotic areas. Chambard also tied the common bile duct in guinea-pigs and described an inter-lobular increase of connective tissue together with bile duct proliferation. He believed that the bile ducts growing into the lobule carry in inflammation from the dammed up bile which so irritates the liver cells that they atrophy. He considered the connective tissue growth secondary to pressure and cell degeneration. Charcot and Gomboult obtained same results and were of the same opinion. Foa and Salvioli tied the common duct in guinea-pigs, rabbits, cats, dogs, lambs and hens, and obtained cirrhotic changes in every case. They considered that the cell necrosis was due to increased pressure in the bile capillaries and that the increase of bile capillaries accompanies the connective tissue proliferation. Likewise Gerhardt in rabbits succeeded
in producing liver changes with both inter- and intra-lobular increase of connective tissue. The same experiments in dogs gave poor results. He believed that the necrosis was due to chemical action of the bile and that pressure was not of importance. He considered the connective tissue increase as secondary to the bile duct proliferation which he thinks can arise from pre-existing bile ducts or from transformed liver cells. Harley tried this experiment in dogs but with indifferent results. With the same operation in rabbits Jagic got the same changes. He believed that necrosis was entirely due to inflammation and that the connective tissue overgrowth was merely a process of repair and that it is called forth by cell destruction which destroys the walls of the blood capillaries especially in the periphery of the lobule, causing the cells to atrophy. DeJosselin de Jonge with these methods in dogs obtained necrosis and liver atrophy and considered that the connective tissue was only relative increased. Likewise Krause got good results in dogs but none in rabbits. Similarly Lahousse tied the common bile duct in guinepigs, rabbits and frogs. He obtained connective tissue increase together with bile duct proliferation and areas of necrosis in frogs, connective tissue increase and necrotic areas
in the rabbit and connective tissue increase only in the guinea pigs. Legg did the same experiment in the cat and got only interstitial increase. He considers the connective tissue increase to be merely an inflammatory process. Others that repeated this experiment were Tsunoda, Tischner, Simmonde, Popoff, and Pick. In rabbits they all found both inter and intralobular connective tissue proliferation with the necrotic areas in the parenchyma and the bile duct proliferation. In dogs Popoff and Tsunoda got only the typical necrotic areas while Pick got poor results. Pick considered that the necrosis was due to pressure, that the bile duct increase can come only from preexisting bile ducts and that they accompany the connective tissue overgrowth. Tischner who also experimented by tying the hepatic artery and with phosphorus administration believed that in the biliary cirrhosis the necrosis was due to anemia of the blood capillaries from bile duct pressure on them. He believed that new bile ducts could grow from preexisting ducts or else be formed from transformed liver cells. Several others tried the same experiment in various animals with various results, viz; Mayer in rabbits and cats got no areas of necrosis but did get in all cases
interstitial increase; Sterling in dogs got only the necrotic areas; Steinhaus in guinea pigs got very little connective tissue increase but got other changes. He believes that the bile duct proliferation accompanies the increase of connective tissue. Litten in guinea-pigs, got slight connective tissue increase but considered that it was all an inflammatory reaction; Kobner, Lowit, Halter and Lauterbacher in the frog got cirrhotic changes, and Szybinski and Leyden in dogs with poor results although the latter obtained cirrhotic changes in frogs.

Ziegler and Oblonsky administered chloroform to animals but obtained no results. However Aufrecht, Howland and Richards and Whipple and Sperry anesthetized animals for a long time and obtained necrosis and fatty degeneration of the liver. Afanassijew gave ethyl and amyl alcohol to dogs, rabbits and guinea pigs and obtained no evidence of cirrhosis, von Kahlden administered alcohol to various animals for a long time also without results. Friedenwald with the same experiments obtained cirrhosis in some animals and not in others. Afr echt, Kronig, Ackermann and Dinkler fed small quantities of arsenic in small doses to animals and obtained interstitial increase of connective tissue.
Ogata performed seventy-one experiments ligating the common bile duct in guinea-pigs, rats, rabbits, dogs, mice, pigeons and frogs. He found typical necrotic areas in guinea-pigs, rats, rabbits and pigeons; could find none in dogs and frogs, although his period of experimental time was long, and in mice there were no results, but might have been due to the fact that they lived but a short time. Histologically the necrotic areas appeared the same in all affected animals. The protoplasm of the cells was clear and contained many vacuoles which showed no fat even with special stains. The protoplasm appeared swollen. Later it seemed to be gathered around the cell membrane leaving a clear zone around the nucleus. Still later the nucleus loses its power of staining but no karyorrhexis could be distinguished. The necrotic cells soon lost their normal cohesion and were seen lying loosely side-by-side or fused in hyalin masses. In these areas of necrosis the blood capillaries could be distinctly seen filled with intact red blood corpuscles. In these areas the connective tissue appeared perfectly normal and the part they played in repair could not be made out even with special stains. Ogata confirms the report of Gerhardt and Joannovics who said that they found a giant-cell formation, phagocytic for the necrotic
cells, and fibroblastic infiltration with bile duct proliferation. He (Ogata) found that the cells of the necrotic areas seemed to be saturated with bile pigment. He believes in the chemical theory of Gerhardt but considers that the vacuoles that appear in the cells are somewhat of the nature of retention cysts. He thinks that the cells store the bile under increased pressure, and that not being able to secrete it these cysts are formed. The pressure on the blood capillaries he also believes prevents absorption by the blood. In such cases no marked jaundice appeared. His evidence of this is the large number of bile pigment granules that he found in these vacuoles. He further considers that the enlargement of the vacuole in the cells brings about in time cell death. He thinks that the connective tissue increase and the bile duct proliferation go together as evidenced by the fact that he found, always on the outside of the bile-duct buds, a row of spindle-shaped fibroblasts with delicate fibrillae. He considers that the new bile ducts can only come from preexisting ones and that their new growth and the new proliferation of the connective tissue is the same process - one of repair due to the destruction of the liver cells.

He carried on further experiments by inoculating dogs, rabbits, and rats, one group with chloroform, a second with chloroform and streptococcus and a third
with chloroform and B. coli. The method of injection was subcutaneous, intraperitoneal and intravenous. His animals all died early and showed parenchymatous degenerations but no cirrhosis.

Dantschakoff-Grigorewsky obtained well described cirrhosis of the liver by the subcutaneous injections of bouillon cultures of staphylococcus in rabbits and guinea-pigs. Herter and Williams with repeated inhalations of chloroform in dogs produced a cirrhotic condition of the liver that showed the degenerated liver cells infiltrated with proliferated fibroblasts.

Krawkow with intramuscular injections of B. pyocyaneous cultures in pigeons, obtained a well marked parenchymatous degeneration and interstitial connective tissue increase and a slight bile duct proliferation.

Sciagliosi obtained a beginning interstitial hepatitis from subcutaneous injections of B. subtilis and B. prodigiosus bouillon cultures in guinea pigs.

Oppel and Manwaring administered phosphorus to various animals and obtained changes in the liver, comparable to acute yellow atrophy in man, but no cirrhosis.

Straus and Blooq administered for long periods alcohol to animals and obtained an infiltration into the portal spaces of various cells. In a few animals
they obtained an increase of connective tissue at
the periphery of the lobule.

Wyssokowitch proved that the endothelium lining
the blood capillaries in the liver could take up bac-
teria injected into the circulation. Welch and Blach-
stein injected B. coli and B. typhosus into the
vein of a rabbit and discovered that it could be found
in the gall-bladder for weeks afterwards. Buxton
and Torrey proved that typhoid bacilli injected
into the peritoneal cavity were quickly taken up by
the blood and deposited in the various organs. They
found that these bacteria would almost entirely dis-
appear from the blood within an hour.

Bischoff in experimenting with alcohol on
animals, while working on experimental heart lesions,
obtained a fatty degeneration of the liver which pre-
ceded the changes in the heart which were also of a
fatty nature.

DISCUSSION OF FORMER EXPERIMENTS.

The question of cirrhosis of the liver as has
been seen by the foregoing has held the attention of
many workers. The problem has had five main distinctive
groups of workers; those who were working on the question
of its being primarily due to degeneration of the liver
parenchyma, those who were applying themselves to the
infection theory, those who believed that it was due to a damming back of the bile, those who believed in the autointoxication theory, and finally those who considered alcohol to be the exciting agent. Early in the research it became evident that the various types of cirrhosis had different causes, so from that time the workers limited themselves pretty much to experimenting with one particular type. It seems as if the infectious type, leaving out the syphilitic type for obvious reasons, had been pretty clearly proved by certain of the foregoing experiments. Also the type due to bile stasis has been proved. The one that is due to necrosis of the liver parenchyma is necessarily somewhat bound up with the others but it seems to me as if it had been shown clearly that liver degeneration, outside of these other causative agents, is certainly at times due to toxins. This is the type that Mallory considers the forerunner of toxic cirrhosis. Whether this toxic type is always due to bacterial toxins or, as Adami thinks, products of gastro-intestinal derangement, or of sub-infection as Opie, and others think, is still debatable. One fact stands out, however, above all others and that is that the injury to the liver cells of whatsoever nature must be a repeated damaging action rather than a sudden severe one. The autointoxication type has been
and certainly can be covered by what has been remarked about the toxic type. The alcoholic type is yet to be proved. The results from alcoholic experiments have varied so much that as yet no decision can be reached but it is safe to say that alcohol per se has not been proved experimentally or otherwise to be the exciting cause of the so-called alcoholic cirrhosis. The clinical evidence is in favor of convicting it, but other factors may yet enter in that will give an entirely new aspect to the whole question.

PERSONAL EXPERIMENTS.

These experiments that follow were undertaken with the purpose of doing two things, viz., to confirm or disprove former results as to the toxic, the infectious, and the biliary types of cirrhosis, and if possible to gain a method by which it might be shown that there is a direct connection between alcohol and hepatic cirrhosis.

EXPERIMENT NO. 1.

Rabbit inoculated by injection in posterior vein of ear, Oct. 23, 1912, with 1 c.c. of twenty-four hours stock culture of staphylococcus pyogenes aureus. Ear became inflamed and rabbit appeared quite sick for
three days. Recovered. November 7, reinoculated in same dose with same organism just obtained from pus that came from an abscess in the vicinity of a man's hip. Animal again became sick. Both times temperature was somewhat raised. Rabbit began to show signs of emaciation and weakness. November 14, developed severe dyspnoea, joints became stiff and animal not able to stand. On November 16, it was no longer able to move, so was killed with chloroform.

Autopsy:— showed small subcutaneous and peritoneal abscesses. Other abscesses were seen on heart, kidneys, and on superior surface of diaphragm. On skinning animal, abscesses were found pretty well throughout the subcutaneous tissue and musculature. There was a large amount of pus in the knee joint. Other joints were not opened. Bacteriological examination showed the presence of staphylococcus and cultures proved it to be the Staphylococcus pyogenes aureus. No bacteria could be recovered from the heart's blood but could from exudate in serous cavities. Sections of the muscle tissue showed typical abscesses; of the spleen showed hyperemia and leucocytic infiltration; of the meninges showed many plasma cells and lymphocytes. Sections of the liver showed considerable increase of fibroblasts in the portal spaces that seemed to be arising from the blood vessel sheaths and also from
the connective tissue surrounding the bile ducts; around the blood vessels particularly in the portal spaces there can be made out a slight infiltration of lymphocytes and endothelial leucocytes. These seem to be invading the parenchyma. The blood capillaries between the liver cells are very much dilated and can be observed filled with blood running between the chains of liver cells which seem atrophied and degenerated. In some places this is albuminuous, in others fatty and in some hydropic, and is scattered pretty well throughout the whole parenchyma. Some of the cells have entirely lost their power of staining and others appear more like empty spaces although it seems as if their cell wall was left intact. Mitotic figures are observed in the nuclei of some cells while other cells have two nuclei, particularly at the periphery of the lobule - showing that active regeneration is taking place. In the walls of the dilated blood capillaries there is some proliferation of the endothelium. The intralobular supporting connective tissue can be easily made out, and I am of the opinion that it is proliferating somewhat. Occasionally one can see, in the spaces formerly occupied by liver cells and now filled with a necrotic mass of debris, an occasional endothelial leucocyte that is acting as a phagocyte in removing this necrotic material. A few bile capillaries
can be made out distended with bile. There seems to be no signs of absorption of bile. The few vacuoles that are seen are apparently perfectly empty and resemble what is known as hydropic degeneration. There is in no part of the liver any evidence of suppuration or of abscess formation - in fact one can see no polymorphonuclear leucocytes at all except as can be normally observed in the blood vessels.

This seems to be a good example of beginning cirrhosis from absorption of toxins. In this case the pyemia was too severe for the animal to recover but had it been less severe it seems reasonable to believe that these liver changes would have advanced until finally we should have obtained the typical picture of atrophic cirrhosis.

EXPERIMENT NO. 2.

On October 24, 1912, a guinea-pig was inoculated with 1 c.c. of a twenty-four hour stock culture in bouillon of B. coli, by intraperitoneal injection. No symptoms developing this was repeated November 7, and again on November 25, using the same dosage. The guinea-pig was then carefully observed and no change was noted except an increase in weight and size perfectly compatible with normal growth. On March 11, 1913 the pig was again inoculated with .5 c.c. of same organism. No symptoms
of any nature were even observed. The animal was killed on April 8, 1913 with chloroform and autopsy showed no gross lesions except a liver pale in color. Histological examination shows a fatty degeneration throughout the whole substance of the liver tissue. This degeneration is so extensive that the normal liver markings are hard to make out. The condition microscopically is comparable to that seen in acute yellow atrophy in man. There is no increase of either bile ducts or connective tissue to be made out in any of the portal spaces but rarely one can see a slight proliferation of fibroblasts and endothelial cells about a central vein. Evidently there is perhaps a slight tendency towards repair in these few locations. No evidence of cell regeneration could be made out in the parenchyma as no mitosis could be found nor any cells with two nuclei. In fact most of the nuclei stained rather indifferently as one would expect with the severity of the cell destruction. Once in a while there is to be observed a proliferation of the endothelium of the blood capillaries and an occasional endothelial leucocyte that has wandered into the necrotic tissue.

How this animal would appear so healthy and vigorous is hard to explain when one thinks of this so almost complete loss of functioning liver substance.
However this can be taken as either an example of toxic degeneration, or perhaps of direct infection. The sub-infection or direct infection idea, seems to me, not applicable to this case, for if we should consider it in this light one would certainly expect to find more evidence of inflammation which would be manifested by an infiltration with lymphocytes or polymorphonuclear leucocytes with the addition of more connective tissue reaction. We have noticed a similar fatty condition in the livers of guinea-pigs that have succumbed to what we believe to be "guinea-pig epizootic". Other animals had been affected and it may be possible, though I think hardly probable that this pig was or had been infected with this disease and that the colon-infection acted as a restraining influence on the action of the infection in question.

The experiment certainly adds nothing to our knowledge of liver cirrhosis.

**EXPERIMENT NO. 3.**

A rabbit was anaesthetized for forty minutes on three successive days with chloroform and then killed in usual manner on the second day after the last anaesthesia. Grossly the liver was paler in color and somewhat mottled. On section was soft and friable. Histologically we find large focal areas of necrosis somewhat irregularly arranged.
and considerable fatty degeneration mostly manifest in
the periphery of the lobules.

EXPERIMENT NO. 4.

A rabbit was treated in exactly the same way as the
one in Experiment No. 3, and allowed to live for twenty-
eight days when he was killed in the usual way and autopsied. Grossly nothing was observed except perhaps the
liver markings were somewhat more prominent than normal. Histologically there is seen considerable proliferation
of the interlobular connective tissue and some of the
intralobular supporting tissue. In the portal spaces
the connective tissue is proliferating from the sheaths
of the blood vessels and from the connective tissue
about the bile ducts. Some of the connective tissue
nearest to both these sites appears as though it had
attained nearly to an adult type as it has a large
amount of dense fibrous intercellular tissue. However,
the size of the cellular elements and their bright
staining reaction clearly show that this is newly formed
tissue. The bile ducts in the portal spaces are larger
and more tortuous than normal and occasionally one can
see a proliferating bud. The intralobular connective
tissue proliferation seems to be both a new ingrowth
from that in the portal spaces and a direct proliferation
of the preexisting intracellular supporting frame-work.
The endothelium of the blood capillaries in the spaces between the liver cells is proliferating somewhat. The liver cells themselves for the most part are not necrotic though scattered throughout the lobule are seen cells that have no nucleus, that show evidence of vacuolization, and that show albuminous degeneration. Once in a while a group of three or four cells can be observed that stain more brightly with eosin and have a very heavy staining nucleus with hematoxylin. These appear to be newly formed cells. Many of the cells have granules of bile pigment scattered throughout their protoplasm. Most of the cells while otherwise normal have the lattice-like arrangement of their protoplasm that has been described by other experimenters. Although areas of necrosis are not common it is fair to presume that this appearance of the protoplasm is not normal and is a degenerative one. It seems as if this condition is one that is seen often in liver cells that have been more or less injured and it also seems to me, as if it was a condition that the cell is able to recover from and reach its former normality without loss of the cell or regeneration by cell division as is necessary when necrosis has taken place.

This liver shows a marked cirrhotic condition, although it is only a beginning one, and does not
agree with the work of others who are of the opinion that chloroform alone would not cause cirrhosis of the liver. However, if we agree with most authorities we will have to admit that cirrhosis is for the most part a change set up primarily by repeated injury to the liver cells. We find that these conditions have been fulfilled by this experiment. In this light it seems fair to presume, at least, that there is no reason why repeated injury to the liver cells from chloroform, or from any other agent acting in the same way, cannot cause cirrhosis and it is certainly evident that it has done so in this case.

EXPERIMENT NO. 5.

Another rabbit was treated in the same way as the ones in Nos. 3 and 4, except that while under the third anesthesia,.5 cc of a twenty-four hour bouillon stock-culture of B. coli was injected into the posterior ear vein. The animal was killed after a three weeks interval in the usual manner. Grossly the liver markings were quite prominent and the liver pulp seemed moister than normal. The surface has a slightly roughened or irregular contour- slightly hob-nailed. Histologically one finds a marked connective tissue proliferation in the portal spaces
which is beginning to infiltrate the periphery of the lobule. This proliferation seems to be coming much more rapidly from the sheaths of the portal vein branches than from the connective tissue around the bile ducts although it is coming to some extent from this location also. Some of the portal spaces show a condition comparable to scar tissue as the connective tissue has developed to an adult type - so much so that the intercellular fibres are firm and contracted down so that the nuclei are thin and elongated. In these particular areas there seems to be equal involvement about both veins and ducts. Here also the ducts show numerous bends and some tortuosity but the connective tissue has closed in so firmly about them that no headway is being made. In a few areas we find this cirrhotic change apparently involving only the portal veins and in such areas the bile ducts appear like objects that had been caught in the process and squeezed up. One would expect from this evidence of bile stasis but none is present nor did the animal have any jaundice. In still other areas the reverse is true that the ducts seem to be mostly involved and the vessels ingulfed. Throughout the whole parenchyma the supporting connective tissue is rapidly proliferating.
In both the inter and intralobular regions there is also more or less endothelial proliferation. The liver cells themselves appear quite granular and appear to be undergoing an albuminoid degeneration. The cells are vacuolated and have the lattice work arrangement of the protoplasm mentioned before. The nuclei mostly take the stain very nicely but once in a great while evidence of karyorhexis is seen. What evidence of necrosis is present seems to be more marked about the periphery. All the blood vessels are dilated as are the capillaries lying between the cells. The latter are very evident but in no place was there any evidence of rupture or hemorrhage.

This experiment seems to show that repeated injury to the liver cells when there is infection or subinfection added will produce very quickly the conditions favorable for the development of cirrhosis. I say sub-infection as the colon culture used was one long cultivated in stock and was very attenuated. The findings from this experiment agree fully with Opie's conclusions, viz., that subinfection with colon bacilli will bring about cirrhotic changes in the liver much more quickly than will injury of the same nature
without the secondary infection.

EXPERIMENT NO. 6.

A rabbit was subcutaneously injected with phosphorus dissolved in oil. Ten days afterwards the animal was killed in the usual way and autopsy showed a light yellow colored liver that was friable and greasy to the touch. Histologically no cirrhotic changes were found and merely an immense amount of fatty degeneration. The results of this experiment are of too little importance to be gone into with any amount of detail here. Possibly if the animal had been allowed to live longer repair would have taken place and the cirrhosis observed. In this connection I would say that this animal and the one in Nos. 1, 3 and 7 were used in the course of pathology for student experimental work, and were not experimented with directly as part of this research.

EXPERIMENT NO. 7.

A cat was operated on under aseptic conditions and the common bile duct ligated with a silk thread. Unfortunately the bandage that was placed around the cat's abdomen became caught on a wire and on the side of the cage and wounded the cat's side so that
infection was set up in the thigh. This reached such an extent that the hip joint was apparently involved and the animal got so weak and emaciated that it had to be killed at the end of four weeks so that the desired experimental period could not be obtained. At autopsy it was found that the infection was one with Staphylococcus pyogenes aureus, and involved the hip-joint and had extended up into the pelvis giving an abscess between the musculature and the peritoneum - one that extended pretty well up over the brim of the pelvis.

The gall-bladder was found enormously dilated and full of bile, and the liver very much bile stained both on the surface and on section. The cut surface was greenish and firmer than normal and the markings very distinct. Histologically the portal spaces are enormously enlarged from the extreme dilatation of the bile ducts, which are very tortuous. The blood-vessels of the portal spaces, the central veins and the intralobular capillaries are also dilated and filled with blood. There is quite an extensive interlobular proliferation of connective tissue coming for the most part from the connective tissue about the bile ducts. The tortuosity of the bile ducts prohibits the observation as to their proliferation.
The epithelium of the ducts and the bile capillaries is much more columnar than normal and can easily be observed even in the intralobular tissue in places. There is some proliferation of endothelium but not at all marked. The whole of the parenchyma shows albuminoid degeneration and an occasional area of necrosis. These areas are round in shape and seen to be mostly central. There are no signs of infection in any part of the liver.

This liver seems to show the customary reaction to biliary obstruction and I fail to see any evidence that the infection of the animal had any effect unless that manifested in the albuminous degeneration. The results otherwise agree with those obtained by Ogata and others with the same technique. It seems as if this experiment confirmed the results of those who claim that biliary cirrhosis can follow bile stasis without infection entering into the question. In this particular case had the infection any marked bearing we would certainly expect to find the connective tissue reaction much more active in the vicinity of the blood vessels and an infiltration with leucocytes or lymphocytes. If the toxins were to be considered we would
expect more evidence of necrosis.

EXPERIMENT NO. 8.

A rabbit was given per os daily except Sunday, before feeding time in the morning, fifteen c.c. of 34% alcohol. This was given with a medicine dropper and after a few administrations no difficulty was found in the rabbits swallowing it. By this method I endeavored to reproduce as closely as possible the conditions in the steady drinker that are supposed to predispose to cirrhosis of the liver, viz., a repeated ingestion of alcohol in the "whiskey proportion" on an empty stomach. This experiment was started November 4, 1912 and the animal killed April 9, 1913—covering a period of slightly over five months. Autopsy showed a liver small for an animal as large as this one (which at the beginning was a full-sized rabbit and which grew fatter and larger in the meantime never showing any signs of being sick). The liver markings were extremely prominent and the organ was lighter in color and firmer. There was some slight roughening of the surface. The heart showed the muscle walls lighter in color than normal, and also showed the endocardium lighter in color than normal and a sort of cluing together of the flaps of the aortic valve—Evidently
there was some aortic stenosis and insufficiency. Histologically sections showed quite a large increase in the interlobular connective tissue in which were many proliferating bile ducts, the buds and new branches of which stood out very distinctly. In several places it was evident that these were trying to penetrate the periphery of the lobules. The blood vessels were not particularly noticeable. The connective tissue seemed to be proliferating from the preexisting connective tissue in these portal and interlobular spaces. Some ingrowth into the lobule had taken place but was not particularly far advanced. More or less increase of the intralobular connective tissue was also observed. The cells of the liver parenchyma showed pretty much throughout a moderate degree of albuminoid degeneration. Occasionally the central part of a lobule and in one place a whole lobule had undergone a more severe change but not far enough to be called true necrosis. With the higher power of the microscope the protoplasm of the cells has the lattice work arrangement of threads as described previously. These threads where they crossed one another seemed to be sort of bunched up in a way similar to the nodal points in fibrin fibres. The protoplasm of the cells had a tendency to gather at the periphery of the cell
along the cell membrane and these threads intervened across the more central portion. The nuclei stained rather irregularly having somewhat of a similar thread-like appearance of their chromatin. Karyokinetic figures were rarely observed but a good many cells were seen with double nuclei. In some cells the nucleus was apparently lost yet the protoplasm had the characteristic appearance already noted. An occasional cell was vacuolated but nothing could be determined as to the contents of the same. Once in a while a cell or a group of cells would stain more brightly than the others. As some of these had double nuclei I took them to be regenerated or regenerating cells. Very rarely in the protoplasm of a cell could be made but a portion, really one of these nodal masses mentioned above, that took a brighter stain with eosin than the others. It seemed to me that this might possibly be a beginning of that hyaline skein-like degeneration that Mallory\textsuperscript{93} believes to be pathognomonic of alcoholic cirrhosis.

The intralobular blood capillaries were dilated and their walls could be fairly well made out. The endothelium of these as well as that of the lymph spaces in the portal regions is actively proliferating.
It seems as if this experiment gives at least suggestive evidence that alcohol, per se, has a deleterious effect on the liver and that as a result of this effect we get cirrhotic changes. It is true that no others have been able to get just this result but I believe that with this same technique others will get the same results. A long series of experiments are necessary before absolute conclusions can be drawn and such experimentation is to be undertaken. Hence the question is still open until the results of further research can be announced.

EXPERIMENT NO. 9.

Another rabbit was started Dec. 11, 1912 on alcohol, using the same methods as used in animal No. 8. On March 11, 1913, 15 cc of a twenty-four hour bouillon culture of attenuated B. coli was injected into the posterior ear vein. The rabbit appeared slightly indisposed for a day or two but showed nothing further. This animal was also killed on April 8, 1913 and autopsy showed a liver with fairly prominent markings and nothing else of note. Histologically there was noted merely an increase of interlobular connective tissue mostly about the bloodvessels and some slight increase of the intralobular about the central vein.
There was much more marked albuminoid degeneration to be seen in the liver cells and considerable necrosis at the periphery of the lobules. The connective tissue mentioned above seemed to be a much younger process than seen in animal No. 8.

The conclusions to be drawn from this is that the subinfection with B. coli had no influence whatsoever on the cirrhosis. I believe that with a longer period under alcohol that there would have been no marked difference in the two alcoholic animals as all the changes noticed were the same as in the former animal except they had not reached the same stage. From this it would seem that the proliferation of bile ducts must be a much later process than that of the connective tissue. This conclusion does not agree with that of others in the cirrhosis that they obtained from chemical or bacterial toxins or from bile stasis.

EXPERIMENT NO. 10.

This animal, a rabbit, died spontaneously and autopsy showed a large retroperitoneal abscess on left side which by bacteriological findings was proved to be due to the Staphylococcus pyogenes aureus. Histologically the blood vessels and
capillaries were very much distended, the parenchyma had undergone albuminous degeneration, the cells were more or less full of bile pigment, and the interlobular connective tissue was just beginning to proliferate. This seems to be merely a reaction to circulating toxins and in the nature of a slight toxic cirrhosis.

Animal No. 11. This rabbit suddenly died while being etherized for experimental work of a different sort. Histological examination shows that the liver cells have undergone a severe albuminoid and fatty degeneration, that the intracellular blood capillaries are distended and have ruptured in places. There is also a slight proliferation of the connective tissue in the portal spaces. The kidney showed a severe tubular and hemorrhagic nephritis. This animal evidently was suffering from some severe intoxication and the connective tissue had just begun to react to the stimulus of the cell injury.

Animal No. 12, a guinea-pig that died spontaneously. Histological examination shows a liver almost completely destroyed by fatty degeneration. At the periphery of the lobules there are small areas that show only a severe albuminoid degeneration; but nowhere can normal liver cells be found. The central
veins are dilated and filled with blood. The hepatic artery seems to have had its wall thickened and appears as though sclerosed. There is no evidence of repair.

This case is mentioned to show that repair is a process considerably later than that of the injury to the liver cells themselves and that when the injury is very severe we may not get any repair.

HISTOLOGICAL TECHNIQUE.

The organs from these various animals were hardened by Zenker's method and sectioned by the paraffin method. The sections were stained with hematoxylin and eosin, Van Gieson's connective tissue method, and Mallory's hematocytin molybdic acid method.
CONCLUSIONS.

In these experiments no attempt has been made to reproduce the types of cirrhosis due to syphilis and tuberculosis, the first for obvious reasons and the second has been too well worked out to need further experiment. An attempt has been made to reproduce the toxic, the infectious, and the alcoholic. It did not seem as if the pigment form needed any more confirmation. The results here given seem to confirm the results of others as to the toxic form. No results have been obtained that could be classified as infectious. These experiments were undertaken not to disprove former attempts but rather to become more familiar with technique and the pathological conditions. It is pleasing to have obtained results similar to more experienced research workers.

However the main desire in starting this piece of research was to try and obtain a method by which it would be shown that there is some direct connection between alcohol and the cirrhotic liver changes that have so long been known, and have so long been clinically considered as going hand in hand. It is my present belief that such technique has been established as can now be used for further experimentation and which it is hoped will lead to results that cannot be questioned. Therefore in this respect this article
is to be considered merely as a review of the literature of the subject and as a preliminary report of a research just begun. At this time we can safely say that cirrhosis of the liver can follow direct injury to the connective tissue by the exciting agents of syphilis and tuberculosis as well as by irritating pigment particles; also it can safely be assumed that any toxic or infectious agent that will bring about repeated injury to the liver cells will stimulate reparative processes. Whether alcohol can fulfill these conditions I believe will be answered in the affirmative, basing my answer on the results of these two experiments reported herein. Although the series is short and although several authors have reported that cirrhotic livers may be found in control animals, yet I believe that the similar results obtained in these two animals is at the very least to be regarded as throwing suspicion on alcohol and giving those that are opposed to this opinion the burden of proof. As to cirrhotic conditions sometimes found in the livers of control rabbits, they must be rare, as in about forty autopsied rabbits during the last year no such condition has been found except in the animals already mentioned where the condition was clearly explainable.
SUMMARY.

In the foregoing article it has been my endeavor to first orient myself and by so doing I have considered the various classifications of cirrhosis of the liver in accordance with various authorities and have accepted as the basis of my experimental work the classification of Mallory which divides cirrhosis into five types, viz:—the toxic, the infectious, the pigment, the syphilitic and the alcoholic. Then briefly an attempt has been made to give the most important historical facts concerning cirrhosis of the liver at the same time going quite thoroughly into the special non-experimental researches that have been made as to its etiology and pathology from Laennec down to the present time.

Following this aspect of the question the next consideration has been a very careful review of all the former experimental work on this subject as it has appeared in the literature. The perusal of this immense amount of experimental work that has been done by the many workers previously in this field shows that the greater part of the work has been done on dogs, rabbits, guinea-pigs, cats, monkeys, rats, and frogs, and that these experiments have been of what we may call three types, one the operative, the second, the injection, and the third the ingestion method. The first type of experiments
have had to do principally with biliary cirrhosis and regeneration - the reaction being caused by tying bile duct, blood vessels, or lymph vessels, or by injuries to the liver parenchyma. The second type has to do with the injection of various substances directly into the circulation or the liver parenchyma so as to bring about primary degenerations which could be followed by regeneration and reparative reactions. The third type has to do with the ingestion of substances by the stomach so as to get similar action as from the last type. Lately anesthesia with chloroform or inhalation of various vapors has been tried but this is an indirect method of the second type. These experiments have resulted in fair proof as to the etiology and histology of the obstructive, toxic and infectious types of cirrhosis and have shown that cirrhosis is more or less of a reparative process that follows primary injury of the liver cells.

A few experiments have been personally undertaken which have confirmed what has been previously claimed as to these types mentioned above. In addition an attempt has been made to obtain a proper technique by which we could get conditions present comparable to the conditions in man that are supposed to at least predispose
to alcoholic cirrhosis of the liver. By feeding 34% alcohol in small quantities to rabbits before feeding time it seems as if these conditions had been realized to some extent. The result of these experiments have given us evidence that these conditions will give some cirrhotic change in the liver. As the experimental time was short and as the series of animals was so small no absolute conclusions are attempted. Rather this is proposed merely as a preliminary report and as such would say that from the evidence at hand the opinion seems at least plausible that alcohol is a factor of direct etiological importance in the causation of the so-called "alcoholic cirrhosis of the liver". The bibliography that follows is, as far as can be found out, complete.
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EXPLANATION OF ILLUSTRATIONS.
PLATE I.

Fig. 1.

Portions of liver lobule from experiment No. 1.
Pyemic rabbit.

Drawn with Leitz, objective 3, ocular 2 (X70)

1- Central vein.
2- Interlobular connective tissue.
3- Chains of liver cells more or less degenerated.
4- Dilated blood capillaries.
5- Fibroblastic proliferation from blood vessel sheath.
6- Fibroblastic proliferation from bile duct sheath.
7- Endothelial proliferation.
8- Blood vessel filled with blood.
Fig. 1.
PLATE II.

Fig. 1.
Portion of liver from experiment No. 2. Guinea pig inoculated with Bacillus coli.

Drawn with Leitz, objective 3, ocular 2 (X70)

1- Central vein.
2- Area about central vein showing some evidence of necrosis.
3- Parenchyma showing diffuse fatty degeneration.
4- Proliferation of endothelial cells and fibroblasts about central vein.

Fig. 2.
Portion of periphery of central vein.

Drawn with Leitz, objective 1/12, ocular 4 (X1000)

1- Liver cells fatty degenerated.
2- Endothelium lining central vein
3- Proliferating endothelial cells.
4- Proliferating fibroblasts.
PLATE III.

From Experiment No. 4.

Fig. 1- Drawn with Leitz, objective 3, ocular 2.

1- Central vein.

2- Proliferating connective tissue in interlobular and portal spaces.

Fig. 2- Leitz objective 5, accular 4.

1- Central vein.

2- Intralobular connective tissue proliferating.

3- Connective tissue proliferating from blood vessel sheaths.

4- Proliferating endothelium.

5- Intercellular substance showing adult connective tissue.

6- Blood in vessel.

Fig. 3- Leitz, objective 7, occular 4. A group of cells from Fig. 2.

1- Necrotic cell with no nucleus.

2- Cell vacuolated with faintly staining nucleus.

3- Newly proliferated endothelial cell.

Fig. 4- Same magnification—another group of cells.

1- Necrotic cells.

2- Regenerating cells.

3- Cell with double nucleus.
Plate III.

fig. 1.
PLATE IV.

Experiment No. 5.

Fig. 1- Leitz objective 3, occular 2. Portal space and portion of adjacent lobule.

1- Adult connective tissue hyperplasia.
2- Connective tissue infiltration of liver lobule.
3- Bile duct capillary.
4- Blood vessels with surrounding proliferation.
4'- Bile duct with surrounding proliferation.
5- Intralobular connective tissue proliferation.
6- Dilated blood capillaries.
7- Parenchyma showing albuminoid degeneration.
   Few nuclei staining.
PLATE V.

Experiment No. 8.

Fig. 1- Leitz objective 5, ocular 2.
1- Central vein.
2- Interlobular connective tissue proliferating.
3- Portal spaces with connective tissue and bile duct proliferation.
4- Budding bile capillaries.

Fig. 2- Leitz objective 1/12, ocular 4. Portions of lobule about central vein.
1- Central vein.
2- Necrotic cell.
3- Endothelial cell.

Fig. 3- (same magnification) showing proliferation about a branch of portal vein.
1- Branch of vein.
2- Proliferating fibroblast.

Fig. 4, Leitz objective 1/12, ocular 2. Proliferation about bile capillary.
1- Bile capillary.
2- Fibroblasts proliferating.
3- Regenerating liver cells.
4- Liver cell already regenerated and about to divide.

Fig. 5, Leitz objective 1/12, ocular 4. A small clump of cells from the periphery of the lobule.
1- Blood capillary
2- Beginning hyaline degeneration.
3- Cell protoplasm with a skein-like appearance.
PLATE VI.

Experiment No. 9.

Fig. 1, Leitz, objective 3, ocular 2.

1- Central vein.

2- Interlobular proliferation of connective tissue.

3- Portal space with increase in connective tissue.
PLATE VII.

Experiment No. 11.

Fig. 1. (X about 50). Section showing central vein and lobule. The dilatation of capillaries and beginning interlobular connective tissue proliferation is shown.

Fig. 2- Leitz objective 7, occular 2. A portion of lobule about central vein.

1- Central vein.
2- Capillaries distended with blood.
3- Liver cells showing albuminous degeneration.