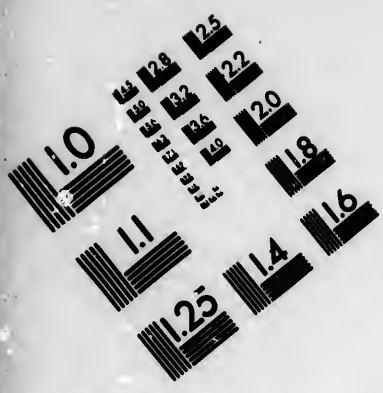

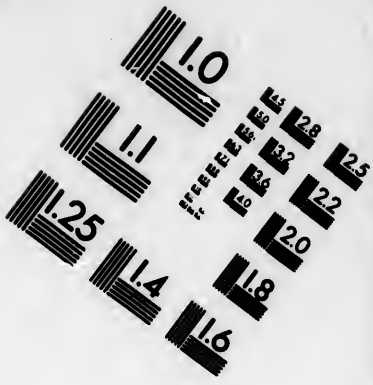

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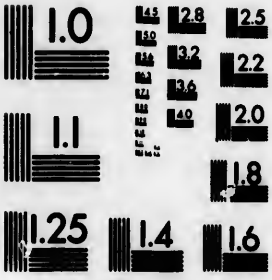

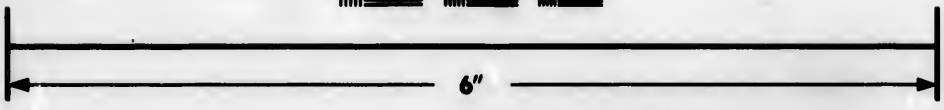

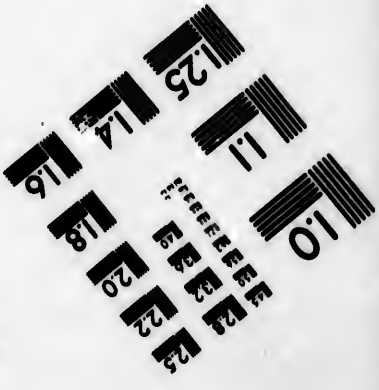

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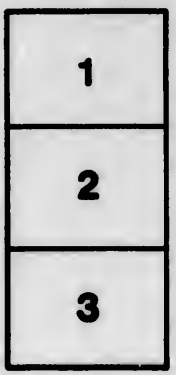

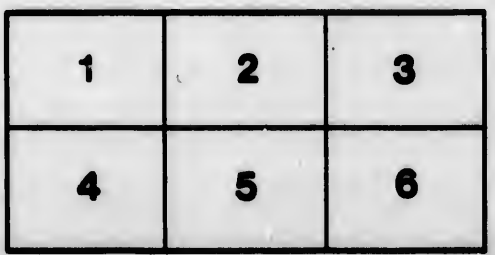

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ON THE

BACTERICIDAL FUNCTIONS OF THE LIVER

AND THE ETIOLOGY OF

PROGRESSIVE HEPATIC CIRRHOSIS.

BY J. G. ADAMI, M.A., M.D., F.R.S.E.,

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ON THE BACTERICIDAL FUNCTIONS OF THE LIVER AND THE ETIOLOGY OF PROGRESSIVE HEPATIC CIRRHOSIS.

BY

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Professor of Pathology, McGill University, Montreal.

Since writing the paper upon Progressive Hepatic Cirrhosis which Professor Osler was good enough to read on my behalf at the Edinburgh meeting of the Association I have been employed without intermission continuing the series of studies therein indicated. These further observations have so expanded and modified the statements already made that I am pleased that what as regards this JOURNAL was the premature publication of my paper elsewhere permits me now to take up the subject in these columns in the light of further researches and further experience.

To recapitulate briefly my Edinburgh paper. I therein pointed out that investigations conducted in Nova Scotia upon a curiously localised enzoötic known as the Pictou cattle disease showed me that in this disease (the most characteristic lesion of which was a peculiar extensive cirrhosis of the liver) there was to be gained from the liver, lymph glands, bile, and occasionally other organs and fluids of the body, a characteristic polymorphous bacillus, which in the earlier stages of its growth outside the body resembles a diplococcus (*vide* Fig. 1) in the later stages became more bacillary, and again when seen in the tissues was present as a diplococcus surrounded by a faint halo. These forms, I may say, are especially abundant in the liver and the glands, and in general a large proportion of them do not take the stain. These non-staining forms are to be recognised as well-defined shadows having a characteristic brownish colour. In the liver the microbes are present, stained and unstained within the liver cells and to a less extent in the newly formed connective tissue. (Fig. II.) The germ is pathogenic for rabbits, guinea-pigs, and mice; and in these animals while death occurs before cirrhosis has had time for adequate development, the microbes are abundant within the liver cells. (Fig. III.)

Struck by many resemblances between this disease and the ordinary cirrhosis in man, I have examined sections from a series of twenty cirrhotic livers, and in them have found constantly an almost identical form present also in the main within the liver cells. These show themselves as minute diplococcus-like bodies surrounded by a faint

halo; they are so small as best to be studied under the one-eighteenth or one-twentieth immersion lens, although they can be made out, yet with some difficulty, under the one-twelfth immersion (*vide* Fig. II.). The method of staining found by me to demonstrate them most surely was by carbolised fuchsin followed by bleaching in the bright sunlight for several days.

As an appendix to my paper, I described a case of cirrhosis which had within the last few days come to the *post-mortem* table at the Royal Victoria Hospital. From the liver and other organs of this case was gained a polymorphous bacillus appearing in early cultures as a diplococcus, and later assuming a bacillary or diplobacillary form (*vide* Fig. IV). I pointed out that while in certain respects it resembled the colon bacillus, I must provisionally assume that it was a distinct species. More especially was I led to this conclusisn from the fact that upon lactose and glucose broth there was no fermentation of sugar, while preparations made to demonstrate the presence of flagella showed one, or at most a pair, of these, appearing to be terminal and not lateral.

Such, in brief, were the main facts gained by me up to the middle of July. Since then the subject has widened greatly, and I can here give but an epitome of my later researches, the full details of which will be published elsewhere.

Further study of the form isolated from the case of human cirrhosis and of cultures from a second case (for the material of which I am indebted to my friends, Professor Wyatt Johnston and Dr. Anderson) have conclusively proved that this form must be regarded as at most a variety of the colon bacillus. The colonies upon agar plates and tubes made during the first days of growth were much smaller than those obtained ordinarily from the colon bacillus. A growth on fresh acid potato, while visible, was less abundant than that formed by the stock bacillus, and was of a very pale fawn colour rather than brown, and, as already stated, sugars were not fermented, and lateral flagella appeared to be absent.

But now, with frequent transfers upon slightly acid glycerinated agar, the colonies have become larger, and after passage through the rabbit they are scarce distinguishable from those of the ordinary colon bacillus. Glucose and lactose broths are now fermented with a development of the proper proportion of gas (one-half the closed limb of a Smith's fermentation tube), films made from young cultures in broth and glycerinated agar, show abundant lateral flagella. No distinction can be made out in the behaviour in milk, litmus milk, and on fresh acid potato.

As with the colon bacillus. mice seem to be relatively unaffected by intraperitoneal or subcutaneous injections of the organism in question; rabbits are affected. In short, so far as I have studied the two forms, the only well-marked remaining difference between them is, that while the colon bacillus causes broth reacting 1.5° acid to phenolpthalein [1] to become generally turbid in from 24 to 48 hours, with the development of relatively little sediment, the forms isolated from the liver, spleen, and kidney of our case of cirrhosis induce so little turbidity of the medium that at the end of 48 hours it is still possible to read print through the test tubes; again the sediment is relatively abundant. Two series of cultures alone, one from the heart blood, the other from the kidney of my case of cirrhosis, still retain some of their original characters; they show feeble growth, imperfect fermentation, and marked liability to present the diplococcus form; but they also with frequent transfer appear to be approximating towards the ordinary colon type.

These facts, with other considerations to be mentioned later, lead me to the present conclusion that the micro-organism in question must be regarded as one of the very numerous varieties of the colon bacillus. A study of Dr. Wyatt Johnston's case has shown me that, along with similar atypical forms, the typical colon bacillus can be obtained directly from the liver in cases of cirrhosis. Again, looking up my previous *post-mortem* records, and Professor Flexner's note upon the bacteriology of the cases of cirrhosis, material from which he courteously sent me, I find that both of us have frequently obtained the colon bacillus from cases of this disease. So also Professor Kanthack concluded that the cultures which I sent to him two years ago from two cases of cirrhosis were those of the colon bacillus, although when first isolated these had certainly been atypical.

It is very possible that under this term "colon bacillus" we include numerous forms which the imperfections of our methods prevent us from properly distinguishing. But balancing all the facts of the case, I cannot lay down that there are adequate grounds for separating this one form from the main group. The only safe conclusion that I can come to is that the cultures of the colon bacillus isolated from the liver of cases of cirrhosis within a few hours after death may be found markedly attenuated or modified, only gaining typical characters after repeated subculture outside the body.

Making a parallel series of inoculations into rabbits with the cirrhosis form and our stock laboratory culture of the colon bacillus, the results tallied very closely. I will here, however, only mention one—but that one all-important for the full comprehension of the subject.

If 0.5 c.cm. of a 48-hour broth culture of either form be inoculated into the marginal vein of the rabbit's ear, within twenty-four hours the liver cells are crowded with forms which are in the main diplococci. Occasional streaks of three dots or of four can be made out; but whereas the individuals in the 48-hour growth prior to inoculation were present in the main as stumpy bacilli and diplobacilli, now in the liver these were present in a diplococcus form (*Vide* Fig. V).

As to the exact method of the passage of these bacilli into the liver cells, I am not fully prepared to make a statement. Dr. Maude Abbott is at present making a series of studies upon the subject. I can only here point out that (1) it is an observation frequently repeated, that the endothelium of the hepatic capillaries possesses pronounced phagocytic properties; (2) that Chiari has recorded similar results following the intravenous inoculation of a closely allied form (the typhoid bacillus); and (3) that the remarkable appearances presented can be easily reproduced, cautious staining with carbol-thionin giving excellent results.

It is clear from this one series of observations that the colon bacilli injected into the blood stream find their way into the liver cells, and, what is more, they are present in these in greater numbers than in the spleen, kidneys, or other organs; but what is equally remarkable is that at the end of sixteen to twenty-four hours in rabbits so inoculated, while the liver is so crowded with the bacteria, if streak cultures be made from the various organs, abundant colonies may be obtained from the spleen, the heart blood, and also, but to a less extent, from the kidney; but taking a considerable amount of liver juice, this provides relatively few colonies. So far, in these early cases, the bile has been found by us to be sterile. It would seem clear, therefore, that the liver cells do not act as excretory agents for the bacilli, but have pronounced bactericidal functions. Save for Chiari's observations upon the similar destruction of the typhoid bacilli, this remarkable and important function of the liver cells has so far, I believe, escaped general recognition.

As I shall proceed to point out, when we consider that the colon bacillus is the commonest form within the intestinal canal, and is present there in enormous numbers, the full significance of these observations becomes evident. The bacilli or diplococci thus seen in the liver within twenty-four hours after inoculation, are clearly in the main, if not dead, at least incapable of prolifcrating outside the body. That they are in the process of degeneration is shown, I am inclined to think, by their form and by the fact that they easily give

up their stain, and again, it seems to me, by the fact that when decolorised they have a peculiar brown tinge.

That the colon bacillus should thus appear within the tissues as a coccus or a diplococcus form rather than a bacillus is somewhat difficult to realise. Certainly one's experience in the bacteriological examination of sections of the appendix removed for appendicitis—an experience which here in Montreal is extensive, and in which again the colon bacillus is the form most commonly to be recognised—had not prepared me to recognise that in these diplococci often surrounded by a faint halo I was dealing with the colon bacillus or some variety of the same. Nevertheless, my observations have made this absolutely certain, that not only in the liver, but in the lymphatic glands, and, indeed, in the kidney and spleen the colon bacillus is liable to assume the above form. I have now been able to recognise in the tissues a series of forms from the easily distinguishable and typical bacillus through the diplobacillus, formed of two stumpy members, to a stumpy bacillus, either alone or still as one member of a pair, in which the main body is unstained and so appears as a halo, while along the main axis are to be recognised two fine spherical bodies giving the appearance, as above mentioned, of a diplococcus. I begin now to understand the very frequent diplococci seen in the routine examination of sections of *post-mortem* material, when cultures have revealed not a single diplococcus lanceolatus or other coccus form.

What is more, I have been able to reproduce a like series of appearances outside the body. It is interesting to note that in the early stages of rapid growth in nutrient broth the bacillus is frequently represented by diplococci of fair size, and where the short stumpy bacterium form predominates, proper staining with fuchsin, and decolorisation, gives the appearance of more intense polar staining with a clearer central space. Again, in older growths the bacillary forms when stained to the proper extent, and when examined under a high power, appear to be composed of an obscure string of spherical bodies united by a common investing substance.

I find, however, that when the bacillus has been grown in broth or upon agar, close to the upper temperature limit of growth and under certain other conditions unfavourable to active proliferation, then these interior bodies, whatever be their nature, are most easily demonstrable, so that a long bacillary form is seen to be composed of an investing relatively colourless ground substance in which are disposed either a row of deeply-stained spherules; or again, each of such spherules, which at first appears to be somewhat oval, can be resolved into a pair of gonococcus-like bodies, the division between which is roughly at right angles to the long axis of the bacillus.

I will not here pretend to lay down what is the nature of these bodies; to call them nuclei or pronuclei would, in the present state of our knowledge, be if not absurd at least indefensible. Their arrangement is, however, very remarkable, and appears to be ultimately related to the vital functions of the bacillus. Here, again, abundant work is necessary before their full meaning is elicited.

Another series of observations begun by me is an examination of sections from some 200 portions of liver preserved from the necropsies at the Royal Victoria Hospital during the last three years. These I have selected from cases in which examination with ordinary stains has shown the absence of anything that could be spoken of as cirrhosis. So far I have already examined only 40 of these, but in this series of sections, to my surprise and, I must acknowledge, temporary confusion, I have found with scarce an exception indications of the presence of these shadows of the colon or allied bacilli. I stained these sections by carbolised fuchsin and bleached them in the sunlight and while I have found in them very rare deeply-staining diplococcus forms and still rarer typical colon-like bacilli, it is the minute brown shadows, namely, the unstaining diplococcus-like bodies tending to be surrounded by a halo that I have almost constantly come across. So common is the appearance, that in the three cases in which I have failed to recognise it I am inclined to ascribe my failure to insufficient study. In other words, not a little of the fine brown pigmentation recognisable in the liver cells apparently healthy (but not all) is an indication that colon—and presumably other—bacilli have been taken up by the liver cells and have there been destroyed. This statement I know will be doubted, but I make it with a full sense of responsibility. I have experimentally, and in the organs, more especially in cases of cirrhosis, observed the successive stages, and I have no longer any doubt about the absolute correctness of the statement.

From this it follows, we must assume (1) that the colon bacilli in small numbers, are, in the healthy individual, constantly finding their way into the finer branches of the portal circulation; and (2) that one of the functions of the liver is to arrest the further passage of these bacilli into the general circulation, and to destroy them through the agency of the specific cell of the organ.

I have not as yet been able to make a parallel series of examinations of the mesenteric glands; I can only point out that in cases of hobnailed liver the appearance of sections of these glands when properly stained by carbol-fuchsin or carbol-thionin is remarkable. (*Vide* Figs. VI. and VII.)

It is scarcely necessary for me to point out the steps by which the

bacilli pass from the lumen of the intestine into the tissues, and so into the lymph and blood capillaries. Observations of Heidenhain, Ruffer, and others have demonstrated with the greatest clearness that leucocytes are continually passing out on to the free surface of the intestinal mucosa, and that a large number of these, laden with fatty particles, bacteria, and other matters, find their way back into the submucous layer.

These observations at first sight would appear to wholly controvert the view that there is any necessary connection between the presence of more or less modified colon bacilli, or varieties of the same, in the liver and the development of ordinary progressive cirrhosis. It may be argued that inasmuch as such forms are constantly to be found in the liver, it is clear that the bacillus can have no power to induce excessive connective tissue formation, for otherwise every living being should suffer from cirrhosis. But there is this to be noticed: in the ordinary liver in which cirrhosis is absent, the forms visible are almost all corpses, and even long action of strong carbolised fuchsin will not lead them to become stained. In cirrhosis, on the other hand, while there are many of these non-staining forms, areas can be made out in which diplococcus-like bodies stain deeply. Either they have only recently entered the organ and are just killed, or they are still alive though in a form so attenuated, that it is only with difficulty that cultures can be gained from the organ. I still cannot but consider that the very great number of these forms found in well-marked advancing cases of cirrhosis is ample evidence that there is a direct connection between these and the process. So, also, in those advancing cases of cirrhosis my observations show me that the mesenteric glands are crowded with a diplococcus form of the bacillus, just as I found them crowded in cases of Pictou cattle disease.

In favour of this contention that there is a relationship between the presence of these diplococcus forms and the development of ordinary cirrhosis, the following considerations appear to be of weight:

1. The very great number of these forms found in the liver in well-marked progressive cases of ordinary hepatic cirrhosis.

2. The coincident great number of the same recognisable in the mesenteric lympathic glands, there being in this a close parallelism to what is seen in the Pictou cattle disease.

3. The parallelism in general between the bacteriology of these cases and that of Pictou cattle disease. Up to the present time my investigations upon the micro-organism of this disease show that, while very closely allied in form and characters to the colon bacillus, it is and remains a distinct species. Repeated subculture during the

last three years has not brought it nearer to type, if I may so express it. The agar colonies of the bacillus are much smaller than those of the ordinary colon bacillus; it does not ferment lactose or glucose, and does not yield the indol reaction, while upon acid potato the superficial growth is so slight as to be almost invisible, and I have found inoculations fatal to mice—animals not killed by the colon bacillus.

The constant discovery of this form in animals afflicted with the Pictou cattle disease renders it a most reasonable supposition that there is a direct relationship between its presence and the development of the disease. At the same time it is to be borne in mind that, so far, by simple inoculation, although rabbits, guinea-pigs, and mice are killed—and that on the average after relatively long periods (fifteen to thirty days in the two former animals)—I have been unable to reproduce the cirrhotic change in the livers of the same. It may be that the time elapsing between inoculation and death is too short to permit extensive fibrosis to develop, but it may well be that some additional factor is necessary to cause the cirrhosis in the cattle as in the inoculated animals, some prior or contemporaneous action upon the liver cells favouring the multiplication of the bacteria, or aiding the pathogenic action of their toxins upon the hepatic tissues.

Had further work upon human cirrhosis confirmed my first impression that the form isolated possessed individual features, and had further study revealed the repeated presence of such specific microbe in cases of the disease, it would also have been reasonable to assume cirrhosis to be due to the action of such a germ. But further study has shown that while one germ is to be detected in the livers of advancing cases of cirrhosis that germ is evidently the colon bacillus, more or less attenuated, it is true, but still a common form, and one which is habitually to be detected in livers presenting not a sign of cirrhotic change. If, therefore, there is any relationship between the presence of such a form and the development of the disease, clearly some other factor or factors must be at work. But beyond the point that there is this remarkable abundance of modified colon bacilli in the liver and in the mesenteric glands in cases of ordinary cirrhosis, I can at the present moment bring no further facts forward. To state positively what is the additional factor or factors requires experiments which necessitate months to bring to a definite issue. One may undoubtedly hazard a very shrewd guess as to what they must be. If the bacilli gain entry into the system through the intermediation of leucocytes, then a subacute enteritis or gastro-enteritis appears to afford the necessary localised determination of leucocytes, and such subacute or chronic gastro-enteritis is a very familiar feature in

the clinical history of cirrhosis in man. If some depression of the functions of the hepatic cells be requisite, then we know that alcohol the main predisposing cause of ordinary cirrhosis, has a direct action upon the hepatic parenchyma. Indeed Ramond[2] would seem already to have had a slight measure of success in the experimental production of hepatic cirrhosis, by giving to animals by the mouth alternating doses of alcohol and bacterial toxins over long periods. It is along somewhat similar lines that I am at present working, employing not toxins but cultures of the colon bacillus alternated with alcohol. The result will show whether this will give to us the solution of the cirrhosis problem.

I am the more inclined to be cautious, in that the conclusions reached in the appendix to my Edinburgh paper, although stated to be provisional, may naturally have raised expectations as to the specific nature of ordinary cirrhosis which this further contribution to the study must overthrow. It is so novel and unexpected a discovery that in the tissues the colon bacillus may be represented by a minute diplococcus-like body that I cannot but feel that for the statements contained in my Edinburgh paper no apology is needed.[3] The recognition of this diplococcus-like modification in itself modifies and expands the subject to such an extent as to open up a very wide field. For the present I shall be amply satisfied if I have adduced evidence favouring the view that after all what—in the absence of adequate classification—I prefer to call "progressive hepatic cirrhosis," is but one of the results of the entry of the colon bacillus and its products into the system.

In conclusion, let me state again that I do not for a moment assume that all extensive fibrosis or cirrhosis of the liver belongs to the same category. I fully admit that syphilis, tuberculosis, typhoid, and perchance some of the exanthemata, may be followed by extensive laying down of new connective tissue in the liver. But in none of these conditions, with the possible exception of syphilis, does the cirrhotic change assume the peculiarly progressive and extensive type seen in the hobnailed liver and the varieties of the same. And, as to Hanot's cirrhosis, never having studied a case possessing the classical symptomatology of this disease I cannot make any statement.

REFERENCES AND NOTE.

[1] Procedures recommended for the Study of Bacteria, *Journal of Amer. Public Health Association*, 1898, p. 75; reprint, p. 38.

[2] *La Presse Médicale*, April, 21st, 1897.

[3] Of my previous observations, but one, I believe, has not been confirmed in my further studies. I refer to the observation that in the microbes isolated from a case of cirrhosis the flagella were terminal. In the earlier specimens, made by the Nicolle-Morax modification of Loeffler's method, I looked most carefully for lateral flagella and found none.

The specimens utilized for my camera lucida drawings appeared so definite that I forwarded it to Edinbuagh, there to be demonstrated. Later specimens stained for flagella by the same method show most clearly the lateral arrangement and abundant flayella. Either the earlier attenuated forms were provided with few flagella, and those close to the ends, or the early preparations were defective. I am ignorant of any observations supporting the former view, and must, therefore, except the latter as the probable explanation. It is, however, interesting to note that the earlier observers upon the colon bacillus made a like mistake and accorded it to terminal flagella.

FIG. I.

Microbe of *Pictou Cattle Disease* from 24 hour old agar culture.
Stained Carbol Fuchsin.
Reichart $\frac{1}{18}$ immersion.
Zeiss camera lucida.

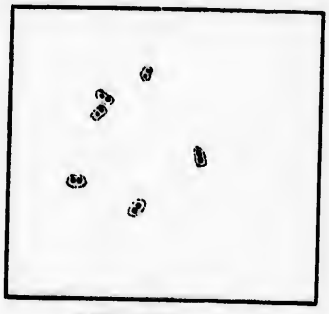

FIGURE IV.

Microbe isolated from case of Progressive Cirrhosis in man. Broth cultures from pipetta of heart blood 24 hours old. Stained Carbol fuchsin ; $\frac{1}{18}$ immersion and camera lucida.

Growth feeble and specimen over decolourised. so that diplococcus forms appear smaller than normal.

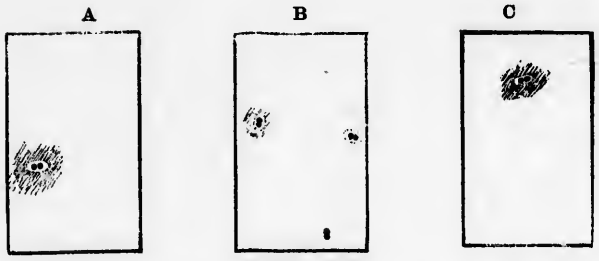

FIGURE VIII.

From slide preparations from agar (glycerinated) cultures made direct from pipettes taken at autopsy 52.98 on case of atrophic cirrhosis. Drawn with Zeiss's camera lucida. Reichert $\frac{1}{18}$th immersion. Stained carbol fuchsin.

A From Kidney juice pipettes, 24 hours growth.
B From Mesenteric Gland " " "
C From Ascitic Fluid " "

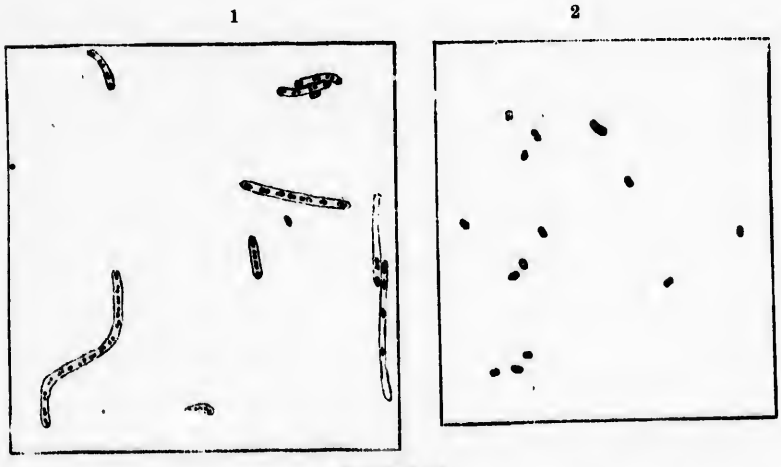

FIGURE IX;

Microbe isolated from case of Progressive Cirrhosis in man. To show extreme polymorphism.

1. From agar plate colony, plate made from isolated colony on glycerinated agar inoculated from spleen juice. Agar plate 5 days old. Shows long filaments composed of series of diplococci imbedded in sheaths.

2. From broth cultures 8 hours old, inoculated from colony on agar plate supplying the filamentous form.