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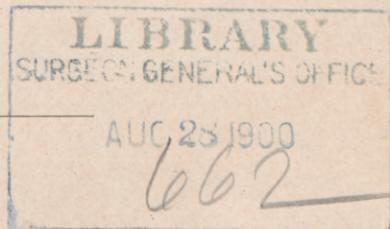
UPON THE BACTERIOLOGY

OF

PROGRESSIVE CIRRHOSIS OF THE LIVER.

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

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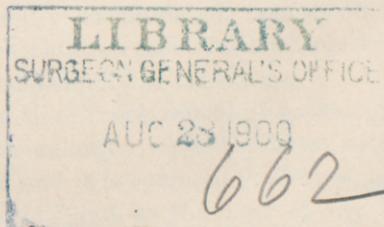


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It is needless for me here to enter into a discussion of the various theories which have been adduced to explain the development of cirrhosis of the liver. I need only say that the experience of a large number of observers, who for weeks and months have inoculated and treated animals with various alcohols has shown that alcohol itself induces at most the fatty liver with, it may be, a very slight amount of fibroid change in the portal areas, and that not a single observer has by this means been able to produce anything at all resembling the extreme deposit of fibrous tissue which we meet with in the hobnailed liver.

Alcohol in the main leads to the fatty liver, while on the other hand the evidence has steadily accumulated, notably in India, that extreme cirrhosis may attack children and adults who have not taken a particle of alcohol either medicinally or otherwise.

Within the last few years, the French school of pathologists headed by Hanot, has regarded the enlarged cirrhotic liver with jaundice as being of infectious origin, and it is common in France, now-a-days, to speak of "*le foie infectieux*," but to the best of my knowledge no one has as yet described any one micro-organism as being found frequently associated with these cases of so-called infectious liver. At

¹ A paper read for the author by Professor Osler, in the Section of Medicine, at the meeting of the British Medical Association at Edinburgh, July, 1898.

most, Levi¹ from a case of cirrhosis in a 17 year old male, with marked periportal fibrosis and enlarged spleen, obtained a diplococcus pathogenic for guinea pigs. The age of the patient, the proliferation of the bile ducts and the absence of ascites, would indicate that his case was one of what is termed "Hanot's Cirrhosis" but the bacteriology is rendered somewhat doubtful in that there were also present bacterial endocarditis of the pulmonary valve and suppurative meningitis. Unfortunately I have not been able to obtain the original paper, nevertheless, this brief statement of the main features of the case, makes it at least possible, as Paltauf has urged, that the endocarditis and meningitis and the presence of diplococci in all the organs, may have been due to a complication.

On the other hand there is a somewhat suggestive relationship between these cases of Hanot's cirrhosis and epidemic jaundice, in some cases of which observers have noticed the presence of a diplococcus or bacillus with polar staining.

To the best of my knowledge, no one has as yet recognised the frequent presence of any one form of micro-organism in the commonest form of hepatic cirrhosis—the so-called portal cirrhosis, tending to the production of the hobnailed liver, with or without marked atrophy of the liver.

On behalf of the Government of the Dominion, I spent the summers of 1894 and 1895 in Nova Scotia investigating a very remarkable disease affecting the cattle in a limited area of the country—the so-called "Pictou cattle disease"—of which the main lesion is a singularly extensive cirrhosis of the liver. The disease is only found in a district spreading along the northern coast of the peninsula, in a tract of country about 40 miles long by from 5 to 12 miles broad. There apparently it has been noticed for some 40 years, now at one end of this area now at another. The disease would seem to be very chronic and not to affect all the cattle on a farm simultaneously, but unless due precautions are taken, in the course of three or four years most of the animals upon a farm will, one after the other, be affected. It would seem further that the disease does not spread directly from animal to animal, for there appears to be no special incidence of cases following upon the long winter sojourn of the animals in the byres, which, with rare exceptions, are miserably dark and ill-ventilated, the attempt being to keep these as warm as possible in consequence of the severity of the winter.

One or two cases are on record in which the disease has broken out in a neighbourhood after the body of a cow affected with the disease

¹ Arch. Gen. de Med., March and April, 1894

has been washed down by one of the streams and stranded upon the farm lands. The gradual extension from farm to farm, through any given district, seems to be largely brought about by the fact that each farm has at the back of it a belt of woodland into which the cattle roam during the summer. The belts are badly fenced off from each other, and here, if an animal is affected, it attempts to wander off into the woodland and there die in some remote corner. Thus, unless precautions are taken, the carcasses of these animals remain uninterred and appear to act as centres of infection. Under the present Government regulations, notification is given of every suspicious animal, and the Government Inspector, if satisfied that it is a case of the disease, immediately destroys the beast and burns the carcass, or has it buried in quicklime. By this means the number of animals affected is rapidly being reduced, so that within the last few years the number of cases occurring annually has sunk from 150 to under 30.

As for the symptoms of the disease, the first symptom which is noticed is that the milk has a somewhat acrid odour upon boiling and acquires a peculiar bitter taste ; within a few days the animal becomes dry, it is weak and restless, the coat stares and the limbs are dragged, the bowels loose, the abdomen a little swollen, the eyes are staring, the conjunctivæ subicteroid. The animal becoming weaker and weaker dies apparently in a condition of complete exhaustion. In some few cases death is preceded by a period of intense excitement almost maniacal in character, the animal rushing about charging at obstacles and then falling into a condition of paresis followed rapidly by death.

I killed and made post-mortem examinations upon some 30 animals during two years and found, as Dr. Osler and Dr. Wyatt Johnston previously determined, that the main lesions are an extreme condition of generalised cirrhosis, not only periportal and pericellular, the organ being somewhat enlarged and having a smooth and rarely a finely granular surface. There is evidently an abundant production of thin bile, for with scarce an exception the gall-bladder was found very full and the fæces well stained. The periportal and abdominal lymph glands in general are large and succulent, there is a moderate amount of ascites, the fluid being perfectly clear and limpid, and together with this there is a rather remarkable condition of a gelatinous œdema of the mesenteries and intestinal walls. A further constant lesion is the presence of numerous follicular ulcers in the 4th or true stomach. These, save in the very acute cases, are found in a cicatrised condition, giving strongly the impression that the earliest

lesion in the case has been gastric and has been followed by infection of the abdominal lymphatic system and the portal area.

From all the animals which I killed, I was more fortunate than my predecessors in gaining a characteristic micro-organism. This may have been due to the fact that I employed a somewhat different method of gaining my cultures. Instead of taking the media and inoculating on the spot, all I employed was a series of sterilised glass pipettes in which I collected relatively large amounts of the juices of the various organs ; ascitic fluid, blood, etc., and then when back in my temporary laboratory, either upon that or the following day, I inoculated my media. By this means, constantly from the lymph juice of the abdominal glands and from the liver juice or bile and more rarely from other organs and fluids, I obtained in each case growths of a characteristic micro-organism ; small, polymorphous, at times appearing as a diplococcus and at others as a diplobacillus which by its polymorphous character gave me a considerable amount of trouble, until I found that employing the same broth tube, at the end of 24 hours I obtained the one form, at the end of 48, the other. Further study showed me that this micro-organism was in reality a short bacillus with polar staining, in this resembling to some extent the micro-organisms of hæmorrhagic septicæmia in the lower animals, but unlike them, possessing a slight capsule. I was able to grow this upon all the ordinary media of the laboratory. Into the character of this micro organism I will not here further enter, beyond stating that I found it pathogenic for rabbits, guinea pigs and mice, rabbits dying in from 15 to 35 days, guinea pigs in from 30 to 35 on the average.

The characteristic features of this disease—the ascites without jaundice, the gastric and intestinal disturbance and the condition of the liver—led me seriously to consider the points of similarity between the course and symptoms of these cases and those present in portal cirrhosis in man, and though it may seem a small matter, I was especially struck by the fact that the first post-mortem which I performed upon a case of atrophic cirrhosis upon my return from Nova Scotia in 1895, presented the same gelatinous œdema of the mesenteries and intestinal walls which was so prominent a feature in the Pictou cattle disease.

Thus on and off for the last three years my attention has been directed towards this possibility of discovering bacteria in ordinary progressive portal cirrhosis. Upon three occasions I have thought that I have gained specific micro-organisms. In two, unfortunately, the growth became contaminated with the colon bacillus, and as this occurred on the eve of my vacation I was unable to continue the

search further. In the third, which occurred a little over a month ago, the growth was very slight, and although pure, it had apparently died out on the fifth day and I was unable to gain any further cultures, if indeed what I saw was anything beyond the frequent presence of diplococci already existing in this liver juice at the time of extraction.

The difficulty that has pursued me in this search has been that which has prevented me from publishing so far any extensive report of my studies upon the Pictou cattle disease, namely, the extraordinary difficulty in staining the micro-organism in the tissues. I have tried a very great number of methods, and while with many I have been able to recognise the bacteria, the results obtained have been so inconstant that I have felt that others following me might very possibly have negative results; thus I have been unwilling to make any full statement until I should be able to state clearly how to be able to recognise the micro-organism. While this micro-organism stains deeply it appears to lose its stain even more rapidly than does the tissue. Sometimes Gram's or Weigert's method shows them perfectly, but while the iodine appears to have a deterrent effect upon the decolorisation of the microbes, the stain is not properly fixed by its means. And while again I have obtained good results by staining with methylene blue dissolved in anilin oil, a momentary passage of sections so stained, through a mixture of anilin oil and xylol and so through xylol into Canada balsam, yet even here the colour appears to fade out rapidly so that in a few days the micro-organisms are unrecognizable. Eventually the thought struck me that bleaching in the sunlight might be a possible means. By this process there would be no diffusion currents set up, and if, as my previous work had shown, the bacilli took up the stain with rapidity then the deeply stained sections would have so much of the dye in the bacilli that, upon bleaching out, the bacilli would be left stained when the tissue itself had become colourless.

My laboratory assistant, Mr. E. W. Hammond, prepared a large series of sections in this way and obtained some excellent results. He found that, as I had suggested, strong staining with carbolised fuchsin followed by bleaching for a short time each day for a period of a fortnight or more, demonstrated the bacteria admirably. While the process is a slow one it has the undeniable advantage that each day the mounted section can be examined to see how far the process of bleaching has progressed. By this means I was able to find out that in the Pictou cattle disease the micro-organisms, while present scattered through the new fibrous tissue, are present also in large numbers

within the liver cells, and in the liver of a rabbit which had been inoculated with the micro-organism isolated by me, although the animal died before any marked cirrhosis had developed, the liver cells were seen to contain these microbes in very great numbers. As to how the micro-organisms enter these cells, that is a point on which at present I can throw no light, but the appearances given, as will be seen in a specimen under the microscope in the Pathological Exhibit, is that these cells contain large numbers of extremely minute diplococci.

Recently, within the last month, a remarkable case of cirrhosis with pigmentation unaccompanied by diabetes has again drawn my attention to the bacteriology of atrophic cirrhosis. Dr. Maude Abbott, who is working in my laboratory at the Royal Victoria Hospital, showed me some sections of the abdominal lymphatic glands, stained by Weigert's fibrin stain in which, under high power, I noticed a peculiar fine granulation, and upon examining under the 18th inch immersion, these fine granules resolved themselves into minute diplococci.

Examining the liver stained the same way, I there noted large numbers of the same micro-organism, and since then I have gone through all my five cases of cirrhosis which I have had during the last three years; through specimens of four well marked cases of hobnailed liver received from Professor Hektoen, of the Rush Medical College, Chicago, and through a series of sixteen livers, some of well marked atrophic cirrhosis, others of milder stages of cirrhosis sent to me by Dr. Flexner from the Johns Hopkins Hospital. So far in every case of well marked portal cirrhosis whether of the small shrunken type or of the large hobnailed type, whether associated with jaundice without ascites or with ascites without jaundice, I came across constantly one characteristic form of micro-organism, obtaining the best and most permanent results by the method already mentioned, of staining in carbolised fuchsin and bleaching or partially bleaching in the sunlight.

This micro-organism is extraordinarily minute; by the trained eye, in well stained specimens, it can be recognised under the 12th inch immersion, but for satisfactory work it is absolutely necessary to employ the 18th or 20th inch immersion lens. According to the depth of the stain, so does it appear either as an ovoid bacillus, which might easily be mistaken for some stained deposit in the liver cells, or as a minute diplococcus surrounded by a halo, the explanation being that with strong staining the the bacillus and capsule are stained throughout, with weak, the body of the micro-organism and the capsule are decolorised, leaving simply the polar staining. Even in the tissues

this micro-organism is somewhat polymorphous, that is to say, that at times one sees the two stained portions so close together as to resemble a minute edition of the gonococcus, at other times they are further apart and appear rounded like the coccus form of pneumococcus, while in the same specimen a rare form may be seen in which the two stained portions are even slightly elongated. These are present in greatest numbers within the cells. A remarkable feature about them, and one which years ago I noted in connection with the Pictou cattle disease, is whether they be seen in the lymphatic glands or in the liver, in the unstained condition many have distinctly a brownish tinge, so that in the liver especially these bodies may at first sight be mistaken for minute granules of precipitated bile pigment. They vary in number; in two cases in which the disease appeared to be of very slow progress but few were present; in those cases in which the bands of portal connective tissue showed an abundance of small round cells and in which the disease according to all indications was active and progressing, the number was remarkable, in fact so numerous were they that it was impossible for me to arrive at any other conclusion than that there is a distinct association between the presence of this micro-organism and the development of the disease.

So minute are these micro-organisms that it is difficult to focus them, and I would strongly urge those attempting to confirm these observations to take, if necessary, from twenty minutes to an hour studying an individual field of the microscope before arriving at any definite conclusion.

Here, perhaps, I should correct myself. In the fully stained condition the micro-organisms while small are not so very much smaller than the ordinary run of pathogenic bacteria. But in this fully stained condition, as already stated, they are very difficult to distinguish from granules scattered through the cell protoplasm; it is in the partly decolorised condition in which the polar staining alone is recognisable and is peculiarly characteristic, that they appear so very minute. Indeed, I know of no form so minute, save that recently described by Nocard and Roux as being the causative agent in the contagious pleuro-pneumonia of cattle, and the strain upon the eyes in studying these microbes is most severe. This adds greatly to the difficulty of photographing the micro-organisms.

I am indebted to Dr. Patrick, the photographer of our hospital, for the accompanying lantern slides, in which despite great difficulties he has managed, I think successfully, to demonstrate the organisms in the tissues; but with regard to this I may say that where he shows

one micro-organism the slightest change of focus would bring others into view, so that his slides show but about 1-20th of the number recognisable in any given field.

As to whether the micro-organisms in the human and in the bovine liver are identical it is impossible for me to affirm. At Johns Hopkins, as in our own hospital and in a large number of foreign hospitals, not to mention the leading hospitals in the old country, it is now the custom to obtain cultures from half a dozen or more regions, including the liver, in every autopsy performed within fifteen hours after death. And, while the methods employed for obtaining cultures are in many cases faulty, to the extent that an insufficient amount of tissue juice is taken, it seems to me unlikely that had this form been cultivable upon the ordinary media, it would not before now have been isolated. Nevertheless, there is this to be said, that very frequently the colon bacillus grows upon cultures made from the liver; isolated colonies of the micro-organisms from the Pictou cattle disease, while growing more slowly, are not unlike those of the colon bacillus, and thus possibly the micro-organism if growing in a manner similar to that of the Pictou cattle disease, might be neglected on account of this similarity in appearance.

It so happens that here in Montreal cases of the disease are few and far between; five cases only have come under me in four years, and in a series of 934 autopsies made during twelve years at the Montreal General Hospital and indexed by Professor Wyatt Johnston, I find that altogether 31 cases of cirrhosis of the liver are recorded and 14 of the cirrhotic and fatty liver, with one recorded as "hypertrophic cirrhosis." I have thus thought it wise to publish these observations, even though I can at the present moment make no absolute statement with regard to the isolated micro-organism and its character, beyond the statement that, as already mentioned, in one agar tube made from the live juice, I was able to recognise the form, although in not very great numbers and with the absence of any visible growth.

Lastly, as to the cases in association with which I have found this micro-organism. If future observers confirm the observations given above, then we must conclude that many of the divisions and distinctions attempted to be drawn between the forms of advanced cirrhosis of the liver, must be broken down for, in the first place, I have found the same appearance in the large liver with moderate granulation or slight hobnailed condition, which some would speak of as "hypertrophic cirrhosis," as again in the small contracted typical hobnailed liver; in cases frankly multilobular with sharply defined bands of connective tissue cutting off relatively large areas of liver substance

and showing relatively few bile ducts ; as again in cases of the more unilobular type with ill-defined edges to the lobules and abundant bile ducts ; in cases which have shown jaundice without ascites, ascites without jaundice, and again both jaundice and ascites ; lastly, in cases with a pronounced alcoholic history and others in which no history of alcoholism could be obtained.

I am far from wishing it to be understood that I am here laying down that all cases of fibroid change in the liver present these minute micro-organisms ; to make any such suggestion would be absurd. Nor again am I prepared to say that Hanot's cirrhosis affecting the young adult and presenting the enlarged liver with smooth surface and accompanying this, crises of icterus, is identical with the more ordinary form of progressive cirrhosis affecting the adult. It may be so or it may not. All that I at present am prepared to lay down is this, that the progressive and extensive cirrhosis affecting the adult at or after forty years of age is, according to my investigations, accompanied by the presence in the liver cells, as again in the newly formed connective tissue, of an extraordinarily minute bacillus, having a polar staining and resembling greatly in its appearance under the microscope a form which I have isolated from cases of infective cirrhosis among cattle.

I cannot conclude without again expressing the debt under which I labour to several helpers. To Dr. Maude Abbott and Dr. Patrick ; to my Laboratory Assistant at the University, Mr. E. W. Hammond, who aided me very materially in Nova Scotia ; Mr. Howell, my assistant at the Royal Victoria Hospital, but for whose energy I should have been unable to prepare and examine all my material ; to Mr. Nicholson, who has most gladly crossed the Atlantic to demonstrate my specimens during the course of the meeting ; and above all, on this occasion am I indebted to a member of McGill University, my predecessor not only in the teaching of Pathology, but also in the investigations in the Pictou cattle disease, than none, not even myself could more willingly or more appropriately, or better, have brought this subject before you.

Appendix, (July 12th, 1898.)

By a remarkable coincidence, upon the afternoon of the day upon which I completed the dictation of the foregoing in order that I might have it complete for Dr. Osler to take with him to Edinburgh, I was called to perform a post-mortem upon a case apparently of heart failure which turned out to be one of atrophic cirrhosis of the liver. I should here add that a diagnosis of cirrhosis of the liver had been

considered and had been left in doubt ; while clearly, from the condition of the heart, death had resulted from failure of that organ.

Not to enter too fully into the details of the case, for the clinical notes of which I am indebted to Dr. James Stewart, I may say that the body was that of a female of 56, who had always lived in Canada, and who, after the diseases of childhood had, until two years previously, enjoyed good health. She had 10 children with no miscarriages, and there was no history of inherited disease. She gave a moderate history of alcoholism, stating that she chiefly drank beer, but if one may base any argument upon the frequent presence of minute whitish plaques which were found scattered along the œsophagus, she was a pronounced alcoholic.

For the last two years she had not been well, dating her impaired health from a fall while out walking, when she injured her back somewhat. For the last year her heart had been very weak and upon exercise her feet and legs became swollen. Since last April, the weakness, swelling of the legs and abdomen, shortness of breath and palpitation have been much worse, and for three days before admission, dyspnœa, sleeplessness and weakness had been extreme, while for months she had steadily been losing flesh.

Upon examination she was found sallow, with moderate anæmia of mucous membranes, the sclerotics were icteroid with distension of the superficial vessels ; the face was emaciated and there was orthopnœa ; the temperature was normal, the pulse rapid and the respirations were 36. The skin, more especially the face, neck and arms, was of a peculiar ashy colour ; this, she stated had been noticeable for some years ; there was slight general œdema, marked œdema of the lower extremities, and definite ascites. The pulse was 100, very irregular in volume and rhythm ; the apex beat was unrecognisable ; there were no murmurs. There was evidence of right-sided pleurisy and numerous coarse and fine râles with expectoration of frothy mucus. There was frequent vomiting and retching, though this had begun only a few days before admission to hospital. The urine was dark, amber coloured, with flocculent sediment, a fine ring of albumin and contained some bile. For a week or more her condition improved ; the heart became more powerful, the ascites diminished. Suddenly upon the 6th, the patient died.

The autopsy was held six hours after death, and showed the following conditions .

Heart.—Large, full, with dilatation of the cavities, the muscle being somewhat atrophied and fibroid. The coronary vessels were atheromatous. All the valves were normal and in both auricles were puri-

form and breaking down ball thrombi indicating a very feeble circulation.

Lungs.—Both showed adhesions to the diaphragm and elsewhere, and on section presented little beyond cedema, save that the lower lobe of the right lung was almost completely compressed and airless as a result of the right-sided pleurisy. The right pleural cavity contained about 700 ccm. of red stained fluid without flocculi.

Abdomen.—The abdomen contained between 900 and 1,000 ccm. of turbid and greenish ascitic fluid. Neither liver nor spleen were visible.

Stomach.—This was long and narrow with mucosa thickened, the cardiac portion having a curious strawberry-like appearance with fine white dots standing out, but not projecting from the generally reddened surface. This faded off in the pyloric portion of the stomach where there was a fair amount of mucus.

Duodenum.—The first three inches presented an identical strawberry-like appearance to that seen in the cardia.

Intestines.—The small intestines in general were congested and the last three inches before the ileo-cæcal valve showed large prominent solitary follicles. The walls of the small intestine had a distinctly brownish tinge as of von Recklinghausen's Hæmochromatosis. The mesentery of the small intestine was distinctly fatty and swollen, and upon section abundant milky lymph poured out wherever it was cut.

The abdominal lymph glands were in general reddened and succulent. The retroperitoneal glands, especially those in the neighbourhood of the portal fissure and of the pancreas, were markedly enlarged.

Liver.—The liver was distinctly small, weighing 1045 grams or a little over two pounds; the two lobes were correspondingly diminished in size, the organ was pale and had a finely granular surface; there were abundant old fine veil-like adhesions over the upper surface to the diaphragm. On section the organ cut fairly firmly more especially along the lower half of the right lobe and the under surface. On the whole the appearance on section was more fatty than fibroid. Microscopically, the organ presented along with fibroid thickening of the portal sheaths, a somewhat diffuse cirrhosis, the bands of fibrous tissue not being sharply cut and being infiltrated with a considerable number of small round cells. The cirrhosis was very obvious but not of the more usual type and the diffuse nature of the change may explain why the surface was finely granular rather than distinctly hobnailed.

Gall Bladder.—This had cedematous thickened walls.

Spleen.—Of normal size and rather soft, although on section the trabeculæ were seen larger and more prominent than usual.

Pancreas.—Voluminous and moderately firm.

Suprarenals.—Of fair size.

Kidneys.—These were the hog-backed type of mixed interstitial and parenchymatous nephritis. This so-called 'hog-backed' appearance, is that which, as I believe, Formad of Philadelphia, was the first to point out, is the more common form of alcoholic kidney in North America. The organs were large with a finely granular surface and full and firm cortex. There were white infarcts both in the kidney and the spleen.

The body was still warm at the time of autopsy and I obtained numerous sterilised pipettes of tissue juice from the liver, spleen, kidneys, abdominal lymph glands, ascitic fluid, pleural fluid, pericardial fluid, blood, and lymph from the mesentery, and with these made a series of inoculations on the surface of sloping glycerinated agar tubes, as also some into broth.

Portions of the collected ascitic and pleural fluid were centrifuged, the cellular debris dissolved by caustic potash and after further centrifugation the deposit examined under the microscope after staining with carbolic fuchsin. This deposit showed rather rare minute diplococcus forms with a slight halo round them, similar in all respects to those recognised in the tissues.

A series of slide preparations were made from the various pipettes direct, fixed in the usual method and stained with carbolic fuchsin, which was warmed until definite vapour was given off, then washed with water just cool enough to bear the hand in it, and mounted. As a result the characteristic diplococcus form was found in the preparations made from the liver, lymph from the mesentery, the ascitic fluid, the heart blood, the left kidney and the mesenteric glands; they were not found in the pleural cavity, the bile or the pericardial fluid.

Next, using a fair amount of the fluid out of each pipette, I allowed this to flow over the surface of slanting glycerinated agar tubes, my experience with the Pictou cattle disease having shown me, that while the fluid in the pipettes could show numerous diplococci, apparently the majority of those are dead, and only a few remain alive. By this means, in 24 hours I obtained fairly frequent growths in tubes from the spleen and left kidney, and rare growths in the mesenteric glands and heart blood. Four colonies only appeared upon the tube prepared from the liver, and two from the ascitic fluid and these latter did not show clearly till the expiration of 48 hours.

Subcultures made again directly upon the glycerinated agar surface,

showed a fine rather thin growth at first of minute discrete colonies; later these ran together into thin waxy lines which upon the expiration of four or five days assumed a faint yellowish tinge. Other tubes were made directly from the pipettes of the kidney and spleen into beef broth. This upon the following day showed a faint turbidity, which, just as in the case of the Pictou cattle disease, if anything, lessened with further growth, while a whitish deposit formed at the bottom. Since then, using the remaining pipettes, I have obtained a great number of broth cultures, the growth being much more free upon this medium than upon the agar, and further, yielding forms which are more characteristic and less liable to cause confusion; for the micro-organism is most remarkable in its characters.

Grown upon broth, in 24 hours it is present in the form of minute diplococci surrounded with a faint halo or capsule. Often these tend to be arranged in irregular chains in which the separate appearance of the dots are not quite regularly arranged, the long axis joining the two dots not of necessity coinciding with the long axis of the chain.

Grown upon glycerinated agar, the appearance is most puzzling, and although I had similar experience when working out the character of the micro-organism of the Pictou cattle disease, these agar cultures have given me a week of profound anxiety, until within the last 24 hours I have solved the problem. A 24 hour culture at 37° upon glycerinated agar, reveals minute forms which upon careful staining with fuchsin, not too deep, are clearly forms of diplococci. One gets every transition from the frank diplococcus form through one in which only very careful focussing shows that the somewhat oval bacterium has at either pole a deeper stained mass, to forms in which the polar staining cannot be made out so that one appears to deal with true short bacilli. Add to this, a certain number of oval forms can be seen still smaller than the diplococcus, in which the distinction between the two ends cannot be made out.

In 48 hours, and still more in 72 hours, the same culture which had given this appearance at the end of 24 hours appears to be contaminated by the presence of long distinct bacilli; that is, if sections be well stained with fuchsin, while this is still more the case after staining with Loeffler's methylene blue. At first sight, a culture from this variety showing these bacilli of irregular length with rounded ends, often lying side by side, appears to be undoubtedly of the colon bacillus or some allied form. And here I gain an explanation of the contrary results obtained by a bacteriological *confrère* and myself in London two years ago. I took to him cultures isolated from two cases of cirrhosis which, on examination in Montreal, seemed to be

diplococci, and his statement that he found only colon bacilli made me cease my investigations for the time. I can now well understand his most pardonable mistake.

Yesterday upon examining the agar plate culture from the spleen which had been made upon the 7th inst, and had been left for 24 hours in the incubator and there apparently yielded no growths and which thus had remained for four days at the ordinary temperature in the shade, I recognised one form of growth alone present, extremely minute, the colonies well separated from each other.

Upon removing one colony and making a coverslip preparation stained with fuchsin, I found that I had to deal with long chain-like bacilli interspersed with some shorter forms of the same breadth. The appearance was so wholly unlike anything that I had previously made out in the broth cultures, that I neglected this colony and made a culture from another identical in appearance and equally isolated, and in this case after staining with fuchsin, and as I thought over-staining, I washed in absolute alcohol. The result obtained was most remarkable. The long bacillary forms could still be recognized in this as in the other specimen and if anything they were longer, but each long filament showed a discoloured sheath in which, scattered at perfectly regular intervals, were pairs of dots deeply stained. In some places individual dots could be clearly recognised elongated and replacing the pairs. These pairs of dots in size resembled the diplococcus-like forms seen in the broth cultures and in the tissues to which I have already so frequently drawn attention. I regret that time has forbidden that I should obtain photographs of this very curious appearance so that they could be demonstrated with the lantern. In the series of preparations which will be found in the Pathological Museum, this form will be placed under the microscope and there will also be a series of sketches made by me under Zeiss's Camera Lucida showing this appearance.

I touched the same colony with a platinum needle and inoculated a broth tube from it and eight hours later, the broth, which had in the meantime been kept at 37°, showed a faint turbidity, and now some specimens treated with carbolised fuchsin in the same way, showed in place of the singularly long filaments, numerous diplococcus forms, fairly large, in which the connecting more colourless portion could be well seen, while here and there a rarer form showed three instead of two dots along the course of the bacillus. These observations satisfactorily explain the curious condition of affairs.

The microbe which is seen in the tissues as a diplococcus surrounded by a faint halo, is in broth after 24 hours present also as a diplococcus

but rather larger, showing or not showing the halo according to the extent of the stain.

Upon agar agar, while first present as a diplococcus form, it gradually extends and each day is seen as a longer bacillary form, but if stained and decolourised with carbolised fuchsin and decolourised to the right extent, each long filament is seen to be made up of diplococcus-like members lying in the common sheath. I made out a similar condition of affairs in connection with the Pictou cattle disease, but do not remember to have come across such long filamentous forms.

We seem thus to be dealing with a form totally unlike any which to the best of my knowledge, has been described; the polymorphism is remarkable. It is most difficult, however, to determine how to describe the appearances seen, and I am at a loss whether to state that we are dealing with an encapsuled diplococcus or with a bacillus having inclusions taking a peculiar deep staining, just as in the ordinary cell, the nucleus stains deeper than the surrounding protoplasm. The general appearance in the tissue is certainly that of an encapsuled diplococcus, but on the other hand, grown outside the body and upon agar and then treated with Loeffler's methylene blue, the whole of that portion which plays the part of a capsule to the diplococci takes on a stain with as great intensity as do ordinary bacteria. On the whole at present, I am inclined to the latter view, because examining tubes in which proliferation is most rapidly proceeding, I find upon staining with carbol fuchsin and decolourising with alcohol, that one has in the youngest ovoid forms what is most suggestive of the polar staining such as one sees in the bacteria of hæmorrhagic septicæmia, that is to say, there is at either pole not a complete coccus form, but a generally deep staining concavo-convex segment, the two parts being separated by a clear space and the membrane joining the ends of the opposite crescents being clearly visible.

If this form coincides in other respects with the micro-organism of Pictou cattle disease, it will grow rather more easily upon slightly acid media, it will grow upon serum and very slowly in gelatine without marked liquifaction, and will be fatal for animals of the laboratory at a relatively long period after inoculation.

It so closely resembles the micro-organism of the Pictou cattle disease that I feel that I may safely prophesy this, for the time taken in unravelling the mutability of growth upon agar agar has prevented me from working out these points till the last few days.

The great similarity in appearance presented by growths upon agar agar under ordinary staining to the colon bacillus may perhaps make

it necessary to say a few words about the relationship of the micro-organism isolated by me, to the bacillus in question.

I have made growths side by side, and find that in broth the colon causes a greater turbidity and appears to grow more freely upon agar agar and also to be endowed with greater motility. While upon staining an 18 hour broth culture of the micro-organism by the Nicolle Morax method, in order to demonstrate flagella, I found that the micro-organism, which are even stumper than the colon bacillus, under similar circumstances to be possessed of terminal flagella, either one or two, and not of lateral. This, if it were necessary, would seem distinctly to prove that the micro-organism is wholly distinct from the colon group. However, I make this statement provisionally, and will give fuller details as to the characters of the micro-organism within the next few months, probably in the *Journal of Experimental Medicine*.¹

I trust, however, that I have said sufficient to prove: 1st, That in at least a very large number of well-marked cases of progressive cirrhosis in man, there is to be found largely within the liver cells, also in the lymph spaces in the newly formed connective tissue, a peculiar and very minute form of micro-organism, present on staining to the proper extent, as a diplococcus surrounded by a faint halo, or when stained deeply, being a rather obscure bacterium, which may easily be mistaken for stained deposits within the cells.

2nd. That in the infective cirrhosis of cattle, a very similar micro-organism is recognisable, present in like positions within the tissues and showing similar appearances when stained.

3rd. That from at least 30 cattle affected with this disease I have been able to isolate the micro-organism—from the liver, bile, abdominal lymph glands, and in some cases from the various organs of the body.

4th That the micro-organism isolated is a polymorphous micro-organism, appearing as a small diplococcus when grown in broth, tending to assume a distinctly bacillary form when grown for a few hours on other media, or in broth for a longer period.

5th. That this micro-organism is pathogenic for the animals of the laboratory, and that in them it is to be recognised within the hepatic cells as in other regions.

¹ August 20th.—Fuller studies have shown me that these statements need amending. While the bacilli at first caused no fermentation of glucose and lactose broths, later growths gave definite gas production, though not so extensive as the atypical colon bacillus. The broth growths also remain atypical, but undoubtedly the bacilli when growing freely have, like the colon bacillus, lateral flagella. The germ belongs to the colon group. Fuller details of its characters will be given in a later communication.

6th. That from a case of distinct atrophic cirrhosis in the human being, I have been able to isolate from various organs of the body a similar micro-organism, which grown in broth has a diplococcus form, grown upon agar, is present as a short or longer bacillus according to the age of growth.

This is not the occasion for me to discuss at length the bearing of these observations upon the nature of progressive cirrhosis in man. It is only necessary for me to say that if they are confirmed, as personally I feel they must be confirmed by everyone who proceeds with sufficient caution to follow the methods employed by me, then cirrhosis of the liver assumes an entirely new aspect. We gain a satisfactory explanation at once of such phenomena as the enlargement of the spleen, which, as has already been noted by more than one observer, may be made out before there is any sign of portal obstruction ; we see why so frequently there should be right-sided pleurisy, and may even find that the question as to whether a case is complicated with ascites or jaundice, depends upon this micro-organism ; depends upon whether it sets up a low inflammation of the peritoneum, or whether it more especially affects the liver cells and bile ducts ; while disturbances which may occur not immediately in connection with the liver, in the pancreas and in the kidney, would seem to gain a possible explanation from the fact brought out by me, that this micro-organism, common in the liver cells, is in an advanced case to be gained from the heart blood and from the kidney.

That the micro-organism only causes cirrhosis, I do not believe ; indeed, we may find that it is the cause of more than one disturbance in the liver, and indeed in other organs. This I base upon the fact that in the case in which I have isolated this allied form from man, the micro-organism shows itself capable of existing in several regions of the body ; in fact of setting up what bacteriologically we regard as a septicæmic condition.

The illustrations referred to together with a further note on the subject, will appear in the next number of the JOURNAL,

