A STUDY OF THE BILE OBTAINED BY NON-SURGICAL BILIARY DRAINAGE, WITH ESPECIAL REFERENCE TO THE BACTERIOLOGY.

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It is not our intention in presenting this paper to attempt a discussion of the composition, chemistry and exertion of the bile. It is our purpose rather to try to point out the information of practical clinical importance that may be obtained from a study of the bile collected by means of duodenal drainage. Until recently such procedure would have been out of the question, for up to 1919, practically all studies upon the composition and bacteriology of the bile were made entirely upon bile obtained from the gall bladder at operations or at autopsy, or through post-operative biliary fistulae.

In 1919, B. B. Vincent Lyon (1) published a paper in which he showed how fresh bile may be obtained through duodenal drainage, basing this work of practical value upon a suggestion made by Meltzer (2) two years before in an article entitled "Disturbances of the Law of Contrary Innervation, as a Pathogenetic Factor in the Diseases of the Bile Ducts and Gall Bladder."

It will be recalled that in this communication Meltzer suggested the possibility of causing by reason of "crossed innervation" the paradoxical situation of relaxation of the sphimcter of the common duct and at the same time stimulation of the gall bladder and bile ducts to contract by the local application to the duodenum, by means of the duodenal tube, of a 25 per cent solution of magnesium sulphate. In making this suggestion Meltzer but elaborated upon the well proved physiological observation made over twenty years before by Dayon and Oddi. To Lyon, however, belongs the credit of first making practical use of these physiological and pharmacological facts. He showed that when through a duodenal tube hyperisotonic solutions of magnesium sulphate are brought in contact with the duodenal mucous membrane and are withdrawn before absorption

takes place, fresh bile may be obtained from the gall bladder and bile ducts, and further that this bile may be separated into three definite fraction, i.e., A, bile which comes from the common duct; B, bile which is of a different character and is derived from the gall bladder, and C, bile which is obtained from the hepatic ducts and bile capillaries and canniculae.

Abundant confirmation of his earlier work has been obtained by Lyon with an enormous series of cases, and has been further confirmed by a large number of other observers. Thus there has been established a comparatively simple technique by which with reasonable certainty, fresh bile from various portions of the bilary tract may be collected through the duodenal tube. It is not too much to say that the method has become firmly established, and must be looked upon as a well recognized and necessary additional method of gastri-intestional examination.

In passing it is perhaps fair to point out that the hypothesis advanced by Meltzer, and its further application by Lyon, has not been accorded uniform approval. There are those who question the validity of applying the law of crossed innervation, to the gall bladder and the muscle of Oddi. There are others who question whether the sphincter action of the muscle of Oddi is definitely established. Again specific action of magnesium sulphate in bringing about this relaxation and stimulation through intraduodenal excitation. Much stress is laid upon the fact that Meltzer's hypothesis is purely theoretical and has never been proven. The careful experimental work of Doyon and Oddi in 1894-1895 seems to refute this latter contention. A thorough discussion of these mooted points would here be out of place, but it is our belief based upon personal observation and experience with the method, in conjunction with careful clinical and experimental evidence, that the principle enunciated by Meltzer and put to practical use by Lyon, is in the main entirely reliable. Admirable clinical discussion of the entire question has been furnished by Smithies and Karshner, and Oleson ([3]).

During the past year transduodenal lavage and stimulation of the flow of bile by the introduction of hyperisotonic magnesium sulphate solution, has become almost a routine practice in our Gastro-intestinal Clinic at the Graduate School of the University of

Pennsylvania. In the course of this work we have met with the difficulties and disappointments from time to time, that have been common to all workers in this field. On the whole, however, our results have been encouraging and rarely has serious difficulty been experienced in introducing a duodenal tube into the duodenum. As a rule not over an hour and a half has been required to get the tube in place. We have not had the difficulties claimed by some, in determining the position of the duodenal tube. Without resorting to fluoroscopic examination we believe that the presence of the tube in the duodenum can be readily determined by the presence of the duodenal tug, i.e., when the tube is in the duodenum the plunger of the syringe attached to the free end of the tube will return to its position when traction is made upon it. Such is not the case when the tip is still in the stomach. Second, the appearance of pure yellow bile on aspiration. Third, failure to obtain water in the syringe after half a glass of water has been taken by mouth and fourth, when the tube is in the duodenum, 30 c. c. of warm water and allowed to run in and are then syphoned off by gravity, the water will return definitely bile stained.

On the whole, we have experienced but little difficulty in recognizing the free bile fraction referred to by Lyon, namely, the A, B, and C bile. Doubt has been entertained by some as to whether the dark bile alleged to come from the gall bladder is actually derived from this source. In this connection observations which are being carried out by one of us in conjunction with Dr. Eimann of the Bacteriological Laboratories of the Presbyterian Hospital of Philadelphia, and which await later publication, are suggestive. Estimation of the cholestrin content of the various specimens of bile conclusively shows that in the dark gall bladder bile, the amount of cholesterin is distinctly greater than that obtained from either the common duct of hepatic duct specimens. This increased cholesterin content results upon the concentration and stagnation of the bile, which takes place in the gall bladder. The remarkable ability of the gall bladder to concentrate the bile, and the restriction of this concentrating ability to the gall bladder, has been admirably shown by the work of Rous and McMaster (4). These authors have shown fluid is not withdrawn from the bile in the bile ducts whereas the

gall bladder is able to reduce the bulk of bile delivered to it as much as 10.8 times in 24 hours.

The technique employed by us for obtaining samples of bile for ordinary study, is essentially the same as that advocated by Lyon and now widely employed. The method is so well known that detailed discussion seems unnecessary.

The accumulated observations of many workers during the last couple of years leave no doubt but that valuable data are obtainable from bile collected by the transduodenal route, among which with a fair degree of accuracy, may be determined the state of the biliary tract. In order to be of value it is generally conceded that the study of the bile must be carried out when the specimens are fresh. These studied may be grouped under three headings. The gross appearance of the bile; miscroscopic appearance and its bacteriology.

Except when such examinations are carried out, the following conclusions are justified as to the state of the biliary passages.

1. If the bile, the A. bile obtained from the common bile duct, is darker than normal and more vicious, and shows the presence of a considerable number of epithelial cells, mucus leucocytes and bacteria, it is suggestive of chronic bile duct infection. When such infection is associated with stones, cholesterin crystals and the bile are increased, the bile frequently feels gritty.

2. When the B. bile derived from the gall bladder shows increased viscosity, is darker than normal and shows increased epithelial cells together with a large number of bacteria and leucocytes, and increased cholesterin crystals, an acute cholicystitis is probably present. When there is marked suppuration of the gall bladder, the number of leucocytes present may be so great as to be looked upon as pus. In violent acute inflammation a large number of red blood cells are frequently found. When in addition to an acute cholicystitis, gall stones exist, small calculi, gritty substances in the bile and a marked increase in cholesterin crystals is usually present. In chroniccholicystitis, the bile derived from the gall bladder is usually clear, shows flakes of suspended mucus, and a moderate increase in leucocytes. Frequently the presence of a number of bacteria may be recognized.

3. Failure to obtain the gall bladder bile after repeated stimu-

lation when the clear yellow bile derived from the bile ducts flows freely, strongly suggests the presence of cystic duct obstruction.

When after repeated stimulation no bile is obtainable, complete occlusion of the common bile duct may be inferred.

Disease of the biliary tract, therefore, depends upon careful observation of the viscosity, color of the bile, presence and absence of cellular elements such as epithelial cells, leucocytes, red blood cells, the amount of cholesterin present, the freedom of the bile from gritty particles, and finally the number and the varieties of bacteria present.

From a diagnostic, prognostic and therapeutic standpoint, obviously great interest centers about the bacteriological examination of the bile. A satisfactory technique for carrying out such examinations is obviously difficult, and many factors must be overcome before the result obtained can be regarded as reliable. In numerous instances we have attempted bacteriological studies of the bile. In an effort to have these observations as reliable as possible the following technique, after the method advocated by Lyon, has been adopted.

The night before the examination is to be made, the patient must brush the teeth thoroughly, the mouth and throat and nose are carefully washed by Lavoris followed by Liquor Antisepticus. From then on no further food or fluid is ingested until the tube is passed on the following morning on a fasting stomach. Prior to the passage of the tube, the teeth are again scrubbed, the nose, throat and mouth are thoroughly disinfected by Lavoried followed by Liquor Antisepticus. The duodenal tube having been rendered sterile by careful surgical sterilization, is then passed by an operator wearing sterile gloves and a sterile gown. As soon as the duodenal tube has reached the stomach, the stomach is carefully washed with distilled water until the return is entirely clear. It is next washed with a solution as Lavoris c. c. being diluted with 200 c. c. of sterile water. This lavage is followed by another lavage of sterile water. Then 250 c. c. of a 1 to 1000 Silvol solution is introduced into the stomach and removed. It is followed by another lavage with sterile water which is continued until the return flow is entirely clear. Such fluid or contents as is then remaining in the stomach is then cultured and the duodenal

tube allowed to pass into the duodenum. Before stimulation with magnesium sulphate a culture is made from the duodenal contents. Then stimulation of the duodenum is carried out as usual, with 75 c. c. of a 25 per cent of sterile magnesium sulphate solution. The bile is then obtained in the usual way, being segregated in the three fractions A, B and C bile from all of which separate cultures are made.

By following this method it is generally possible to render the upper alimentary tract as sterile as possible, and by taking cultures from the stomach and duodenum and possibly the mouth and throat before stimulation is made and the bile is aspirated, one is enabled to know which if any organisms are present in these structures before the flow of bile begins. Exceptionally the only organism obtained from the bile, is the same as those obtained from the stomach or duodenum before the bile has entered it. The organism found cannot be regarded as in any way peculiar to the bile. The cultures obtained from bile contain only the commoner mouth saprophytes such as the steptococcus salivarious, micrococcus catarrhalis, or the bacillus subtlis. No significance can be attached to the findings which must be looked upon as due to contamination. The organisms which ,however, are of importance and which have been repeatedly isolated by numerous observers, include streptococci, especially hemolyticus, the staphylococcus aureas and albas, the bacillus typhosis and the colon bacillus. From the somewhat meagre published results of bacteriological examination of the bile thus obtained, it is surprising to note the frequency with which some observers hemolytic streptococci. It is also interesting to note that pneomococci have been reported by some. It seems somewhat incomprehensible in view of the well known bile soluble property of pnumococci.

It must be admitted that the technical difficulties, as well as the rather meagre knowledge at present available as to the bacteriology of the stomach, duodenum and biliary passages often makes the bacteriological study of the bile distinctly unsatisfactory.

Elaborate and careful experimental studies on the bacterial statis and germicidial properties of the bile, have been carried out by Neilson and Meyer ([5]). Their experiments were largely carried out

with test tubes and included a variety of biles from different animals, such as cats, goats, rats, dogs, oxen, sheep, pigs and a few observations on bile obtained from man. The human specimens were obtained wholly from cholicystectomized bladders by aspiration from laparotomy. Their observations were made largely upon organisms of the typhoid dysentery paratyphoid group, and the ziblio of chlorea. In the specimens of bile obtained from man they found that the typhoid bacilli grew well and remained visible for more than ten days, whereas in the ziblio cholera, it required eight days before the sterilization was complete. They concluded that the rigor of bacterial growth in average human cystic bile, is noteworthy. They further discovered that proliferation is somewhat influenced by artificially inducing hydronion concentration, but that complete suspension of growth only occurs at a PH which in all probability never occurs in a human body. The importance of their paper, so far as man is concerned, has largely to do with the influence of cystic bile on the human typhoid carrier state.

It was originally our intention to discuss the bacteriological findings in a series of cases that exhibited disease of the biliary tract or gall bladder, but in the earlier portion of our work so much difficulty was experienced with the technique, that the results obtained were regarded as too unreliable for permanent record. We have had an opportunity of carrying out some successful and interesting bacteriological observations on a limited number of cases, and in order to demonstrate the importance of the bacteriological findings in the bile, these cases will be briefly reported.

Case I.

Mr. C. P., Jr. Male, aged 60. The first of January this case suffered from gastro intestinal disturbances, associated with marked jaundice. A diagnosis was made by his physician of acute catarrhal jaundice. In the course of two weeks the jaundice entirely cleared up.

The patient first came under observation three weeks after the onset of his jaundice. At that time there was no evidence of jaundice on physical examination. Stools contained bile, and the urine was free of bile. He exhibited, however, considerable pallor. There was some elevation of blood pressure, he suffered from a distinct asthenia,

coated tongue, and loss of appetite. Examination of the urine showed the presence of considerable quantities of albumin, numerous hyaline and granular casts, and red blood cells. He was admitted to the hospital apparently suffering from acute exacerbation of a chronic.

Haemateria became more marked, and the anemia progressed. Careful search was made for a focus of infection that might be held responsible for the acute renal manifestations. All posible foci of infection were eliminated except the gall bladder, over which there was a slight persistent tenderness. With the history of a recent attack of jaundice ,it was decided that the gall bladder was in all probability the focus of infection responsible for his progressive anemi and his physical symptoms. Three weeks after coming under observation his haemoglobin was 55%; his red cells 2,860,000 and his leucocytes 17,100. At this juncture duodenal drainage was instituted. A considerable quantity of dark green gall bladder bile was obtained. In this bile was found numerous flakes of mucus and sediment, epithelial cells, in three of the specimens of gall bladder bile, pus was present. On culture on two separate occasions, staphylococcus aureus and albus were found in pure culture and the A, B, and C bile, but these organisms were not present in the cultures taken from the stomach or from the duodenum before stimulation was resorted to. It was also interesting to note that several specimens of urine collected at about the same time and examined at a different laboratory, also gave pure cultures of the same organism. There seemed, therefore, to be some justification for the assumption that the bladder and renal infection were the result of the same organism. Owing to a steadily falling blood count, the patient was given a transfusion of 500 c. c. of blood. Some slight improvement followed this procedure, although the blood count did not noticeably increase. The most marked subjective improvement was a biliary drainage, which was practiced once a week. A vaccine was prepared from the strains of streptococcus aureus and albus isolated from the bile. During the past month the patient has been receiving the vaccine every four days. The haematuria has cleared up, the renal symptoms have subsided, the blood count has shown marked improvement and subjectively the patient shows every indication of recovery.

Case II.

Mr. E. D., aged 52, in whom a diagnosis of duodenal ulcer and cholicystitis was made. The condition of the gall bladder was discovered in the course of doing a drainage, during a course of two weeks treatment with duodenal feeding. In this patient the microscopic study of the bile showed a considerable number of pus cells, bile stained epithelial cells, many short motile bacilli, a few pigment crystals and quantities of amorphous bile salts. Bile from the gall bladder was obtained by the aseptic technique of Lyon cultures showed a luxurious growth of colon bacilli. An autogenous vaccine of colon bacilli was prepared, and injections were given at intervals of four days up 1,000,000,000 bacilli. At the same time weekly drainage was practiced. The original culture was obtained on January 13th. Following the above outline treatment, on March 23rd a second culture was obtained by the same technique. At this time the patient was symptomatically greatly improved. Gastro intestinal symptoms had disappeared.

Case III.

Mrs. G. B., aged 35. A patient in the service of Dr. Pemberton suffering from chronic arthritis. After exhaustive search for the foci of infection in the course of which the tonsils were removed, and some teeth extracted without improvement, non-surgical drainage of the biliary tract was instituted. The B bile was a deep black. Was vicid, contained many shreds and particles and on culture showed many streoptococcus hemolyticus and staphylococcus albus in luxurious growths. In view of these findings, the gall bladder was regarded as the probable focus of infection and was removed. At operation no stones were found but a definite chronic cholicystitis existed. Cultures taken from the gall bladder at operation showed hemolytic streptococcus and staphlyococcus albus. Confirmation by surgical means of the findings obtained by the transduodenal drainage are interesting and significant of the value of the latter method. Bi-weekly biliary drainage was instituted in this case. Two months after the original culture was obtained from the bile, bile cultures were again made following the Lyon antiseptic technique. This time the staphylococcus albus alone was present. Streptococcus had dis-

appeared. An autogenous vaccine was prepared and given. A month later the biliary drainage was discontinued and the patient at that time was showing marked improvement, her temperature was practically normal and she was able to be up and walk about.

CASE IV.

Miss K., aged 60. This patient was suffering from a chronic arthritis. Her tonsils had been removed without result. Biliary drainage was instituted, cultures were obtained according to the Lyon technique. A non-hemolytic steptococcus was isolated from the B bile. A vaccine was made and is being administered. The patient is still under observation and although improving it is too early to know the ultimate result.

CASE V.

Mr. M., aged 53. The patient gave a history of recurring attacks of slight jaundice and so-called biliousness. These latter he had ever since his youth. Biliary drainage was instituted and cultures made from the bile. In this instance although the bile showed staphylococcus albus in the bile, non-hemolytic streprotocci and unidentified micrococci were also found in the stomach cultures. The results therefore obtained from the biliary culture were too uncertain to justify any therapeutic procedure based upon these findings without further checking the results. This case is cited to emphasize the importance of culturing the stomach, even though it is believed to be sterile, since even though the same organism has been discovered in the bile, they could not be regarded as significant since they had also been isolated from the gastric culture.

These cases are cited in order to point out the possibility of accurate bacteriologic study of the bile by means of biliary drainage, and also to again emphasize the tree frequently forgotten rôles that the gall bladder plays in producing focal infection.

The checking by operative findings of the results of biliary drainage, in Case III, is a gratifying evidence of the reliability of bacteriological studies made by this technique. A much more striking example, however, of the value of pre-operative duodenal findings in comparison with the lesion found at operation, is furnished by Whipple ([6]) who in a series of 27 cases on whom careful studies

of the biliary tract were made by means of duodenal drainage before operation, found a striking coincidence in the operative findings with those obtained by a study of the bile before operation.

In carrying out bacteriological studies of the bile, a caution which has been rightly emphasized by Lyon, should be pointed out. Lyon insists upon the importance of replanting from the original cross culture if one is used, in six hours. If this is not done, a rapidly growing colon bacillus may render it extremely difficult to isolate the slower growing steptococcus, should such an organism be present. In order to render the bacteriologic examination doubly safe, bile is planted on gauze cultures during the biliary drainage, and is transplanted within twelve hours. In addition to this all the bile withdrawn is collected in twelve bottles separated into the A, B, and C biles, and streaks are made from these biles on solid media at once. By following this method the isolation of these organisms will be facilitated.

Conclusions.

1. We believe that the method of biliary drainage instituted by Lyon, and based upon the hypothesis of Meltzer is a useful and practical procedure. That there is every reason for the belief that the biles obtained in this way are derived from the common bile duct, the gall bladder, the hepatic duct and biliary capillaries.

2. In our experience the diseased condition of the gall bladder and bile ducts can be recognized in this way by a chemical and bacteriological study of the bile, which in a pathological condition show alterations which are significant.

3. The bacteriological study of the bile derived in this way, is feasible and is of importance, but in order to be successful, a rather elaborate, time-consuming technique is essential.

4. If such a technique is carried out, the bacteriological findings are reliable and are a guide to therapeutic procedure.

Finally, however, it should be emphasized that it is our feeling that the information obtained by transduodenal lavage is only a diagnostic adjunct, and in no sense should take the place of careful history taking and complete physical examination. It is only when the findings derived from biliary drainage are taken in conjunction with a complete clinical study of the case that accurate conclusions

can be drawn. Our knowledge of the bacteriology of the biliary and upper gastro intestinal tract, is still so meagre that a great deal of work and much careful observation will be required before this phase of the subject is placed upon an entirely satisfactory basis.

REFERENCES.

1. LYON. Jour. A. M. A., Vol. 73, Sept. 27th, 1919, pp. 980.
2. MELTZER. Am. Jour. Med. Sci., Vol. 153, April 1917, pp. 469.
3. SMITHIES, KARSHNER AND OLSEN. Jour. A. M. A., Vol. 77, No. 26, Dec. 24th, 1921, pp. 2036.
4. ROUS AND MCMASTER. Exper. Med., Vol. 34, No. 1, July 1, 1921, pp. 7.
5. NEILSON AND MEYER. Jour. of Infect. Dis., Vol. 28, 1921, pp. 542.
6. WHIPPLE. Annals of Surgery, Vol. 73, May 1921, pp. 556.

DISCUSSION.

THE PRESIDENT: The paper is now open for discussion.

DR. DALAND: Mr. President and gentlemen, I congratulate Dr. Piersol in having summarized in such a satisfactory way the status of gall bladder and biliary drainage, from the various standpoints to which he refers. I believe there are quite a number of examples illustrating what he said.

Referring to the difficulties in regard to the bile, in regard to its effects upon microorganisms, the effect upon pneumococcus, and from what he states his conclusion is most encouraging, opening up an entirely new avenue of information, diagnosis, and is an additional adjunct, as he states, to our existing knowledge.

I have nothing to offer. I am interested in the subject, and I would like to ask Dr. Piersol one question, and that is, in these cases of gall bladder disease, where he recovered a specific organism, and the gall bladder was the focus of infection, as to whether or not there existed elsewhere a focus that preceded the infection of the gall bladder?

THE PRESIDENT: Is there further discussion on this paper?

A MEMBER: I have gained a good deal from the discussion of Dr. Piersol, and I think his is a very able paper.

A year ago before this Association I reported some thirty cases in which biliary drainage was done, which was followed by operation. In these thirty cases there were twenty-six cases in which bile was absent, and in all of those cases we found either an obstruction to the cystic duct, and the gall bladder was full of stones, and did not contain any of this bile. That was helpful in quite a number of ways, as we not only got a better idea of diagnosis, but we were more easily able to handle a patient and convince him that an operation was necessary.

I am very glad to say that in the past year our experience has been the same, practically the same, that is, that the absence of B bile convinces us that the cystic duct, the gall bladder itself—great care must be exercised—this must

be done very carefully, and the less we check ourselves up by giving large doses of belladonna and repeating the test, we could very easily be misled, because sometimes we do not get the B bile at the first sitting, and we have gone over these cases two and three times, also seeing from the x-ray whether our tube is in the proper place, and in checking up in that way we felt that we have accomplished something. I am very glad to have heard this paper, and I have the assurance that it is worth while going on with the work.

THE PRESIDENT: Is there further discussion of Dr. Piersol's paper?

Dr. Piersol, will you close the discussion?

DR. PIERSOL: In answer to Dr. Daland's question, so far as we were able to determine, we were not able to discover any foci of infection other than the gall bladder.