DUODENAL INTUBATION

SIGNIFICANCE OF THE CELLULAR CONTENTS OF BILE IN THE DIAGNOSIS OF DISEASES OF THE BILIARY TRACT

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In cases of suspected disease of the biliary tract three methods of investigation are available. The first is a thorough clinical examination, the second the radiological investigation of the biliary tract, and the third the examination of the bile obtained by duodenal intubation after the use of magnesium sulphate or some other biliary stimulant. The bile is then submitted to cytological, bacteriological, and chemical examination, reliance being placed mainly on the former, since the most important diagnostic criterion is held to be the presence of cells and flocculi in the bile. It is with this particular aspect that this communication is mainly concerned.

The method of duodenal intubation was introduced into clinical medicine by Lyon in 1923. The underlying basis of the test was the observation by Meltzer in 1917 that the entrance of magnesium sulphate into the duodenum caused a relaxation of the sphincter of Oddi. Following the publication of Lyon's monograph the test was widely used throughout the world, though probably to a greater extent in America and on the Continent than in Great Britain. Although reference to the method is still found in many standard works dealing with diseases of the biliary tract, improvements in the technique of cholecystography have resulted in the test being less frequently carried out than formerly. Nevertheless in some clinics duodenal intubation is still retained as part of the full investigation for all types of disease of the biliary tract. In Great Britain, however, it is held to be of particular value for the diagnosis of simple catarrhal cholecystitis in which structural changes in the gall-bladder wall have not developed to such a degree as to be demonstrable by radiology. As recently as 1937 McNee, writing on catarrhal cholecystitis, states that it cannot be diagnosed by simple clinical methods or by cholecystography, and that "duodenal intubation is at present the only diagnostic method on which we can positively rely. It is too little used in Britain, but is a routine method in America and elsewhere."

During an investigation into the possible value of sulphonamide drugs as biliary antiseptics we noticed that many cells were present in the bile recovered when magnesium sulphate was used as a biliary stimulant, whereas cells were absent in the same patients when certain other stimulants were employed. This observation suggested that magnesium sulphate might have produced the cells by irritation of the mucous membrane of the duodenum and

that in consequence their presence did not necessarily indicate pre-existing inflammation in the biliary tract. To test this hypothesis we thought it necessary to carry out the following procedures:

1. Intubation of cases with no clinical evidence of biliary or gastro-intestinal disorder, using magnesium sulphate as the stimulant. The cases in this group would act as a control series, since for the purposes of this investigation these patients may be considered as normal persons. The finding of cells in the bile would suggest that the magnesium sulphate was acting as a local irritant.

2. Intubation of the same cases, using as stimulants substances which appeared unaltered in the duodenum in the course of normal digestion, such as peptone or olive oil. The absence of cells in the bile after the use of these physiological stimulants, under exactly similar circumstances and in the same cases, would suggest a local irritant action of the magnesium sulphate.

3. Intubation, prior to operation, of patients with disease of the biliary tract, and examination of the bile aspirated at operation from the gall-bladder, and, where possible, from the common duct.

4. Observation of the effects of a similar solution of magnesium sulphate on the mucosa of different parts of the human gastro-intestinal tract exposed at operation, or in fistulae.

Methods

The tube was introduced into the stomach and the contents were evacuated. The patient was then made to lie on the right side with the pelvis elevated on pillows. Usually within one hour the first sample of bile, so-called Bile A, appeared and was collected. One of three stimulants was then injected through the tube. These were 30 c.cm. of 33% magnesium sulphate, 30 c.cm. of 10% peptone, and 30 c.cm. of olive oil warmed to body temperature before injection. Lyon (1923) recommended the introduction of 75 c.cm. of a 33% solution of magnesium sulphate, but since 30 c.cm. of a 33% solution has been found by ourselves and others to produce a good biliary reflex and to yield a free flow of dark bile (Bile B), we are satisfied that the smaller quantity is adequate. After injection, 20 c.cm. of air was blown through the tube to ensure that all the stimulant reached the duodenum, and then the tube was clipped for three minutes to prevent reflux. After this the bile was allowed to flow freely into test-tubes.

Where possible, intubation was performed in each patient on three occasions, but in some cases only one or two

examinations could be undertaken. Nearly all the patients were intubated at intervals of two days, a different stimulant being employed on each occasion, but in five cases magnesium sulphate was used immediately after the reaction following peptone had finished.

We considered as Bile B (so-called gall-bladder bile) the dark bile, which was collected until its colour returned to that seen before the stimulant was introduced. In the few cases in which this dark bile did not appear, the bile collected within forty-five to sixty minutes after injection of the stimulant was called Bile B.

Estimations of the bilirubin and protein content of the various samples of bile were made, but as the results do not affect the subject of the present report they are not given here.

Where flocculi suspended in the bile were visible to the naked eye they were removed by pipette for examination. Where flocculi were absent the bile was centrifuged and the deposit examined microscopically. Whereas the cells in the bile can be easily seen in the deposit of fresh specimens, considerable difficulty was experienced in staining them. We note that Lyon and most other workers report only on such unstained material. Attention is drawn to the fact that digestion of the cellular elements in bile takes place rapidly on standing, hence the need for early examination of the deposit.

Through the courtesy of Prof. J. R. Learmonth the opportunity was taken of observing the direct action of magnesium sulphate on the mucosa of the human stomach, duodenum, jejunum, and ileum exposed during the course of various surgical procedures. A small glass cylinder containing the test solution of magnesium sulphate was placed on the mucosa, and samples were removed at intervals of three, five, eight, and ten minutes. Controls with saline or peptone were first carried out.

Material

Duodenal intubation was performed on 33 subjects. Of these, 21 were chosen as control cases because they suffered from no disease of the gastro-duodenal or biliary tract, while 12 were proved cases of chronic cholecystitis with cholelithiasis. Of this latter group 8 were submitted to cholecystectomy subsequent to duodenal intubation. In all, 68 intubations were performed. Details of the

TABLE I.—*Duodenal Intubation of Subjects with no Symptoms or Signs of Biliary or Gastro-intestinal Disorder, showing Relative Numbers of Cells in Bile obtained after Various Biliary Stimulants*

Case	Mag. Sulphate	Peptone	Olive Oil
1	+++	0	0
2	++	No bile obtained	0
3	+++	+	0
4	No bile obtained	+	Not done
5	+	0	" "
6	0	0	0
7	+++	0	0
8	+++	0	Not done
9	+++	0	0
10	+++	0	0
11	+	0	0
12	+	0	Not done
13	+	0	" "
14	+	0	" "
15	Not done	R.B.C.s only	" "
16	+++	0	" "
17	+++	0	" "
18	+++	0	" "
19	+++	Not done	" "
20	+++	" "	" "
21	+++	" "	" "

0 = Clear bile. No flocculi seen. Centrifuged deposit shows no cells, or only a very few small flecks of mucus containing small groups of cells.
+ = Clear bile. No flocculi seen. Centrifuged deposit contains moderate number of cells occurring in groups with mucus.

++ = Flocculi seen in small numbers.
+++ = Flocculi seen in large numbers.

Flocculi vary in size from a pin-head to a match-head, and consist of a mass of round cells bound together by mucus. Each flocculus usually contains very large numbers of cells.

TABLE II.—*Cases of Cholecystitis and Cholelithiasis. Showing Relative Numbers of Cells in Bile obtained after Various Biliary Stimulants by Duodenal Intubation and by Aspiration at Subsequent Operation*

A.—Bile removed by Duodenal Intubation				B.—Bile aspirated at Operation	
Case	Mag. Sulph.	Peptone	Olive Oil	Gall-bladder	Common Duct
1	+++	0	0	No operation	
2	0	0	Not done		
3	Not done	0	" "		
4	++	0	" "		
5	+++	0	0	No bile (a)	0
6	Not done	+	0	0	0 (b)
7	+	0	Not done	No bile (c)	—
8	Not done	0	" "	0	—
9	+++	Not done	" "	0 (d)	—
10	++	" "	" "	(e)	+
11	++	0	" "	0	+
12	+++	0	" "	0	0

(a) = Gall-bladder full of stones with no bile.

(b) = Bile contaminated with blood from operative trauma.

(c) = Gall-bladder full of stones, with no bile.

(d) = A few R.B.C.s only.

(e) = A few columnar epithelial cells not seen in previous medical drainage.

Symbols indicating number of cells as in Table I.

biliary stimulants employed and the results obtained are shown in Tables I and II. In the 8 cases in which operation was performed bile was aspirated from the gall-bladder, and in 4 of them it was possible to obtain bile from the common duct as well. The direct effect of a solution of magnesium sulphate on the human alimentary mucosa exposed at operation was observed in 5 patients, seven tests being carried out.

Results

1. *Cytology of the Bile obtained by Duodenal Intubation Before the Use of Biliary Stimulants.*—Cells were usually absent from the centrifuged deposit of Bile A of all patients, though in a few cases occasional flecks of mucus containing small groups of cells were seen.

2. *Cytology of the Bile obtained by Duodenal Intubation in Normal Cases After the Use of Biliary Stimulants.*—From Table I the following results may be summarized:

Of 19 cases in which bile was obtained after magnesium sulphate 12 showed numerous flocculi containing large numbers of cells, 5 showed no flocculi but the centrifuged deposit contained a moderate number of cells occurring in groups with mucus, and in the other 2 cases no flocculi and only very scanty cells were found on centrifuging.

Of 17 cases in which bile was obtained after peptone 14 showed no flocculi and only very scanty cells in the centrifuged deposit, while 2 cases had no flocculi but a moderate number of cells in the centrifuged deposit.

In all 8 cases in which bile was obtained after olive oil no flocculi were seen, and only very scanty cells were found in the centrifuged deposit.

3. *Cytology of the Bile obtained by Duodenal Intubation in Cases of Chronic Cholecystitis and Cholelithiasis After the Use of Biliary Stimulants.*—From Table II, A, the following results may be summarized:

(a) *Cases not submitted to Operation (Cases 1 to 4).*—In 2 cases out of 3 (Cases 1, 2, and 4) of cholecystitis and cholelithiasis in which magnesium sulphate was used as a biliary stimulant numerous flocculi containing many cells were found. In all 4 cases (Cases 1 to 4) in which peptone was used, and in Case 1, in which olive oil was employed, no flocculi were seen and only very scanty cells were found in the centrifuged deposit.

(b) *Cases submitted to Operation (Cases 5 to 12).*—In the 6 cases (Cases 5, 7, 9, 10, 11, and 12) in which magnesium sulphate was used as a biliary stimulant before operation numerous flocculi containing a large number of cells were found. In only 1 case out of 6 of this group in which peptone was used were there a moderate number of cells in the centrifuged deposit.

In the 2 cases (Cases 5 and 6) in which olive oil was employed only very scanty cells were seen in the centrifuged deposit.

4. *Cytology of Bile aspirated at Operation from the Gall-bladder or Common Duct of Patients in Group 3 (b).*—From Table II, B, the following results may be summarized:

In 5 cases of cholecystitis and cholelithiasis (Cases 6, 8, 9, 11, and 12) confirmed at operation and by subsequent pathological examination of the gall-bladder no cells were found in the bile aspirated directly from the gall-bladder at operation. In Case 10 only a few columnar epithelial cells were present in the gall-bladder bile, such cells not having been recovered previously by medical duodenal drainage. The failure to obtain bile from the gall-bladder in two cases (Cases 5 and 7) at operation was attributable to the fact that it was completely filled with calculi.

Common-duct bile was obtained from 4 cases (Cases 5, 6, 10, and 12) of this same group. In two of these (Cases 5 and 12) no cells were found in the bile; in Case 6 there was contamination with blood but no excess of leucocytes or epithelial

FIG. 1.—Photomicrograph of wet preparation of cells in flocculi in bile obtained by duodenal drainage after the use of 30 c.cm. of 33% magnesium sulphate. ($\times 400$.)

cells; and in Case 10 only a few columnar epithelial cells were present, though these had not been found on previous medical biliary drainage.

It is of interest to note that in Cases 5, 9, 11, and 12 no cells were present in the bile aspirated from either the gall-bladder or the common duct, whereas large numbers of cells were found in the bile obtained by duodenal intubation after magnesium sulphate.

5. *Effect of Magnesium Sulphate on the Human Alimentary Mucosa exposed at Operation.*—In the 5 cases in which the investigation was made it was noted that a marked congestion of the mucous membrane with exudation of red blood cells occurred after a few minutes' exposure to magnesium sulphate, whereas normal saline and peptone solution had no such effect. In one experiment involving a portion of ileal mucosa of a patient, samples studied after three, five, and eight minutes contained an increasing number of cells similar in appearance to those recovered from the duodenum by intubation after magnesium sulphate.

MORPHOLOGY OF CELLS IN THE BILE REMOVED BY DUODENAL INTUBATION

In all cases an attempt was made to establish the nature of the cells observed in the bile. In the fresh preparations

FIG. 2.—Cells as in Fig. 1 stained with Leishman, simulating polymorph leucocytes. ($\times 400$.)

FIG. 3.—High-power photomicrograph of cells as in Fig. 2, showing that the cells which appear to be polymorph leucocytes are in reality epithelial cells with disintegrating nuclei. ($\times 1,000$.)

the vast majority of cells appeared to be round cells (Fig. 1), and only a very small number were recognizable as columnar epithelial cells. The round cells were approxi-

mately the same size as leucocytes, and to begin with we considered them to be leucocytes. This first impression received support from examination of the deposit stained with methylene blue, neutral red, and Leishman, as many of the nuclei appeared to be lobulated. The stained preparations were, however, far from satisfactory, and accordingly we decided that it was necessary to remove the cells from the influence of bile, digestive juices, and hypertonic magnesium sulphate solution by washing the flocculi immediately in saline and then suspending them in serum. A considerable improvement in staining effects was thus obtained, which enables us to say with confidence that the vast majority of round cells seen in the wet preparations were in reality cuboidal epithelial cells whose cytoplasm had largely disappeared and whose nuclear chromatin was rapidly undergoing degenerative changes (Figs. 2 and 3). Examination under the oil-immersion lens clearly reveals the difference between the detached broken-up portions of nuclear chromatin and the lobulated nucleus of a leucocyte. Bile-stained cells were noted in about 40% of our cases. The occurrence of fatty acids and salts in the deposit of bile obtained after olive oil must be remembered, as they may be mistaken for cellular elements.

Summary of Results

1. Bile removed from normal persons by duodenal intubation before the administration of biliary stimulants contained a negligible number of cells.

2. Bile removed from normal persons after the administration of magnesium sulphate usually contained many cells; whereas bile removed from the same patients after the administration of peptone or olive oil contained a negligible number of cells.

3. Bile removed by duodenal intubation from cases of chronic cholecystitis and cholelithiasis, following the administration of magnesium sulphate, usually contained many cells, whereas after other biliary stimulants cells were absent or scanty; furthermore, bile aspirated from the gall-bladder and common bile duct of the same patients at subsequent operation contained a negligible number of cells.

4. The irritant effect of a concentrated solution of magnesium sulphate on the human alimentary mucosa exposed at operation was demonstrated.

It can be concluded from the above experiments that bile removed by duodenal intubation, following the administration through the tube of 30 c.cm. of a 33% solution of magnesium sulphate, may contain large numbers of cells produced by the local irritation of this concentrated solution on the duodenal mucous membrane.

Discussion

It is not the primary object of this communication to evaluate the Meltzer-Lyon test in the diagnosis of disease of the biliary tract. Its purpose is to draw attention to a finding not previously recorded so far as we are aware—namely, that magnesium sulphate can cause desquamation of duodenal epithelial cells in large numbers and that a real danger exists of mistaking such degenerating cells for pus cells, as will be clearly realized from a study of the photomicrographs depicted in Figs. 2 and 3. We do not deny that pus cells and columnar epithelial cells may appear in the bile obtained after the introduction of a biliary stimulant in cases of acute or subacute inflammatory conditions of the biliary tract. We do suggest, however, that a concentrated solution of magnesium sulphate is not a suitable stimulant for use in the Meltzer-Lyon diagnostic

test, because the interpretation of the cytological findings in the bile is complicated by the presence of cells produced locally in the duodenum. It would appear that 30 c.cm. of a 10% solution of peptone would be a satisfactory non-irritating biliary stimulant to employ.

The reasons for our belief that the duodenal mucosa is the source of the cells present in the bile obtained after the administration of magnesium sulphate are based on the experiments and cytological studies already described. In addition it should be noted that the appearance of the cells shown in Fig. 1 would seem to be identical with the cells shown in photographs 23 and 25 in Lyon's *Atlas of Biliary Drainage Microscopy* (1935), which are stated to be cuboidal duodenal cells obtained by intubation of a case of duodenitis. No mention is made by Lyon of the possibility that magnesium sulphate might itself give rise to a duodenitis with a consequent desquamation of epithelial cells.

Lyon states (1923, p. 319): "In all normal gall tracts all three of these different-coloured bile fractions (A, B, and C) should be perfectly clear and transparent and contain no microscopical cytology in the uncentrifuged specimen." He also writes that the first criterion as regards diseases of the biliary tract is furnished by the gross observation of the amount of floccules which can be seen in uncentrifuged specimens. If these criteria are accepted, then the majority of our control cases were suffering from disease of the biliary tract.

Lyon also lays stress on the type of cells in the specimens of bile as indications of the site and degree of inflammation. Thus he holds that columnar epithelial cells alone are indicative of catarrh of the superficial layers of the mucosa of the biliary tract and are associated with leucocytes when the deeper layers are affected. Moreover, he maintains that the cells must be bile-stained before their origin from the biliary tract can be accepted, although he admits that if there is dysfunction of the sphincter of Oddi desquamated epithelial cells and pus cells from the stomach and duodenum may be bile-stained. If these criteria are accepted, then in the majority of our cases the flocculi observed in Bile B and Bile C (flowing after Bile B and lighter in colour), which contained large numbers of round cells unstained with bile, could not have come from the biliary tract.

The absence of flocculi and cells in the yellow Bile A obtained before the administration of magnesium sulphate and their presence in the dark Bile B (gall-bladder bile) after the administration of magnesium sulphate naturally suggest that the site of derivation of the cells must be the gall-bladder or the bile ducts. We believe that this assumption is not necessarily justified, because similar results would occur if the time required for the development of the irritant action of magnesium sulphate on the duodenal mucosa coincided with the time needed to cause a relaxation of the sphincter of Oddi. In our experience this is precisely what happens in most cases. In some instances, however, duodenal irritation develops rapidly, and in others slowly, after the injection of magnesium sulphate, as is illustrated by the following cases:

In two cases (Cases 7 and 17, Table I) a white opalescent fluid, strongly alkaline and showing no visible trace of bile pigments, was collected five minutes after the injection of magnesium sulphate and was followed by Bile B. In Case 17 the absence of bilirubin was confirmed by chemical examination. In both cases flocculi containing a very large number of cells were present in this white bile-free fluid. In another case (Case 12, Table II) a large number of flocculi containing many cells first appeared in Bile C, which flowed after Bile B. Thus the time of appearance of flocculi and cells in the bile after

the injection of magnesium sulphate would appear to vary, the above cases being the extremes in the present series.

A study of the literature available to us suggests that only two groups of workers have undertaken the necessary control observations—namely, examination of the bile aspirated at operation from the gall-bladder and common duct in patients who had previously been intubated, concentrated magnesium sulphate being the biliary stimulant employed. Knott and Bowell (1924) investigated five cases, and their reports do not show any constant parallelism between the findings in the bile removed at operation and that obtained by medical drainage. In parenthesis it should be noted that Venables and Knott (1924) introduced into the duodenum only 10 c.cm. of a 25% solution of magnesium sulphate instead of 75 c.cm. of a 33% solution as recommended by Lyon. It is not surprising, therefore, that they were unable to confirm Lyon's claim that three distinct types of bile could be recovered (Bile A, B, and C). The small amount of the stimulant used may also explain why the number of cells present in the specimens was often much less than was found by us. Mateer and Henderson (1926) could not find any leucocytes or epithelial cells in the bile aspirated direct from the gall-bladder at operation in 54 cases, though in 51% of the same group they found bile-stained cells in the bile recovered by duodenal drainage after the administration of magnesium sulphate before operation. They regarded these cells as evidence of inflammation of some part of the biliary tract other than the gall-bladder; but this assumption lacks proof, since they did not examine bile obtained from the common or hepatic ducts.

The work of the above authors strongly supports our contention regarding the local irritating effect of magnesium sulphate on the duodenal mucosa and that the cells do not come from the gall-bladder. It should be noted that the necessary comparison of the cytological effects of other biliary stimulants with that of magnesium sulphate in the same patients was not undertaken as an additional control. Our repeated failure to find cells in the bile removed by intubation after the administration of peptone and olive oil, in cases in which many cells were present in the bile obtained after magnesium sulphate, suggested at once a possible fallacy in this diagnostic method.

Summary and Conclusions

A study has been made of the cellular content of: (a) bile removed by duodenal intubation after the administration of magnesium sulphate, peptone, and olive oil in patients with and without disease of the biliary tract; (b) bile aspirated at operation from the gall-bladder and common duct in patients suffering from cholecystitis and cholelithiasis; (c) fluid removed from the mucosa of the human stomach, duodenum, jejunum, and ileum, exposed at operation, which had been submitted to the local effect of saline, peptone, and magnesium sulphate.

From the above experiments it is concluded that the cells present in the bile obtained by duodenal drainage after the administration of magnesium sulphate may be produced by local irritation of the duodenal mucosa, and hence do not necessarily indicate pre-existing inflammatory disease of the biliary tract. Attention is drawn to the fact that the degenerating oval or cuboidal duodenal epithelial cells may closely simulate pus cells.

We wish to express our thanks to our surgical colleagues in the Royal Infirmary for permission to investigate their cases, and in particular to Prof. J. R. Learmonth for his advice and help in the experiments connected with the action of magnesium sulphate on the exposed mucous membrane of the alimentary tract. One of us (J. I.) was in receipt of the McCosh Bursary and Crichton Scholarship in Medicine during the period of this investigation.

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[NOTE.—Since the completion of this paper for publication we asked some of our medical colleagues on the staff of the Edinburgh Royal Infirmary to select patients in their own wards who were not suffering from disease of the gastro-duodenal or biliary tracts, in order that we might demonstrate the irritant effects of magnesium sulphate as used in duodenal intubation. Our colleagues watched the technique being carried out and personally made a microscopical examination of the specimens of bile recovered. The results obtained were similar to the findings shown in Table I.]

OBSERVATIONS ON SOME NORMAL AND INJURIOUS EFFECTS OF COLD UPON THE SKIN AND UNDERLYING TISSUES

III. FROST-BITE

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Frozen Skin

Skin can be frozen in the living subject very readily by bringing it into contact with sufficiently cold metal or by allowing a jet of very cold air to impinge upon it. These methods I used extensively to study the effects of freezing (Lewis and Love, 1926). Freezing of the skin in a current of cold air is recognized by an observer the instant it happens. The patch of skin affected seems at once to become pale, dull, opaque, and of a yellowish colour. The attentive subject recognizes freezing at once when it is due to metallic contact, the formation of ice crystals in the skin being accompanied by a very definite pricking sensation. In a jet of cold air freezing may happen without subjective sensation; or it may be signalled by a little short stinging pain that is felt abruptly in the part. All these observations of the laboratory conform with the experience of sufferers from frost-bite in the open air. Skin that is frozen without involvement of the subcutaneous tissue forms a hard plaque in the skin, with edges which are sharply defined and by which the plaque can be lifted. It feels like a coin embedded in the skin. When the frost extends deeply the frozen tissues are solid and immovable.

Freezing-point of Skin

Although very accurate determinations of the living skin's freezing-point have never been made, this point is known accurately enough for our purposes. It is certain that it lies below the freezing-point of water, and it is equally certain that it is not far from -1° C. The freezing-point of blood serum is -0.53° C., from which that of skin is unlikely to be far removed. If metal bars at exactly known temperatures are applied to the human skin, freezing can be shown to occur on occasion at a surface temperature as high as -2° C. (Lewis and Love, 1926). Such an observation fixes a temperature between which and 0° C. the actual freezing-point of skin lies.

Supercooling

It is now to be said that skin when cooled very rarely freezes at its true freezing-point. It exhibits in very