EXPERIMENTAL OBSERVATIONS ON THE PATHOGENESIS OF GALL-BLADDER INFECTIONS IN TYPHOID, CHOLERA, AND DYSENTERY.

By HENRY J. NICHOLS, M.D.

Major, Medical Corps, U. S. Army.

(*From the Laboratory Service of the Letterman General Hospital, U. S. Army, San Francisco.*)

(Received for publication, July 24, 1916.)

The fact that the chronic carrier in the typhoid group of diseases is a result of infection is becoming of more and more importance in epidemiology. In most cases the microorganisms are known to be carried in the gall-bladder or gall-passages. In cholera, the infection seems to produce nearly the same result. At least semichronic intestinal carriers have been found and their occurrence has been shown by Kulescha (1), Greig (2), Schöbl (3), and others to be due to infection of the gall-bladder. This discovery is the most important recent addition to the pathology and epidemiology of the disease. In the case of bacillary dysentery, fewer cases have been studied from this point of view, but intestinal carriers have been reported, and in a few instances dysentery bacilli have been recovered from the bile (4), and analogy and experiment suggest a similar set of circumstances. In short, in this whole group of diseases, one of the most important problems of preventive medicine seems to be the prevention and cure of gall-bladder and gall-passage infections.

The subject has been approached from the experimental side by making use of gall-bladder infections in the rabbit. In some respects, at least, the lesion in the rabbit is a counterpart of that in man, and it seems probable that, in working out means of preventing and curing carriers, the experimental lesion in the rabbit will be an important factor. The present paper deals with experimental observations on the mechanism of gall-bladder infection in typhoid, concerning which our knowledge at present is still uncertain; the mechan-

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

ism of infection in cholera and dysentery by a portal system septicemia is suggested, and the antiseptic properties of rabbit bile are emphasized.

Mechanism of Gall-Bladder Infection in Typhoid.

Logically, as has often been stated, the gall-bladder lesion might be due to descending infection of the bile from the liver, to an ascending infection of the bile from the intestines, or to a transverse infection through the gall-bladder wall from the blood-vessels. The theory of descending infection from the liver was most generally accepted up to 1908 when Koch (5) and Chiarolanza (6) claimed to have proved that transverse infection through the gall-bladder wall is the usual method. Their views are more or less accepted at present (7). The theory of ascending infection from the intestines was early abandoned on account of the rarity of clinical gall-bladder infections with *Bacillus coli* and on account of positive results following intravenous injections in animals with or without ligation of the common duct.

It is desirable to settle this question if possible, because from the point of view of therapeutic attack, at least for prophylaxis, it makes a great deal of difference whether the infection occurs from the bile or from the blood vessels of the bladder wall. The writer is convinced that Koch and Chiarolanza's work is not conclusive and believes that we should go back to the theory of descending infection from the liver as an explanation of the usual method of infection. Occasionally, of course, infection might occur in any one of the three ways, but it seems reasonable that there is some regular method.

HISTORICAL.

The observations on which the theory of descending infections rests date back to our early knowledge of the elimination of microorganisms by the liver. Apparently the first work done was that of Fütterer (8), who, in 1888, in connection with the study of cases of typhoid with cholecystitis, reported that he injected a culture of *pyocyaneus* into the left ventricle of rabbits and recovered the organism in the gall-bladder at least 1½ hours later. In 1895 Fütterer (9) reported that he made a gall-bladder fistula in a dog and recovered *Staphylococcus aureus* from the bile 40 minutes after subcutaneous injection. He em-

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

phasized the importance of the liver in freeing the blood from microorganisms, and also referred to bacilli in the gall-bladder as a possible cause of relapse in typhoid, but of course, at that time, the possibilities of chronic cholecystitis in the spread of the disease were not realized. In 1899 Fütterer (10) recorded further experiments with dogs in which he established a common duct fistula and gave a portal vein injection of a suspension of *Bacillus prodigiosus* and recovered the organism from the bile 2 minutes after injection.

The work of Fütterer was confirmed by several workers, especially by Biedl and Kraus (11) who, in 1896, took up the question of elimination of bacteria by the bile in the course of a general study of the elimination of microorganisms by the liver, kidneys, and salivary glands. They made common bile-duct fistulas in dogs and recovered staphylococci after intravenous injection in 13 to 45 minutes. In 1905 Doerr (12) made a more particular study of the typhoid gall-bladder problem and in investigating the method of infection tied the cystic duct in rabbits; after several days he gave an intravenous injection but obtained no infection. However, when he ligated the common duct and gave an intravenous injection, an infection of the gall-bladder resulted.

These observations established the excretory powers of the liver and seemed to afford a natural explanation for gall-bladder infections in a septicemia like typhoid. In 1908, however, Koch and Chiarolanza published their articles in which they claimed to have demonstrated that infections occur through the gall-bladder wall and not through the bile. Koch drew his conclusions from the histological picture in a human case of typhoid cholecystitis in which he found emboli of bacilli in the folds of the mucous membrane. Chiarolanza tied the cystic duct and immediately injected typhoid bacilli intravenously and recovered them from the gall-bladder. He concluded that the usual method of infection was through the gall-bladder wall. However, as will be shown later, his experiments form a slender basis for adopting this theory, although many recent articles assume that the gall-bladder wall infection method is the proved method of infection (7).

Technique.

A number of experiments have been made by the fistula method. At first large Belgian hares, weighing 10 kilos, were used in order to make the operation easier, but it was found that smaller animals, even guinea pigs, can be easily used. Under ether anesthesia, a median incision is made from the ensiform cartilage to below the umbilicus, the stomach and duodenum are drawn down by an assistant, putting the common duct on the stretch; the mesentery over the duct is cut, avoiding the vessels, and a threaded aneurysm needle is passed under the duct about 1 inch from the duodenum. The thread serves

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

to cut off the bile while the capillary tube is being inserted and to tie the tube in place. The thread is put on the stretch by an assistant to constrict the duct, a small transverse incision is made below the constriction with a small pair of scissors, the capillary tube, which rapidly fills with bile, is inserted, the thread is tied on the neck of the tube, the rubber tubing attachment is brought out by a small opening in the right side of the abdominal wall, and the wound is closed. The rubber tube is attached to a glass tube in a two-holed stopper and the stopper is inserted in a graduated centrifuge tube. Bile soon begins to drop and accumulates at a rate of 1 cc. every 5 to 8 minutes, depending on the size of the animal. The capillary and rubber tube hold about 0.5 cc. of bile. If care is used in inserting the tube and in tying the duct securely, no blood enters the duct and brilliant green bile can be obtained in large quantities. With proper asepsis few contaminations are found in the plated bile. After injection, the bile is plated in 0.5 and 1.0 cc. quantities, and the colonies are counted after 48 hours' incubation. Most of the typhoid, cholera, and dysentery colonies were typical, but in case of doubt, they were identified by staining, by the double sugar tube, and by agglutination. Mesenteric injections were made into the veins of the appendix; some bleeding occurs when the needle is withdrawn, but it stops spontaneously and no gangrene of the intestine follows.

Most of the animals were about the same weight (3 kilos). The time of collection of bile was limited to about 1 hour, as it was found that cultures taken after 1 hour up to 24 hours were almost always sterile, even when many bacilli came through in the 1st hour.

The writer realizes, of course, that bacilli may appear in the bile from foci in the liver after the 1st hour, but the short observation time brings out points of importance and there is a limit to the experimental method on account of emaciation of the animal and infection of the field of operation. Moreover, the process of elimination seems to resemble that of filtration, and Bull (13) has shown that the blood after intravenous injection is usually sterile in less than an hour. Most of the animals were chloroformed in 1 or 2 days but two animals lived over a week before they were killed. Altogether twenty-four rabbits and two guinea pigs were successfully operated on.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

EXPERIMENTAL.

If the theory of descending infection is accepted as a working hypothesis and we attempt to test it experimentally it is evident that two conditions must be met; first, the bacilli must get into the bile, and second, they must be able to multiply in the bile. Infection of the rabbit's gall-bladder with typhoid bacilli has been produced many times by intravenous injection since the first work on this subject by Blachstein (14) and Welch (15) in 1891, but the results are uncertain and on an average not more than 50 per cent of inoculations are successful. In order to secure 100 per cent of infections which are necessary in therapeutic tests many authors have used the direct inoculation of the gall-bladder. This method is almost always successful, but it introduces a new element of traumatism and escape of blood into the gall-bladder and throws no light on the ordinary method of infection. If an explanation of the failure of intravenous injections to produce 100 per cent of infections can be found it may furnish a clue to the solution of the whole problem.

Presence of Bacilli in the Bile.—In the first set of experiments, the cystic duct was tied in order to prevent the possible entrance of organisms from the gall-bladder; a common duct fistula was then made, which, incidentally, prevented any organisms coming from below, and an injection was made into a mesenteric vein in order to favor concentration of the organisms in the liver. 1 cc. of a 24 hour broth culture was used for injection; the bile was collected every 5 to 15 minutes and poured into mannite media or over the surface of a double sugar tube, or Loeffler's media, depending on the organism used. In this way I obtained positive evidence of the presence in first specimens of bile of typhoid, paratyphoid A, and dysentery Y bacilli, and cholera vibrios. These experiments convinced me that microorganisms regularly enter the bile from the liver if they are present in the blood in sufficient numbers. In the rest of the experiments the cystic duct was not tied and the bile was plated in order to obtain quantitative results.

Relation of the Number of Bacilli Injected to the Number of Bacilli Appearing in the Bile.—It is known that, in general, larger intravenous doses produce more positive results in the gall-bladder and

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

several experiments have been carried out to determine what dose is necessary for bacilli to appear in the bile. Fistulas were established and different doses of a 24 hour broth culture of a recently isolated typhoid bacillus were injected into the ear vein. Bile was collected and plated, and the colonies were counted after 48 hours' incubation (Table I).

TABLE I.

Number of Colonies in Plated Bile after Injection into Ear Vein of Different Quantities of Typhoid Bacilli.

Bile.	1 cc.	2 cc.	3 cc.
cc.			
0.5	—	—	—
0.5	—	—	50
0.5	—	—	25
0.5	—	—	6
0.5	—	—	10
1.0	—	1	4
1.0	—	—	2
1.0	—	—	—
1.0	—	—	3
1.0	—	—	2
Total colonies in 7.5 cc. of bile.....	0	1	102
Time of collection in min.....	65	45	45

These experiments show that a fairly large dose is necessary for bacilli to appear in the bile in any number after ear vein injections, and may explain why some injections fail to produce lesions. There is probably some individual variation in elimination, but four other rabbits given 1 cc. and one other rabbit given 2 cc. failed to show any colonies, so the figures given above may be taken as fairly typical.

When bacilli appear, the first plates show the largest number of colonies; as the capillary and rubber tube hold about 0.5 cc. it is evident that the bacilli begin to appear in the bile very quickly and can be demonstrated in collected bile after 2 or 3 minutes. If a large enough dose is given, bacilli will therefore appear in the bile, but they may not be able to multiply and produce a lesion as will appear later when the antiseptic action of rabbit bile is considered.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

Relation of the Place of Injection to the Number of Bacilli Appearing in the Bile.—Further evidence along this line is furnished by injection into the mesenteric veins. If gall-bladder infections are due to bacilli in the bile it should be possible to produce more infections by mesenteric than by ear vein injections and more bacilli should appear in the bile in the former instance. Such is the case; after intravenous injection into mesenteric veins seven out of eleven rabbits, or 63 per cent, had gall-bladder lesions at the end of a week, while of twelve controls given the same dose into an ear vein, only five, or 41 per cent, showed a lesion. The number of bacilli appearing in the bile after mesenteric vein injection was larger than after ear vein injection, although there was more variation than in the ear vein injection experiments given above. 1 cc. of a 24 hour broth culture injected into the portal system resulted in thirty-one colonies in the plated bile as compared with none in the control experiment, but in a second experiment no colonies appeared. Similarly, in the case of cholera vibrios, 1 cc. produced twenty-eight colonies in one instance and none in the control, but in a second experiment no colonies appeared. In general, however, on the basis of considerable experience with the two methods, I can state that more organisms appear in the bile after mesenteric vein injection than after ear vein injection. The evident bearing of this fact on production of gall-bladder lesions in cholera and dysentery will be discussed later.

Relation of Immune Animals to the Number of Bacilli Appearing in the Bile.—Evidence is also furnished by the observation of Dr. K. F. Meyer, soon to be published, that immune animals show a somewhat higher percentage of infections of the gall-bladder than normal animals. This condition has been investigated by the same technique mentioned above with results shown in Table II. Meyer immunized the animal with three intravenous injections. Its agglutination was strong at 1: 10,000. The test dose was 1 cc. of a 24 hour broth culture with one control not immunized.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

TABLE II.

Number of Colonies in Plated Bile in an Immunized Animal and in a Normal Animal.

Bile.	Normal animal.	Immunized animal, agglutination, 1:10,000.
cc.		
0.5	—	—
0.5	—	50
0.5	—	90
0.5	—	45
0.5	—	11
1.0	—	20
1.0	—	—
1.0	—	1
1.0	—	—
1.0	—	1
Total	0	218
Time of collection in min	50	50

In this experiment the bacilli in the immunized animal came through in large numbers, while the control showed none. In two other vaccinated animals with an agglutination of only 1: 6,000, only a few colonies appeared. These results can be best explained in accordance with the recent work of Bull (13) by the rapid agglutination *in vivo*, the deposition of bacilli in the liver, and corresponding elimination. In other words, gall-bladder infections in vaccinated animals are not necessarily an index of lack of immunity, but may, in part, be an indication of a rich amount of immune bodies in the blood. Greig (16) has reported the occurrence of gall-bladder lesions in rabbits highly immunized with cholera-like vibrios. These results confirm my previous contention (17) that the gall-bladder lesion in the rabbit cannot be used to test general immunity from vaccination, and conclusions based on gall-bladder infections in immunized animals must be revised with this fact in mind.

The first condition necessary to confirm the descending infection theory, namely, the presence of bacilli in the bile, is fulfilled by the work stated above; more bacilli appear with larger doses, more bacilli appear with mesenteric vein injection than with ear vein in-

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

jection, and more bacilli appear in immunized animals. In regard to the theory of infection through the gall-bladder wall, on a critical examination of the work of Chiarolanza who tied the cystic duct and secured infection after intravenous injection, it is found that his protocols state that the bile is bloody even in animals examined in 24 hours, and he obtained 100 per cent of infections of the gall-bladder with the cystic duct tied and only 74 per cent of infections among normal animals.

Chiarolanza apparently assumed that the bloody appearance of the bile was due entirely to infection. As a control on this experiment, I tied the cystic duct of two rabbits but gave no injection; the next day the animals were chloroformed and the bile in the gall-bladder was examined. It had a reddish color and contained desquamated epithelium. It appears, therefore, that in Chiarolanza's work the bile was simply a diluted blood culture, as in tying the cystic duct the cystic artery is also tied and hemorrhagic infarction occurs from collateral circulation. As has been mentioned above, Doerr performed the same experiment of tying the cystic duct, but waited several days before giving an intravenous injection, and under these conditions no infection occurred. In other words, the bladder wall had recovered to such an extent that no blood and consequently no bacilli entered the gall-bladder. Doerr's experiments are therefore much more conclusive than Chiarolanza's and they support the descending infection theory.

The mechanism of infection in paratyphoid infections is in all probability the same as that in typhoid. Carriers are well known in paratyphoid A (18) and B (19, 20) infections, and acute and chronic cholecystitis has been described in both cases. In the rabbit Doerr (12) has reported persistent gall-bladder lesions after intravenous injections of both organisms. In four of fifteen animals I have obtained gall-bladder lesions with intravenous injections of a recently isolated paratyphoid A bacillus.

Gall-Bladder Infection in Cholera and Dysentery as a Result of Portal Septicemia.—As has been shown above, the evidence points to descending infection of the bile from the liver as the cause of gall-bladder infection in a septicemia like typhoid.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

In cholera, Schöbl, favors the ascending route from the duodenum and cites the vomiting of cholera vibrios as evidence that they may be present in the duodenum and stomach. Greig (2) refers to a lymphatic infection, but it is not clear how the lymph reaches the gall-bladder from the intestines. In a later article (21) Greig apparently also accepts the direct infection of the gall-bladder wall. Kulescha favors the theory of descending infection on account of foci of infection found in the liver. While cholera is not usually classed as a septicemia and cholera organisms have been recovered from the blood rarely, Greig (2) reports their presence in the lungs, and they have been found in the urine a number of times (22).

These facts point to an occasional invasion of the blood and it seems likely that if blood cultures could be made from the mesenteric veins positive results would be more frequently obtained. Experimental evidence favors this mechanism of gall-bladder infection as it has been shown above that more organisms appear in the bile after mesenteric vein injection than after ear vein injection. The gradual development of immunity would also favor the deposition of organisms in the liver and their increased elimination by the bile.

In dysentery, blood cultures are occasionally successful (4) and dysentery bacilli have been found in various organs, including the gall-bladder, at autopsy. In cholera and dysentery it seems likely, therefore, that gall-bladder infections are due to a portal or general septicemia with elimination of organisms in the bile as in typhoid. The occurrence of liver abscess in amebic dysentery is suggestive along the same line.

In experimental animals persistent gall-bladder lesions have apparently not been produced by intravenous injections of cholera vibrios. A considerable amount of work has been done with animals by Greig (21), Cano (22), Schöbl (23), Baroni and Ceaparu (24), and others, but in each case in which the vibrios have been found in the bile after intravenous injection the animals died or were killed in a few hours. In cases of survival after injection, Schöbl found no lesions in guinea pigs, and Greig found no lesions in one rabbit.

A possible explanation of the failure in guinea pigs will appear later. In three rabbits given ear vein injections of 1 cc. of a 24 hour broth culture, I found no lesions and no vibrios in the bile at the end of 1 week. In three rabbits given the same dose by a mesenteric vein, one animal had bloody bile, but no vibrios were found in it. Further work on the rabbit with more careful dosage is indicated.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

Direct inoculation of the gall-bladder of the guinea pig was uniformly successful in Schöbl's hands, and in the rabbit I have produced three persistent lesions in three trials.

With dysentery bacilli less work has been done. Doerr (12) found the Flexner type in the bile 24 hours after intravenous injection in the rabbit, but not after 4 and 14 days. With the Y type I have produced three lesions after four trials by mesenteric vein injection and one after three trials by ear vein injection. By direct inoculation of the Flexner type, out of five trials, five lesions were present at the end of a week. Further experimental work with intravenous injections is needed. Cholera vibrios and dysentery bacilli may be less able to produce gall-bladder lesions in the rabbit than the organisms of the typhoid and colon groups. The clinical percentage of chronic carriers in cholera and dysentery is apparently less than that in the typhoid group; but this may in part be due to a small percentage of cases with invasion of the blood.

Antiseptic Properties of Rabbit Bile.

The second condition to be determined in testing our working hypothesis, namely, multiplication of the organisms in the bile, evidently depends on the qualities of bile as a culture medium. It seems to be generally assumed that ox bile is a standard of bile and that bile is an excellent culture medium for the organisms of the typhoid group. As a matter of fact both these conceptions are more or less incorrect. There is no standard bile, as bile from different animals differs in its action on microorganisms. Even ox bile and human bile may have a distinctly bactericidal action on the typhoid bacillus, as has been shown by Corrado (25), Talma (26), Pies (27), Fornet (28), Meyerstein (29), and others. It is only by the addition of organic matter such as blood or pus that bile regularly becomes a good culture medium. A suggestive recent finding in this connection is that the lactose bile is inferior to the plain lactose broth for the detection of colon bacilli in water (30, 31).

In the following work the difference between the bile in the gall-bladder and the bile directly from the liver has not been considered, but this may be of some importance as the former is known to be richer in solids and, as Okada (32) has shown, may be less alkaline.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

In the case of the rabbit and guinea pig the antiseptic action is strongly marked, as may be seen in Tables III and IV. One loopful of a broth culture of different organisms was put in 1 cc. of different kinds of bile. One loopful was plated immediately and one loopful was plated after 24 hours.

TABLE III.

Antiseptic Action of Bile.

1 loop plated immediately.

Bile.	Typhoid.	Para-A.	Para-B.	Cholera.	Dysentery (Flexner).	Colon.
Ox	240	320	160	10	160	640
Human	1,000	1,600	1,600	50	400	1,600
Rabbit	400	500	800	40	500	In.*
Guinea pig	400	400	500	5	640	800

* In. indicates innumerable.

TABLE IV.

Antiseptic Action of Bile.

1 loop plated after 24 hours' incubation.

Bile.	Typhoid.	Para-A.	Para-B.	Cholera.	Dysentery (Flexner).	Colon.
Ox	In.	In.	In.	In.	In.	In.
Human	"	"	"	"	"	"
Rabbit	—	—	—	—	—	—
Guinea pig	—	—	—	—	—	—

This experiment shows that typhoid, cholera, and dysentery organisms grow well in human or ox bile, but are killed after 24 hours in guinea pig and rabbit bile. All specimens of guinea pig bile killed all the organisms tested. In the rabbit there was some variation especially in regard to cholera vibrios; human and ox bile also vary in effect, but the above result is typical of a certain number of specimens. A fairly long exposure is necessary as there is not evidence of antiseptic action at the end of 1 hour. These facts are plainly of considerable importance in many ways. They may explain failure of infection even with doses large enough to insure the passage of

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

bacilli through the bile. They also furnish a concrete instance of a difference between an experimental animal and man. As a side issue an example may be cited of failure to appreciate this difference. Greig (21) in endeavoring to find some means of preventing cholera carriers, gave rabbits urotropin and tested their bile for vibricidal properties. As a control he used ox bile and while the vibrios grew well in ox bile they failed to grow in the treated rabbit bile. Greig notes the unusual fact that urotropin seemed to be active in an alkaline medium. It is clear from the above results that the vibrios might not have grown in a control of normal rabbit bile.

The reaction of bile from different species varies as much as does the color, and the reaction seems to be of considerable importance in explaining its action on microorganisms. Table V shows the reaction to phenolphthalein and lacmoid; a satisfactory, simple method (32) of titrating the reaction of all specimens of bile has not been found on account of the difference in color. Phenolphthalein is a good indicator for rabbit and guinea pig bile, but it is difficult to use on human and ox bile. With lacmoid the end-point is also difficult to determine exactly.

TABLE V.

Reaction of Bile.

Bile.	Color.	Phenolphthalein.	Lacmoid.
Ox	Dull brown.	Acid.	2*
Human	Golden "	"	Neutral.
Rabbit	Bright green.	Neutral.	3–6
Guinea pig	" yellow.	1 per cent alkaline.	8

* The figures for lacmoid show the number of cc. of N/1 acid required to neutralize 100 cc.

These analyses taken together with the bactericidal tests given above suggest that the alkalinity is responsible for the antiseptic action; guinea pig bile, for example, is 2 per cent more alkaline than usual culture media. If guinea pig or rabbit bile is neutralized by hydrochloric acid or sulphuric acid, the antiseptic properties disappear, as is seen in Tables VI and VII, in which the same technique was employed.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

TABLE VI.

Antiseptic Action of Fresh Bile.

	Rabbit.		Guinea pig.	
	1	2	1	2
	1 loop plated immediately.	1 loop plated after 24 hours.	1 loop plated immediately.	1 loop plated after 24 hours.
Typhoid	50	—	400	—
Para-A	240	—	400	—
Cholera	10	1,600	5	—
Colon	800	—	640	—
Dysentery	480	—	800	—

TABLE VII.

Absence of Antiseptic Action of Bile When Neutralized.

	Rabbit.		Guinea pig.	
	1	2	1	2
	1 loop plated immediately.	1 loop plated after 24 hours.	1 loop plated immediately.	1 loop plated after 24 hours.
Typhoid	200	In.	800	In.
Para-A	200	"	500	"
Cholera	75	"	15	"
Colon	750	"	400	"
Dysentery	480	"	480	100

The alkalinity of the bile may not be the only factor concerned in its inhibitory action, but it must be an important factor in view of these results. It is instructive to note that of all the organisms, the only one which grew in fresh alkaline bile was the cholera vibrio which is known to grow best in an alkaline culture medium.

Prophylaxis.

These facts naturally suggest that an alkaline therapy might be of value in preventing or curing carriers. The reaction of rabbit bile can be influenced by injections of alkalies. A common duct fistula was made and 20 cc. of a 5 per cent solution of sodium bicarbonate, or 1 gm., were given intravenously and the reaction of the bile was

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

tested at different times. At the end of an hour the reaction was nearly twice as alkaline as at first and then it fell again.

In order to make a preliminary test of alkaline therapy in preventing gall-bladder infections, four animals were given 1 gm. of sodium bicarbonate in 5 per cent solution, intravenously, and after 1 hour they, with four controls, were given 2 cc. of a suspension of typhoid bacilli, equal to one 24 hour agar slant. At the end of a week they were chloroformed and examined; the controls showed 50 per cent of lesions, the treated animals showed 25 per cent of lesions. In other words, some prophylactic effect was apparently demonstrable. Further work is necessary but the practical suggestion for the trial of alkalies in the prevention and treatment of carriers is obvious.

It is known that gall-bladder infections in the rabbit tend to spontaneous cure, and further work on this subject should be done with a view to determine the part played by the bile in this process.

DISCUSSION.

The results of the experiments recorded above support the theory of descending infection of the gall-bladder through the bile from the liver. Infection of the gall-bladder wall cannot be absolutely ruled out and probably occurs at times, but the bile-ducts seem to be the regular avenue of infection. This conclusion suggests that prophylactic measures and possibly curative measures should be directed toward the bile rather than toward the blood stream and tissues. Vaccination, for example, appears to have little effect in the prevention and cure of experimental or clinical lesions and in fact may favor the production of lesions by increased elimination of organisms in the bile. A great deal of experimental work has been done with various drugs and synthetic substances, but the subject of the natural defences of the bile and the possibility of increasing them has been neglected. Human bile must have some antiseptic action, because, in any septicemia, some microorganisms undoubtedly pass through the bile-ducts and gall-bladder, but in only a comparatively few cases do they produce a definite cholecystitis. *In vitro*, some specimens of human bile are bactericidal or inhibitive, while others are not. Experiments into the nature of this inhibition with a view to increasing it have been done with the rabbit.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

Rabbit bile is more alkaline than human bile and is more antiseptic for all the organisms considered in this paper, except in some instances for cholera vibrios. Rabbit bile has some effect in preventing lesions, because doses large enough to insure the passage of organisms through the bile and large enough to make an animal sick or even kill it often show no organisms on culture of the bile. Gall-bladder lesions in the rabbit are probably due, first, to a sufficient dose to insure a considerable number of microorganisms in the bile; second, to a low margin of the inhibitory action in the bile or to an increased resistance on the part of the strain employed; and third, to an injury of the liver cells or mucous membrane of the gall-passages and bladder with desquamation of cells and oozing of serum. Pies (27) showed that *in vitro* the inhibitory action of the bile could be abolished by the addition of serum or pus. In the direct inoculation of the gall-bladder this factor probably is important in the almost invariable success of the method. In man the production of gall-bladder lesions probably depends on the same factors as in the rabbit. The difference between rabbit and human bile is only a question of degree, and valuable lessons may be learned from the rabbit, especially in regard to the cause of failure of intravenous injections to produce 100 per cent of lesions.

It is a debatable question whether a patient suffering from an acute infection should be subjected to additional treatment in order to prevent him from the possibility of being a danger to others, provided he recovers, but if a mild therapy were of any value it would be justified. In cholera, for example, alkaline therapy is of value in preventing uremia, which is the greatest danger to the individual, next to collapse (33). If this treatment also helped to keep the bile clean, this fact should be kept in mind. However, as the cholera vibrio grows best in an alkaline medium, this line of treatment may favor cholecystitis. These questions are subjects for further investigation. When the individual recovers from an acute attack, the best practice requires that he shall not be discharged as cured until the carrier question is considered. Aside from the examination of the stool, the use of Einhorn's duodenal tube (34) seems to be a valuable aid in determining the condition of the bile.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

SUMMARY.

1. The theory of the production of gall-bladder lesions in typhoid, by descending infection of the bile from the liver receives support from investigations with the common duct fistula method in the rabbit.

More bacilli appear in the bile with increased doses and more gall-bladder infections are obtained by increased doses.

More bacilli appear in the bile after mesenteric vein injection than after ear vein injection and more lesions result under the first condition.

More bacilli appear in the bile after injection of the same dose in immunized animals than in normal animals and more lesions also result in immunized animals.

In cholera and dysentery the same mechanism is suggested with the additional factor of a portal system septicemia.

2. After the appearance of microorganisms in rabbit bile, their fate is apparently largely determined by the antiseptic properties of the bile.

100 per cent infections cannot be secured by intravenous doses large enough to insure the presence of microorganisms in the bile.

Rabbit bile *in vitro* may be antiseptic to the microorganisms considered.

The antiseptic action is largely due to its alkalinity.

It is apparently possible to protect the rabbit to some degree against gall-bladder infection by a previous injection of sodium bicarbonate.

3. Alkaline therapy is suggested in the prevention and cure of gall-bladder carriers.

BIBLIOGRAPHY.

1. Kulescha, G. S., *Klin. Jahrb.*, 1911, xxiv, 137; *Centr. Bakteriol., Ite Abt., Orig.*, 1909, l, 417.
2. Greig, E. D. W., *Indian J. Med. Research,* 1913–14, i, 44, 59; 1914–15, ii, 1, 28.
3. Schöbl, O., *Philippine J. Sc., B*, 1915, x, 11.
4. Ghon, A., and Roman, B., *Wien. klin. Woch.*, 1915, xxviii, 579.
5. Koch, J., *Z. Hyg. u. Infectionskrankh.*, 1909, lxii, 1.
6. Chiarolanza, R., *Z. Hyg. u. Infectionskrankh*, 1909, lxii, 11.
7. Lange and Roos, *Arb. k. Gsndhtsamte.*, 1915, l, 57.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022

8. Anton, B., and Fütterer, G., *Münch. med. Woch.*, 1888, xxxv, 315.
9. Fütterer, G., *Medicine*, 1895, i, 279.
10. Fütterer, *Berl. klin. Woch.*, 1899, xxxvi, 58.
11. Biedl, A., and Kraus, R., *Centr. inn. Med.*, 1896, xvii, 737.
12. Doerr, R., *Centr. Bakteriol., Ite Abt., Orig.*, 1905, xxxix, 624.
13. Bull, C. G., *J. Exp. Med.*, 1915, xxii, 475.
14. Blachstein, A. G., *Bull. Johns Hopkins Hosp.*, 1891, ii, 96.
15. Welch, W. H., *Bull. Johns Hopkins Hosp.*, 1891, ii, 121.
16. Greig, *Indian J. Med. Research*, 1916, iii, 259.
17. Nichols, H. J., *J. Exp. Med.*, 1914, xx, 573.
18. Kayser, H., *Deutsch. med. Woch.*, 1904, xxx, 1803.
19. Forster, J., and Kayser, H., *Münch. med. Woch.*, 1905, lii, 1473.
20. Irons, E. E., and Jordan, E. O., *J. Infect. Dis.*, 1915, xvii, 234.
21. Greig, *Indian J. Med. Research*, 1914–15, ii, 907.
22. Cano, U., *Centr. Bakteriol., Ite Abt., Orig.*, 1914, lxxii, 124, 125.
23. Schöbl, *J. Infect. Dis.*, 1916, xviii, 307.
24. Baroni, Y., and Ceaparu, V., *Compt. rend. Soc. biol.*, 1912, lxxii, 894.
25. Corrado, B., *Centr. Bakteriol.*, 1892, xi, 696.
26. Talma, S., *Z. klin. Med.*, 1901, xlii, 354.
27. Pies, W., *Arch. Hyg.*, 1907, lxii, 107.
28. Fornet, W., *Arch. Hyg.*, 1907, lx, 134.
29. Meyerstein, W., *Centr. Bakteriol., Ite Abt., Orig.*, 1907, xliv, 434.
30. Cumming, H. S., *Bull. Hyg. Lab., U. S. P. H.*, 1916, No. 104, 105.
31. Obst, M. M., *J. Bacteriol.*, 1916, i, 73.
32. Okada, S., *J. Physiol.*, 1915, l, 114.
33. Sellards, A. W., *Philippine J. Sc., B*, 1910, v, 363.
34. Einhorn, M., *J. Am. Med. Assn.*, 1916, lxvi, 1908.

Downloaded from http://rupress.org/jem/article-pdf/24/5/497/1174244/497.pdf by guest on 27 September 2022