

SUPPURATIVE CHOLECYSTITIS WITH CHOLELITHIASIS
IN A HUMAN "CARRIER" OF THE *BACILLUS*
ENTERITIDIS OF GAERTNER.

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CHOLECYSTITIS and cholelithiasis are of themselves conditions of great interest from the surgical and pathological standpoint, but the comparatively recent demonstration of the relationship existing between them and the "carrier" state in typhoid fever has made them subjects especially worthy of further study.

In the course of a number of examinations of normal and pathological gall bladders the following observations were made, and seem for several reasons to be of sufficient importance to be put on record. Before entering on these I shall discuss briefly certain questions which have a bearing on the present case.

In spite of the fact that the gall bladder is in communication by means of the bile ducts with the alimentary canal which contains numerous micro-organisms, the bile, under normal conditions, remains sterile, not because it possesses bactericidal properties, but simply on account of its movement towards the gut.

Two views are held as to the paths by which the infective agent finds its way into the gall bladder. The one, whose chief supporter is Chiari, is that where the downward movement of the bile is interfered with by any cause, bacilli ascending the ducts find their way into the gall bladder where they multiply, and, in the case of pathogenic organisms, give rise to pathological changes. The other is that the bacilli find their way into the gall bladder from the blood stream. This view, in favour of a haematogenous origin, has many supporters, and evidence has been adduced in its favour from the appearances found in infection of the gall bladder in man by Joseph Koch (1908) and from experimental

results obtained in animals by Dörr (1905), Chiarolanga (1908), and others. The present case, in regard to this point, affords no evidence in favour of one view more than the other.

In regard to the micro-organisms which have been found associated with cholecystitis and cholelithiasis the most important is *Bacillus typhosus*. Round this subject there has accumulated a great mass of literature which has been recently reviewed by Ledingham (1910).

The organism most frequently present in such cases is the *B. coli communis*. Laubenheimer (1908) out of 36 cases where operation had been performed for empyema of the gall bladder found it 21 times (58·3 %); 18 times in pure cultures (50 %). The same observer found among the 36 cases *B. pyocyaneus*, *Streptococci*, *Staphylococci*, *B. influenzae*, "Capsule" bacillus and *B. typhosus*. To these must be added the *Pneumococcus* (Etienne) and certain anaerobes (Gilbert and Lippmann (1907)). It is possible that the paracolon bacilli of some of the earlier workers on this subject belonged to the paratyphoid or Gaertner group.

Blumenthal (1907) holds that the *B. coli communis* is probably a secondary invader, the original cause of the condition having died out. He believes that certain organisms of the *coli* group which may have a causal relation to the condition are not identical with the *B. coli communis* of the gut. This view is combatted by Laubenheimer who examined 11 strains of the colon bacillus from cholecystitis and found that in cultural and biological characters they were identical with the *B. coli communis* of the gut.

In regard to cholecystitis due to the paratyphoid bacilli, the group of bacilli most closely allied to the organism found in the present instance, only a few cases are on record: Blumenthal (1904) isolated *B. paratyphosus* A from the material obtained from the gall bladder of a female patient who had been operated on for cholelithiasis. In this case the wall of the gall bladder was thickened and contained gall stones and a small quantity of bile but no pus. Blumenthal found the same bacillus in the stools on one occasion, and the serum of the patient agglutinated the bacillus in a dilution of 1 in 300.

Forster, J., and Kayser, H. (1905) examined post-mortem the contents of the gall bladder in 148 cases. Eight of these had died from typhoid fever, and in seven typhoid bacilli were found. In the eighth case, in which the body had been dead a long time, only *B. coli communis* was found; but typhoid bacilli were found in the blood of a niece who had acted as nurse to the case. Among 140 cases which had died from

diseases other than typhoid fever were two individuals the subjects of cholelithiasis in whom no history of typhoid fever could be ascertained. In the one, aged 84, *B. typhosus* was present, and in the other, aged 71, the *B. paratyphosus* of the type "B." In this case the gall bladder contained abundance of brown bile and two gall stones.

Gaechtgens (1907) found *B. paratyphosus* B in the stools of two female patients who had the symptoms of cholelithiasis, and Dudgeon (1908) found the same organism in a case of suppurative cholecystitis.

Evers and Mühlens (1909) reported an acute case of suppurative cholecystitis with commencing peritonitis following an acute illness lasting three weeks, which was characterised by gastro-intestinal symptoms and the presence of foul-smelling stools. After the operation slimy purulent fluid escaped, and later numerous faceted gall stones. From the gall bladder contents *B. paratyphosus* B was isolated, identified and tested for virulence by Lentz who found it highly virulent. The fluid from the gall bladder gave positive cultural results on several occasions, and the urine and stools were found to contain the same bacillus. Cultures of the bacillus were obtained from the biliary fistula two-and-a-half months after the operation. The blood serum agglutinated the paratyphoid bacillus in a dilution of 1 in 400 and was negative to the typhoid bacillus to 1 in 25.

Hamilton (1910) from 24 cases of cholecystitis isolated 12 organisms, in two cases *B. typhosus*, in three cases *B. coli* and in seven cases *B. paratyphosus* of which four appeared to belong to the "B" and three to the "A" type.

With regard to the association of the *B. enteritidis* of Gaertner with cholecystitis, as in the case about to be recorded, I have not been able to find a single case in the literature available to me. It seems to me that this may be due, not so much to the rarity of the condition, as to the absence in a number of cases of sufficient tests having been applied to enable the determination of the nature of the organism.

In regard to the formation of gall stones it is probable that in the majority of cases, perhaps in all, a bacillary infection is the primary cause. The evidence in favour of this view has been derived not only from observations on operative and post-mortem material from the human subject, but also from experimental results obtained in animals.

Typhoid and paratyphoid bacilli have been found within the gall stones in a number of cases, e.g. Blumenthal (1904-1907), Forster and Kayser (1905) and others. In the case to be recorded *B. enteritidis* Gaertner was cultivated from the gall stones.

As pointed out by Forster the mere finding of the bacilli within the gall stones does not prove that they have a causal connection with the gall stones, for their presence might be interpreted in another way, viz. that they had grown into the gall stone after its formation; indeed Gilbert and Bacmeister have proved experimentally (the former in porous cholesterine stones, the latter in calcium-cholesterine stones) that secondary invasion of the calculi can take place. Aschoff and Bacmeister have, moreover, several times found typhoid bacilli in the centre of large gall stones in cases of typhoid fever in the second or third week. The writer had recently in his laboratory a similar experience. They maintain that the bacilli must have wandered into the already formed calculi. No evidence, however, is adduced that these were not second attacks, the bacilli having persisted in the gall bladder and calculi from an earlier infection.

The formation of calculi with an accompanying first attack of biliary colic soon after an attack of typhoid fever is strongly in favour of the view that the gall stones are of bacillary origin. A number of such cases have been recorded. Hilgermann (1909) reports a case where calculi were found within two months of the recovery from typhoid fever. The results obtained by animal experiments strongly support the bacillary origin of the calculi. As early as 1899 Richardson found that an injection of typhoid bacilli into the gall bladder of a rabbit was followed by the formation of calculus, and Cushing (1899), Dörr (1905) and others obtained like results from the same animals by intravenous injection of typhoid bacilli.

It is unnecessary to refer to the extensive literature on this subject. It is sufficient here to say that evidence will be adduced in this paper which appears to indicate that the *B. enteritidis* Gaertner stands in relation to the production of cholecystitis and cholelithiasis on the same footing as the typhoid and paratyphoid bacilli.

The present investigation.

History of the case. Case of Mrs M. C. aged 61. No previous illness except inflammation of the lungs when young. There was no history of acute food poisoning. The patient when 22 years of age had "gastric fever." Before her present illness she had frequent attacks of indigestion. The present illness commenced about ten years ago with pain in the region of the gall bladder. The pain came in spasms, and was very often severe, shooting through to the back and up to the right shoulder.

It lasted about a fortnight at first, then went away and recurred again and has kept on recurring at intervals till five years ago when it ceased and at the same time she passed a calculus about the size of a hazel nut. For the next four years she had no trouble at all. About one year ago the pain again commenced to trouble her, and every time it was severe she became of a yellow colour which went after cessation of the pain. This occurred several times during the year.

Physical examination. On inspection of abdomen nothing unusual to be seen. On palpation there is distinct tenderness and resistance over the gall bladder region, which is worse when deep palpation is performed, especially during inspiration. There is no enlargement of the gall bladder to be felt, and no tumour. Liver extends to about half-an-inch below the costal margin.

She was admitted to the Aberdeen Royal Infirmary on November 3rd, and was operated upon on the 5th, cholecystotomy being performed, and the patient was discharged on the 21st. On February 9th she had an attack of pain over the left lobe of the liver and on the following day had marked jaundice of the skin and conjunctiva, and bile was demonstrated in the urine.

I am indebted to Dr Fowler, Woodside, and to Mr H. M. Gray, Aberdeen, for the above history, to the former for samples of blood and faeces and to the latter for the pus, gall stones, and part of the gall bladder.

Examination of part of the wall of the gall bladder. The fundus, which alone was available for examination, was considerably thickened, reaching from 5 to 7 mm. The mucous membrane was folded and covered with an admixture of blood, pus and bile.

Histological examination. The gall bladder showed, in addition to the folding of the mucous membrane, a new formation of epithelial bud-like processes. The vessels in all the layers were dilated, and this was especially marked in the mucous membrane. The vascular dilatation was accompanied by numerous haemorrhages, some large enough to be visible to the naked eye on section. There was present a round-cell infiltration of all the coats which was most pronounced in the mucous membrane, submucosa and muscularis. The cells consisted chiefly of lymphocytes and fibroblasts, but a few polymorphs were present. There was some fibrous thickening of the serous coat. The appearances, therefore, are those associated with recurrent or chronic cholecystitis. Gram-negative bacilli were demonstrated in the wall by Unna's polychrome-methylene-blue method.

Bacterial examination. With the fundus of the gall bladder removed during the operation, some of the contained fluid and six gall stones were received for examination. Some of the fluid (a mixture of bile, pus and blood) was plated on MacConkey's neutral-red bile-salt agar; only non-lactose fermenting colonies appeared. The gall stones were about the size of large peas, and were of the calcium-cholesterine type. One of these was dipped in methylated spirit, which was burned off, and the calculus was then ground up with some broth in a sterile mortar. The mixture was plated as before and gave the same result.

Samples of faeces taken a week after the operation, and three months after the operation, gave non-lactose fermenting colonies. The non-lactose fermenting organisms obtained from these four sources were submitted to further examination and appeared to be identical.

Characters of the bacillus isolated.

The bacillus was a Gram-negative rod, slightly motile, did not liquefy gelatin and did not produce indole. It gave the following fermentation reactions: acid and gas were produced on glucose, mannite, dulcitol, maltose, mannose, galactose, arabinose, sorbitol, whereas no marked change took place in lactose, saccharose, raffinose, inulin, dextrin, salicin, erythritol.

Litmus milk became slightly acid in the 24 hours, then alkaline, and in 15 days this alkalinity was very pronounced. From these reactions, therefore, the organisms appear to belong to the group comprising *B. suispestifer*, *B. paratyphosus* B, *B. enteritidis* Gaertner, *B. typhi murium*, *B. aertryck*, and at first it was suspected that one had here a carrier of the *B. paratyphosus* B, as several such cases had already been recorded.

A study of the pathogenicity of the bacillus, further agglutination and absorption tests, and a comparison with well authenticated strains proved, however, that the bacillus was the *B. enteritidis* Gaertner from the original strain of which it was indistinguishable.

Pathogenic effects. Two guinea-pigs were inoculated intraperitoneally with the bacillus. One received 250 mgrm. of a broth culture (24 hours) and died within 48 hours of acute peritonitis and general infection. The second animal received 10 mgrm. and died in 10 days. In this case there was a milky deposit on the peritoneum, pale yellow or almost white nodules about the size of millet to hemp seed in the liver and in the spleen which was enlarged. From both animals the bacillus was isolated in pure culture. *Rabbits.* One of these died during immuni-

sation and presented post-mortem appearances closely resembling those described in the second guinea-pig. *Rats.* Three of four young rats, which were fed with cultures of the bacillus, died with the symptoms and post-mortem signs of acute enteritis.

Agglutination tests.

I was fortunate in possessing a number of sera for which I was indebted to Dr Bainbridge, enabling me at once to carry out the primary agglutination tests. The results of agglutination tests with these sera correspond to the various members of the group shown on Table I.

TABLE I.

Serum	Pus	Gall stone B.	Faeces B.
<i>B. paratyphosus</i> A (titre 20,000)	<100	<100	<100
<i>B. paratyphosus</i> B (titre 2000) Also for <i>B. aertryck</i> , 1000	<100	<100	<100
<i>B. typhi murium</i> (titre 4000)	<100	<100	<100
<i>B. aertryck</i> (titre 5000)	<100	<100	<100
<i>B. Gaertner</i> (titre 8000)	6400	6400	6400

All the agglutination tests were carried out by the macroscopic method in small test tubes.

C = Complete sedimentation.
 ++ = Marked agglutination.
 + = Slight agglutination.

TABLE II.

Bacillus	Dilution...	Serum (Faeces Bacillus).							
		100	200	400	800	1600	3200	6400	Control
<i>B. Gaertner</i> (original)	C	++	++	+	+	0	0	...	
Faeces B.	C	++	++	+	+	0	0	...
		Serum (Pus Bacillus 16. 1. 11).							
<i>B. Gaertner</i> (original)	C	C	C	C	++	0	0	0	
Gall-stone B. ...	C	C	C	C	++	+	0	0	
B. from faeces 23/1/11	C	C	C	C	++	+	+	0	
Pus B.	C	C	C	C	++	+	+	...
		Serum (Gall-stone Bacillus).							
<i>B. Gaertner</i> (original)	C	C	C	++	0	0	0	...	
Gall-stone B. ...	C	C	C	++	+	0	0	...	
Pus B.	C	C	C	++	0	0	0	...
Faeces B.	C	C	C	++	0	0

The serum of the patient was also tested against its own bacilli and against the original strain of Gaertner, and in both cases gave distinct agglutination in a dilution of 1-40.

Rabbits were immunised with three of the races of bacilli isolated from the case and a number of the agglutination tests were carried out with these. Some of the results of these tests, at different stages of agglutination, are shown on Tables II, III, IV.

The result of an absorption test is shown on Table V.

TABLE III.

Bacillus	Dilution...	Serum (Gall-stone Bacillus).					Control
		10000	20000	40000	80000	160000	
Gall-stone B.	...	++	+	0	0	0	...
Faeces B.	...	++	+	0	0	0	...
Pus B.	...	++	+	+	0	0	...
<i>B. Gaertner</i>	...	++	+	0	0	0	...

Bacillus	Dilution...	Serum (Pus Bacillus).					Control
		10000	20000	40000	80000	160000	
Gall-stone B.	...	++	++	0	0	0	0
Faeces B.	...	++	+	0	0	0	0
Pus B.	...	++	++	+	0	0	0
<i>B. Gaertner</i> (original)	...	++	+	0	0	0	0

TABLE IV.

Agglutination of Bacilli isolated from Faeces on 25/2/11, i.e. about three months after operation.

Dilution...	Gaertner Serum (titre 8000).						Control
	100	200	400	800	1600	3200	
C	C	C	C	C	++	+	0

TABLE V.

Agglutination at dilution of 1-80 after absorption.

Serum	Absorbed by	<i>B. Gaertner</i>	Pus B.	Gall-stone B.	Faeces B.
<i>B. Gaertner</i>	0	8000	6400	6400	6400
	<i>B. Gaertner</i>	0	0	0	0
	Gall-stone B.	0	0	0	0

DISCUSSION.

The *B. enteritidis* Gaertner, the organism with which we are dealing, is a member of the group of bacilli associated with the production either of outbreaks of acute food poisoning, where the dose or the virulence of the infective material has been great, or of a less acute typhoid-like gastro-enteritis where the dose has been small, the virulence low, or the resistance great.

The other more important members of the group are *B. paratyphosus* B, *B. suispestifer*, and *B. aertryck*, the two latter being regarded by many authorities as identical.

The outbreaks of food poisoning due to *B. enteritidis* Gaertner have usually followed the ingestion, in some form or another, of the flesh of the horse, ox or pig, and not infrequently it has been found that the animals from which the material originated had been in a diseased condition at the time of slaughter. This, however, has not been invariably the case and the observations here recorded are of interest from the standpoint that they may help to explain how in certain cases sound meat may become contaminated by human handling.

Some scepticism has been shown by certain workers as to the pathogenic importance of this group of bacilli. They claim to have found them in non-toxic food stuffs and also in healthy individuals, and as a result show a tendency to regard them as ubiquitous. If this view were correct, the observations in this communication would be deprived of their chief significance. This sceptical view, however, is not borne out by the work of Hilgermann (1910) who holds that the majority of cases of infection due to *B. paratyphosus* B. can be traced to infected individuals. The work of Otto Meyer (1909) is also opposed to the view, for out of a large number of examinations he found *B. paratyphosus* B. only twice in the stools of healthy individuals and both proved to be contacts; the one with an "acute" and the other with a "carrier" case. In the stools of over a hundred healthy soldiers he did not find it once.

The *B. enteritidis* Gaertner has been proved to be a cause of certain epizootics among rabbits, guinea-pigs and rats. This may have some significance in relation to human infection in view of the widespread use of rat viruses which contain this bacillus (Bainbridge (1909)). Apart from these, the bacillus is by no means widespread among human beings or the lower animals, and endeavours to find it in the

discharges, etc. of normal man and animals have almost invariably failed.

There is much evidence confirming the earlier observations in regard to *B. enteritidis* Gaertner made by van Ermengem who failed, out of a large number of examinations, to find the bacillus in the stools of healthy individuals, in the organs from bodies advanced in putrefaction, in putrefying food stuffs, etc. Among recent evidence in this direction may be quoted the work of Aumann (1911), working under Dunbar and Trautmann, who, after an elaborate investigation, asserts that the bacilli of the paratyphoid and Gaertner group are not found apart from disease in man or animals. When they occur in healthy animals they must be regarded as "carriers" in the same sense as human "carriers."

Zweifel's (1911) researches led to the same conclusion. The case reported is of interest in this connection for it goes to prove that in certain cases *B. enteritidis* Gaertner may have its habitat in the gall bladder and be shed out periodically in the faeces in a manner exactly analogous to that which occurs in the case of the typhoid and paratyphoid bacilli. In this case there must be the same risks of infection as have been proved to exist in the case of typhoid and paratyphoid.

Infection may be by contact but, as has been shown in those conditions, the danger is greater where the "carrier" is an individual dealing with food stuffs.

SUMMARY.

In a case of suppurative cholecystitis with cholelithiasis a bacillus was isolated from the pus, gall bladder, and stools which has the morphological, biological and cultural characters of *B. enteritidis* Gaertner. This bacillus is pathogenic for the rat, rabbit and guinea-pig, and gives rise to the characteristic lesions produced by the Gaertner bacillus. The serum of the patient agglutinated the three strains of bacilli isolated from the above mentioned sources and the original Gaertner strain in a dilution of 1 in 40. A serum, prepared by injecting *B. enteritidis* Gaertner (original strain) into a rabbit, agglutinated the three strains isolated from the case and the homologous bacillus in almost the same dilutions.

A serum prepared from any of the three bacilli agglutinates the homologous organism, the other two strains and the original Gaertner bacillus in almost the same titre. The sera homologous to allied organisms do not agglutinate these bacilli. Absorption tests confirm these results

and prove that the bacilli isolated are identical with the Gaertner bacillus.

The chief points of interest in the case are that :

1. It forms a link in the chain of evidence showing that as in the case of the paratyphoid bacillus the *B. enteritidis* Gaertner may give rise, not only to the more acute toxic form of poisoning, but also to a sub-acute paratyphoid type of the disease.

2. It establishes the association of *B. enteritidis* Gaertner with suppurative cholecystitis and with cholelithiasis.

3. It proves that this bacillus may have its habitat in the gall bladder and be shed out at intervals into the faeces, in a manner exactly analogous to what occurs in the case of typhoid and paratyphoid infections.

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