

AN INVESTIGATION INTO THE ACID-FAST BACTERIA
 FOUND IN HUMAN FAECES WITH SPECIAL REFER-
 ENCE TO THEIR PRESENCE IN CASES OF TUBERCU-
 LOSIS.

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UP TO the year 1906 tubercle bacilli were considered to occur in the faeces only in pulmonary or abdominal tuberculosis, due to ingestion of the infected sputum or to ulceration of the intestinal tract.

Wood (1905) for instance writes that tubercle bacilli are to be found in the faeces of persons suffering from pulmonary tuberculosis because in the majority of cases the bacilli are swallowed in small masses of sputum. Sahli (1906) states that they may occur in the faeces in intestinal tuberculosis and that "the stools may contain the bacilli even though there is no intestinal tuberculosis (if the patients swallowed their sputum)." He gives the warning that "the tubercle bacillus must be carefully distinguished from the smegma bacillus which is said to occur at the anal orifice, and might have become mixed with faeces." Lichtheim (1883) and Shaw (1897) are in agreement with these opinions, Boston¹ and Simon¹ favour the idea that the appearance of the bacilli in the faeces is strong evidence of intestinal ulceration. The former believes that their presence "points conclusively to the existence of tuberculous ulceration of the intestines"; the latter agrees with this statement if clinical symptoms point to the same conclusion.

Emerson (1906) showed that numerous bacilli in the faeces did not necessarily imply ulceration of the intestine. He demonstrated the

¹ Cited by Rosenberger (1907).

presence of acid-fast bacilli in 57 cases of pulmonary and abdominal tuberculosis. The post-mortem examinations of 33 of these revealed no ulceration of the intestine. Bodo (1892) is quoted as having examined the faeces of nine persons suffering from pulmonary phthisis, finding tubercle bacilli in three.

These observations show (1) that acid-fast bacilli may be present in the faeces in cases of pulmonary and intestinal tuberculosis, and (2) that their presence even in relatively large numbers does not necessarily imply ulceration of the intestine. They do not show that these acid-fast bacilli are tubercle bacilli.

Rosenberger (1907—1909) has made further progress in these investigations. He showed, firstly, that acid-fast bacilli could be demonstrated in the blood of tubercular patients, especially in cases of miliary tuberculosis. This demonstration of acid-fast bacilli in the blood-stream by staining methods has not been confirmed by other observers. It is certain however that they must be of frequent occurrence if the organisms are to be found in the faeces in any numbers. In this connection Rosenberger's observations upon cases of acute miliary tuberculosis are interesting. He found acid-fast bacilli in the faeces in every case. It is only by reference to the circulating blood that it is possible to explain the presence of these bacilli in the alimentary canal when the tubercular focus is in a distant region of the body. If the affected area be situated in the hip-joint or in the dorsal vertebrae, as was the condition in two of Rosenberger's cases, the blood-stream must eventually if not primarily, have been the path by which the specific bacillus reached the intestine. It becomes apparent, if these things be true, that not only must the old ideas regarding the appearance of the bacilli in the excreta be abandoned but also the conception of the bacilli in the blood must be changed and it be admitted that they occur far more frequently than was hitherto deemed possible.

Rosenberger showed secondly that acid-fast bacilli could be found in the faeces of patients suffering from tuberculosis other than that of the lungs or intestine.

He examined in all 672 cases of various forms of the disease and found an acid-fast bacillus in 120 of them. Of these 120 positive cases, 60 had been previously diagnosed as tubercular, the remaining 60 were suspicious cases.

Rosenberger never produced tuberculosis in animals with the acid-fast bacillus which he observed in the faeces. He therefore never proved that they were tubercle bacilli.

So far as the present writer can discover human faeces have never been examined with the view of establishing the identity of any acid-fast bacilli they might contain, except in three cases of typhoid to which reference will be made later.

Animal faeces have been examined twice with this object, by Schroeder and Cotton (1907) and by Griffith (1909). Naturally tuberculous cows were examined, and in a large percentage the faecal matter proved capable of producing infection when injected into guinea-pigs and rabbits, or fed to swine.

The Objects of the Research.

(1) Primarily to prove or disprove the identity of acid-fast bacilli in faeces with the tubercle bacilli. As this could only be accomplished by animal inoculation preliminary investigations were necessary to discover:

(a) What quantity of human faeces could be inoculated into guinea-pigs without producing fatal septicaemia.

(b) Under what conditions of the faecal contents acid-fast bacilli were most numerous.

After ascertaining these it remained to show that:

(2) Acid-fast bacilli are not found in the faeces under normal conditions or in diseases other than tuberculosis.

(3) The acid-fast bacillus present in the faeces of tuberculous persons were tubercle bacilli.

Observations were also made regarding:

(4) The proportion of cases of tuberculosis in which acid-fast bacilli could be demonstrated by staining methods in the faeces.

PRELIMINARY INVESTIGATIONS.

1. *Dilution of the faeces.*

The amount of the injection proved a great difficulty at first. It was considered necessary to find the largest amount of diluted faeces which the animal could survive. It was feared that with too high a dilution, the bacilli which were never present in any numbers would be lost. After several unfortunate results it was found that 0.02 gramme was the largest amount of human faeces that could with safety be injected

subcutaneously into a guinea-pig of average weight. The animal survived occasionally after the injection of 0·04 gramme. Death ensued occasionally after the injection of 0·01 gramme. The variations in the virulence of the faecal organisms and the resistance of the animals evidently swung within wide limits. It was deemed advisable to inoculate at least two animals from each sample. One received 0·02 gramme, the other 0·01 gramme. The routine employed was as follows:

A gramme of faeces was carefully weighed out on a watch glass, rubbed up in a mortar with normal saline and further diluted until the final volume reached 99 c.c. This gave a dilution of one in a hundred. (All apparatus used had been previously sterilised and the operations were carried out as rapidly as was consistent with accuracy.) Of this dilution 1 c.c. was injected into one animal, 2 c.c. into a second.

2. *The conditions under which the faeces contained the greatest number of acid-fast bacilli.*

It was noted that the demonstration of acid-fast bacilli was difficult or impossible in such stools as were hard and constipated and a reference to Rosenberger's paper made it plain that he had experienced a similar difficulty. He states that the bacillus was found "in solid stools 28 times; in semi-solid stools 40 times and in fluid stools 52 times." No mention is made of the use of purgatives so it must be concluded that none were employed. It is evident that there are circumstances connected with the formation of constipated stools which may lead to one of two conditions.

1. The bacilli are present but may not be demonstrated.
2. The bacilli are not present.

In discussing the probabilities of the first supposition it will be remembered that the *viable* bacteria of a loose motion and those of a constipated stool differ greatly in number. Whereas in the former 10—15 % of the total organisms may be living, in the latter only 2 % of the bacteria seen in a stained smear may be induced to form colonies in the most suitable medium known.

Are then the forces at work in a constipated stool powerful enough to kill the tubercle bacillus or can they destroy its staining reaction?

To elucidate this question an attempt was made to produce constipation *in vitro*, if such a term may be used.

Stools were taken from advanced cases of pulmonary tuberculosis. These were examined microscopically and shown to contain the organisms in numbers. They were allowed to remain in the bottles or jars in which they had been received, for varying periods under varying conditions of temperature. At the end of the time they were softened with normal saline, gently ground up in a mortar and fresh smears examined.

Table I shows the results of these experiments.

TABLE I.

Case	Faeces before	Period days	Conditions	Faeces after
A	++	7	Ice chest	+
B	++	10	„	++
C	+++	15	Incubator 37°	++
D	++	15	Ice chest	+
E	++	19	„	+
F	++	20	Room-temp.	+
G	+	20	„	+
H	+++	20	Incubator 22°	++
I	++	20	Ice chest	+

+++ = very numerous. ++ = numerous. + = present.

Acid-fast bacilli were demonstrated in every specimen after it had become hard and dry.

There is a noticeable diminution in the numbers that could be demonstrated.

Are the bacilli present in constipated stools?

Constipation is usually accompanied and frequently caused by lack of bile. Lack of bile is one of many causes of constipation but a recollection of Calmette's and Guérin's work (1909) on the liver drew attention in this direction. The conditions which govern the excretion of the typhoid bacillus are intimately connected with the flow of bile and the excretion is intermittent.

An intermittency in the excretion of the tubercle bacillus, if present, would suggest, among other things, that the bile had some connection with its excretion.

But first this intermittency had to be looked for. The consecutive motions of two men, both advanced cases, were examined. During the period over which these investigations extended no purgative was administered.

Acid-fast Bacteria

Case *A* had both lungs affected. Motions were easy and normally regular and he rarely required a purgative.

Case *B* suffered from constipation and frequently asked for a purgative. The results are given in the following Tables (II and III).

TABLE II. *Case A.*

Stool	Passed	Consistency	Fæces
1	a.m.	semi-solid	+
2	"	"	+
3	"	solid	—
4	"	semi-solid	+
5	"	"	+
6	"	"	—
7	"	"	+
Total	7		Total 5

TABLE III. *Case B.*

1	a.m.	solid	—
2	p.m.	"	—
3	a.m.	semi-solid	—
4	"	"	+
5 & 6	a.m. & p.m.	fluid (diarrhoea)	+ a.m. & p.m.
7	a.m.	semi-solid	+
8	"	solid	—
Total	8		Total 4

Case *A* excreted the bacillus more regularly than *B*, in whose faeces the bacillus only appeared with the onset of diarrhoea.

Both *A* and *B* were given half a grain of calomel at night and a seidlitz powder in the morning. The results are given below.

Case A. Calomel and Seidlitz.

Stool	Description of stool	Fæces
1	semi-fluid	+
2	"	++
3	"	++
4	"	+
5	"	+
6	"	+
7	"	+

Case B. (Same treatment.)

Stool	Description of stool	Passed
1	semi-fluid	++
2	"	+
3	"	+
4	"	+
5	"	++
6	"	+
7	"	+

This purgation, which might seem severe, produced a considerable improvement in the condition of the patients. Their evening temperature was lowered and they confessed to feeling much better in every way. The faeces of "B" were now sent for examination after administration of Mist. Alba.

Stool	Description of stool	Purgative	Faeces
1	soft formed	Mist. Alba	+
2	"	"	+
3	semi-solid	"	—
4	soft formed	"	+
5	"	"	—
6	"	"	+

This was not so effective as the calomel.

Are acid-fast bacilli normally present in faeces?

It has been suggested, apparently without any authority that could be quoted, that acid-fast bacilli occur in normal faeces. Only two references have been found after an extended search in the literature to anyone who has demonstrated acid-fast bacilli in faeces other than those from a tuberculous individual. Sahli has been quoted before in this paper as mentioning that the smegma bacillus "is said to occur at the anal orifice" but even he affords no confirmation that they do actually occur. Jousset (1903) found acid-fast bacilli in the stools of two typhoid patients. They were easily distinguishable from tubercle bacilli by their shape, arrangement, growth on potatoe, and by failure to produce lesions in guinea-pigs. Mironescu (1901) also found an acid-fast bacillus in a case of suspected typhoid. It was ultimately proved to be the acid-fast bacillus found in butter.

It has been the author's custom for over two years to stain smears of every specimen of faeces, which came for examination, in at least three ways: (1) by gram, counterstaining lightly with weak carbol-fuchsin, (2) with Leishmann's stain, and (3) with Ziehl-Neelson.

Hence at hand there are numerous pathological conditions of the intestinal tract the excreta of which have supplied samples, all of which have been examined for acid-fast bacilli. In all there are records of 129 faeces so examined. Of these 69 have been examined especially for this research and all have undergone a careful search.

The 129 include 18 healthy normal faeces, 9 epileptic faeces, 28 suspected tubercle, and smaller numbers of such cases as typhoid, acne, mucous colitis, acute osteo-myelitis, diabetes, cholēlithiasis, and one vegetarian stool.

It may be mentioned here that there occurs numerous in some faeces and not at all in others, oval bodies which are acid-fast. These are homogeneous in structure and in size about the length of *Bacillus aerogenes capsulatus*. The oval is usually perfect in shape, but occasionally it tends to become pear-shaped. In the adult they occurred most numerous in the faeces of a vegetarian and in a case of acne. They are found however in greatest numbers in the stools of children. In an investigation made last year into the meconial stool, these bodies were discovered in nearly every specimen examined.

No conclusion has been reached as to their nature. Herter (1908) is the only author who has described them and he is unable to pronounce upon their identity. He thought at first they were yeasts, but being unable to procure a culture in any media, finally suggested that they were bodies ingested with the food.

The identity of acid-fast bacilli in the faeces.

The faeces of 24 persons suffering from advanced pulmonary phthisis were inoculated into guinea-pigs in the amounts determined by the preliminary investigation.

The faeces of two cases of lupus were also inoculated, in neither of these were acid-fast bacilli seen under the microscope and none of the animals inoculated showed signs of tuberculosis. Twenty-three of the twenty-four samples from advanced phthisis produced general tuberculosis in guinea-pigs and in all of these acid-fast bacilli were seen under the microscope. The twenty-fourth sample contained no acid-fast bacilli by microscopical examination, and no lesions were produced in

the inoculated animals. It is interesting to note that a sample from this last case procured a week later by the administration of a purgative revealed acid-fast bacilli.

The proportion of cases of tuberculosis in which acid-fast bacilli could be demonstrated by staining methods in the faeces.

Various techniques have been suggested for the examination of the faeces for this purpose. Hamburger, quoted by Sahli, recommended the method of Strassburger for solid stools. This consists of a fractional sedimentation in the centrifuge by which the larger particles of the faeces are first thrown down from normal saline and a final sediment procured by centrifugalisation in alcohol.

Page¹ and Park (1905) also consider this method the most suitable. Rosenberger made no use of the centrifuge in his work.

Technique.

The examination of constipated stools was soon abandoned for reasons already stated.

A sterile glass rod was employed to remove a small mass or drop from soft or liquid stools on to a glass slide. A second slide was used to smear out this mass and a film was made in a similar fashion to that by which a blood-film is produced.

This was allowed to dry in the air and fixed by heat in the Bunsen flame. It remained in steaming carbol-fuchsin for five minutes, was washed and treated with 25 % sulphuric acid until only a faint pink appeared on re-washing. It was then lightly counterstained with Loeffler's methylene blue.

Early pulmonary cases (14).

These cases all exhibited very early symptoms such as haemoptysis only, winter coughs, loss of flesh and a few with signs at one apex. Fourteen suspicious cases were examined, of these twelve were ultimately diagnosed as tubercle and nine were passing acid-fast bacilli in their faeces.

¹ Quoted by Emerson.

*Acid-fast Bacteria**Bones and joints (9).*

These included one case of Pott's disease and one case of psoas abscess.

Nine altogether were examined, seven were finally diagnosed tubercle, two presented acid-fast bacilli in the stool.

Lupus (6).

Six cases were examined, kindly sent to me by Dr F. P. Wilson. No acid-fast bacilli were found.

Tubercular meningitis (5).

Five cases examined, four ultimately proved to be tubercular, and an acid-fast bacillus found in the faeces of all four.

Miliary tubercle (4).

Four genuine cases all exhibited the bacillus in their motions.

Glandular enlargement (6).

Six cases examined, all tubercular. The bacillus was found on the two occasions in which the glands were very caseous.

Chronic pleurisy (6).

Six cases were examined. Four of these proved to be tubercular. Acid-fast bacilli were found in two. The non-tubercular cases consisted of one case of sarcoma of the lung and a case of chronic pneumococcal pleurisy.

Cases of pulmonary phthisis with small area of lung affected (7).

Seven examined. Acid-fast bacilli were found in the sputum in all seven, in the faeces six times.

Table summarising cases examined.

Type of lesion	Number examined	Positive examination	No. of cases of T.B.
Early pulmonary cases	14	9	12
Medium " "	7	6	7
Advanced " "	24	23	24
Bones and joints	9	2	7
Lupus	6	0	6
Meningitis	5	4	4
Miliary	4	4	4
Glandular enlargement	6	2	6
Pleurisy	6	2	4
Total	81	52	74

CONCLUSIONS.

The faeces of 23 cases of pulmonary tuberculosis, in which acid-fast bacilli had been demonstrated by the microscope, have been proved capable of infecting guinea-pigs with tubercle.

One hundred and twenty-nine samples of faeces from non-tubercular individuals have been examined microscopically and no acid-fast bacilli demonstrated.

The inference follows that all acid-fast bacilli in the faeces are tubercle bacilli.

Acid-fast oval bodies were found frequently but their nature is unknown.

The tubercle bacillus was discovered 52 times in the faeces of 74 cases, certainly tubercular.

There is an intermittency in the appearance of tubercle bacilli in the faeces, which may be connected with the flow of bile. This explanation is supported by the action of the cholagogue, calomel.

It is a pleasant duty to acknowledge the kind assistance and advice received throughout this investigation from Dr Stenhouse Williams, and to thank him for permission to work in his Laboratory.

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