Preface

In placing this paper before the Faculty of Medicine of the University of Edinburgh, I beg to state that the series of experiments would have been much extended but for the fact of the many difficulties which beset me in the earlier part of the work, my inability to obtain a copy of Parietti's paper, the severe frost during the past winter which prevented me from obtaining satisfactory samples of sludge for bacteriological examination, and illness.

Before concluding I must express my thanks to Dr. Hunter Stewart for his kindly suggesting the subject to me, and Dr. J. Buchanan Young for his assistance in preparing the photographs accompanying this paper.

John Penny.

April 30th, 1895.
Experimental Research
upon the relation of the
Bacillus Typhosus to Sludge.

At the Congress of Demography of 1891 held in London, H.R.H. the Prince of Wales in an introductory address used a phrase which is likely to become historic.

In speaking of "Zymotic Diseases" he referred to them as being also called "Preventable", and continued: His Royal Highness "If preventable, why not prevented?"

Undoubtedly of late years the one outstanding development of medicine and public health has been in the direction of the prevention of disease and especially of zymotic disease. Notwithstanding all that has been done however, so little of a practical nature has yet been accomplished that the query of H.R.H. the Prince might seem indeed justified.

Among the zymotic diseases Enteric or Typhoid Fever holds a peculiar position. It is not contagious in the ordinary sense of the word, and it does not spread with the awful rapidity of Measles or Smallpox. Moreover it is the disease of this class with the immediate etiology of which we are longest and best acquainted and yet it is a constant factor in the disease and mortality statistics of all our great towns. Unlike the other zymotic diseases its greatest incidence is not in children but in adolescents and adults so that its visitations strike the workers and bread winners of a nation and thus bring about a greater economic loss to the commonwealth than almost any of the other diseases of this class. Again unlike typhus, relapsing and other fevers which especially attack the poor in overcrowded and unhealthy districts, Enteric or typhoid fever strikes down all classes of Society from the members of the Royal family to the street urchin, and
the robust as well as the sickly.

The exact nature therefore and the etiology of typhoid fever is a matter of the supreme interest to the state.

Many investigations have been undertaken in this direction and different micro-organisms have been described as the exciting cause.

It was in 1880, that Eberth of Berlin described a bacillus which he had found in the stools, intestines, and spleen of those dying of this fever.

Gaffky in 1884, made a special study of the morphology and reactions of this special bacillus so that its recognition was greatly facilitated. Since that time numerous other observers have confirmed the original statements of Eberth and Gaffky. One of the latest researches is that of Dr. Klein, 1893, who has fully shown

(a) Mittheilungen aus dem Kaiserlichen Gesundheitsamt. 1880.

(b) " " " " " " 1884.

(c) 22nd Annual Report of Local Government Board 1892-93. Supplement to Report of Medical Officer. Appendix B. page 345.
that this particular bacillus has a constant relationship to the disease. It now may be taken as absolutely proved that in all cases of typhoid fever this bacillus of Eberth-Gaffky is present.

The bacillus can usually be demonstrated in the faeces, in the enlarged adenoid follicles of the intestine, in the mesenteric glands and spleen; also it has been found in the eruption spots and in the blood.

The Bacillus Typhosus (Eberth-Gaffky) presents itself as slender rods 2 to 3 μ in length and 0.5 to 0.8 μ broad; its ends are

(a) Dressfeld has found it in the urine and sputum of typhoid patients. British Medical Journal Vol. 1. page 869. 1895.

(b) It has been obtained from the blood of eruptive spots by a great majority of investigators. Wurtz Precis de Bacteriologie Clinique. 1895.

(c) From blood generally negative results except Ottlinger who found it. R. Wurtz. Precis de Bacteriologie Clinique. 1895. page 368.
rounded and it sometimes grows into long threads upon potato-slices. Oval refracting granules may be seen in the protoplasm of the bacillus: these have sometimes been mistaken for spores. The consensus of opinion is that this organism does not form spores.

It possesses a high degree of motility dependent as Löeffler has found upon the possession of flagella which are present in such numbers that the bacilli when subjected to the proper staining processes take on the appearance of spiders. Cultures can be grown at the ordinary room temperature on a variety of media. Gelatine is not liquefied and the growth is most abundant in the presence of free oxygen although the micro-organism may develop in its absence being a facultative anaerobe. It differs from most bacilli inasmuch as it can grow in acid media, a fact which has been taken advantage of by Paroti.

(1) *Metodo di ricerca del Bacillo del tifo nelle acque potabili.*
*Rivista d'igiene e sanità pubblica Roma. 1890.*
to devise a method for its separation and detection. Its growth on slices of potato having an acid reaction is characteristic. In 3 or 4 days at room temperature, and in as little as two at that of the incubator there appears on the surface a moist shining appearance but there is no visible growth.

Notwithstanding the easy identification of this organism and its constant presence in cases of typhoid fever, it must be confessed that the exact relationship between the bacillus and the disease has not been determined. Koch formulated four conditions which must be fulfilled before a disease could be certainly ascribed to a definite bacillus:

1. That the micro-organism be constantly present in the disease,
2. That it be capable of pure cultivation outside the body,
3. That injection of pure cultures of the same micro-organism causes the disease,
iv. That the same micro-organism be found in the animal inoculated.

Conditions one and two are fulfilled as regards Typhoid Fever by the Bacillus Typhosus (Eberth-Gaffky). With regard to the third condition, a difficulty is experienced inasmuch as typhoid fever does not attack the lower animals. Injections of pure cultures have failed to cause this disease in them, and have merely induced symptoms of septicaemia resembling those produced by large doses of other micro-organisms.

From all this it follows that for the present at least such absolute demonstration as is required by Koch cannot be forthcoming in the case of the Bacillus Typhosus (Eberth-Gaffky).

At the same time however the unvarying characters of the bacillus typhosus and its constant association with the disease have led to its being regarded as the proximate cause of this disease.

Taking it for granted that the
bacillus typhosus (Eberth-Gaffky) is the causa causans of typhoid or enteric fever we have yet to learn the exact manner in which the organism is conveyed from the diseased to the healthy. 

(a) The direct infection by contact has seldom, if ever, occurred. This has led to various theories. It has been assumed for instance that the bacilli when they leave the body have almost lost their virulence which they only regain after passing through a cycle of changes outside the body. Be this as it may there can be no doubt that direct infection if possible is exceedingly rare. Instances have been recorded in which the disease was caused by exhalations from drains, sewers, or waterclosets, but probably this mode of infection is exceptional.

(b) Cases noted by Dr. W. Budd (1839) and Sir W. Jenner (1879) can probably be otherwise explained than by direct contact.

(f) Parry, Laws and Andrews have never found any typhoid bacilli in sewer air. (Report to London County Council 1894.)
The method par excellence in which the disease is spread is by the contamination of drinking water by infected sewage.

A consideration of the various epidemics of typhoid fever in this country points undoubtedly to the fact that through the water supply the disease has been spread.

Here, however, we are met by the difficulty that the Bacillus typhosus (Eberth-Gaffky) is not easily separated from the other micro-organisms in water and even when every precaution is taken failure to detect the bacillus is the rule. Thus Bassedebat, examining the drinking water supplied to Marseilles, which is a very hot bed of Typhoid fever, was not able to find the characteristic bacillus in any one of 250 cultivations made of 70 specimens from the water. (a)

Moreover, even when the bacillus (Eberth-Gaffky) has been intentionally added to ordinary water it is found (a) *Annals de l'Institut Pasteur* Oct. 25, 1890.
that it perishes rapidly unless the colony is renewed from time to time.\(^{(2)}\)

Coming to the question of the sewage itself, which has contaminated the water, the demonstration of the bacillus therein has been by no means satisfactory; for instance Parry Laws and Andrews were unable to detect any typhoid bacilli in the sewage coming from the typhoid fever block at the Eastern Hospital, Homerton.

At the time of examination there were forty cases of typhoid fever in the block; many being acute cases suffering from diarrhoea. For two days before the examination of the sewage no disinfection of the typhoid stools had taken place. The sewage was collected from the drain connecting the fever block with the sewer, and therefore gave the best opportunity for the detection of this organism. The results however were entirely negative even under these favourable circumstances.

(a) D. S. Bernheim. Immunisation et Serumtherapie. Paris. 1895.
As these experimenters point out also the mathematical improbability of detecting typhoid bacilli in ordinary sewage is exceedingly great; and therefore it may be that the negative results of the examination of sewage may be altogether due to this fact. Accordingly it becomes of the first importance to ascertain how far sewage is capable of supporting the life of this micro-organism.

The latest researches on the subject contain the following declaration:

"It seems however clear that sewage does not form a medium in which much, if any, growth is possible for them under natural conditions, and their death is probably only a matter of a few days or at most one or two weeks."

not yet been examined viz. Sludge, i.e. the deposit obtained from the bed and sides of the channel through which the sewage flows.

It may be that this forms a suitable medium in which the organism can grow and from which it might be detached from time to time in such amounts as to cause a sudden and widespread epidemic.

The recent report of Dr. Barry on the Epidemic of Enteric Fever in the valley of the river Tees (a) would lend some support to this view.

The incidence of the fever was practically confined to an area supplied by water pumped from the River Tees. The condition of this river above the intake was of the filthiest nature. The foreshores, in particular, had been used for depositing midden refuse and privy

discharge so that in many places the river flowed between banks of stinking abominations. Cesspools also existed on the foreshores in such positions that they would be washed out during floods. In this district cases of Typhoid Fever occurred from time to time and the discharges found their way to the foreshores of the river.

The area supplied by water pumped from the Tees is one in which the prevalence of Enteric Fever is normally high, but on two occasions in the months of September and December 1890, excessive outbreaks of this disease occurred. Dr. Barry associated these with a flooding of the River Tees which had taken place about a fortnight before each outbreak. He considered that the flood waters had washed down the accumulated filth from the foreshores and with it the specific contagion.

In a report upon an outbreak of Enteric or Typhoid Fever at King's...
Lynn, Norfolk (Aug. 31st 1892). Dr. Bruce Low considered the causation to be due to a rapid thaw accompanied by rain which had washed into a lake the typhoid evacuations of eight persons which had been spread on a market garden at Gaywood; the lake being used as an intake for the water supply of the affected district.

These two reports suggest that although the Bacillus-typhosus (Eberth-Gaffky) does not live for any time in sewage, nevertheless it may find in the sludgy solid deposit a medium of ready and rapid growth.

During the last six months, at the suggestion of Dr. Hunter Stewart, I have undertaken an investigation on this subject to see how far sludge offers itself as an agent in preserving the vitality of the Bacillus. The experiments were conducted in the Public Health Laboratory of the University of Edinburgh under the supervision of Professor Sir Douglas MacLagan.
Materials and Media Used.

In no science probably do slight variations of method give rise to more startling differences of result than in Bacteriology. It will be well therefore if I begin by detailing succinctly my methods of working and preparation of the various media.

Glass-ware. All glass flasks, test-tubes and pipettes were thoroughly washed out, plugged with cotton-wool and thereafter placed in a hot air chamber for two hours at 170 °C. Petri's glass capsules were sterilised in a similar manner.

Boxes. Foster's boxes for holding the capsules were cleaned with boiling water and afterwards rinsed out with 1 in 500 solution of corrosive sublimate. A layer of filter paper soaked in the same solution was laid on the bottom of the tray and
on it the capsules rested.

**Water.** The sterilised water was obtained by running distilled water into sterile plugged flasks which were afterwards put in the steam autoclave at \(105^\circ\) extra pressure for one hour.

**Platinum Wires.** All platinum needles and eyes were sterilised by heating in a Bunsen flame till white hot.

Prior to inoculation the plugs in the test-tubes were scorched and the mouth of the tube "flamed" at a Bunsen-burner.

**Media.**

The Peptonised Bouillon was made in the following manner:

One pound of fat-free beef was minced, stirred up with a litre of distilled water and set aside under cover in a cool place for 24 hours.
Then all fat having been skimmed off the contents of the vessel were filtered through a clean towel and the liquid well squeezed from the meat by the hands and afterwards by a meat-press. The expressed fluid was then rendered alkaline by the addition of a dilute solution of caustic potash till it turned yellow turmeric paper brown and red litmus paper blue. It was then brought to the boil and kept at this temperature, with constant stirring, for ten minutes. The liquid was left to cool, and then filtered through ordinary filter paper. The filtrate was tested and when necessary rendered slightly alkaline by the addition of a dilute solution of caustic potash, and made up to one litre with distilled water. The fluid was placed in a sterilised flask which was transferred to the steam autoclave and kept at 15 tons extra pressure for one hour. The
pressure was gradually lowered until it had returned to normal when the autoclave was opened and the flask removed. After cooling the contents were filtered to remove the precipitated phosphates and albumen, and the clear fluid collected in a sterile flask. To this filtrate 10 grammes of peplone and 5 grammes of sodium chloride were added, and after alkalizing when necessary, the whole was made up to a litre with distilled water. The flask was then heated at 100° C., for three-quarters of an hour and the fluid filtered and collected in a sterile flask and again made up to one litre with distilled water.

This peptonised bouillon was sterilised by heating in the steam autoclave on three successive days, for half-an-hour, at a temperature of 100° C.
ii Peptonised Gelatine

Peptonised bouillon was prepared as above, and to a litre of it 100 grammes of finely cut up gelatine was added. The flask was then removed to the steam autoclave and left for half-an-hour at a temperature of 100°C. The solution was then rendered alkaline by a dilute solution of caustic potash made up to a litre with distilled water and filtered through a prepared hot-water funnel. The clear filtrate was collected in a sterile flask and the whole sterilised by heating in the autoclave on three successive days at 100°C, for half-an-hour on each occasion.

iii Agar-Agar

To a litre of peptonised bouillon, made as above described, fifteen grammes of finely cut agar-agar was added. The flask with its contents was heated in the autoclave
at 100°C, for one hour with vigorous shaking every ten minutes. The fluid was allowed to cool to 50°C, and then the white of two fresh eggs previously beaten up with sixty cubic centimetres of distilled water was added. The whole was heated over a steam-bath until the temperature reached 80°C, after which it was transferred to the autoclave at 100°C, for ten minutes. The fluid was then filtered through a properly prepared hot-water funnel and the filtrate collected in a sterile flask, and sterilised on three successive days at 100°C, for half-an-hour on each occasion.

iv. **Potato Media**

(a) **Cylinders.** Potatoes were carefully cleaned in water with a brush, and after being freed from dirt were laid for one hour in a solution of corrosive
sublimate (1 in 100). The sublimate solution was then poured off and the potatoes were washed free of it with distilled water. They were then allowed to partly dry, after which the core was punched out with a Roux' potato-knife. By this means two semi-cylinders are obtained. From the centre of these, lengths suitable for the potato test-tubes were cut out, washed in distilled water and dried in folds of filter paper. One such semi-cylinder was placed in a sterile plugged potato tube and the whole sterilised for half-an-hour in the autoclave at 100°C.

(b) Potato Gelatine

After thorough cleansing as in (a) the potatoes were peeled and grated to a pulp through a potato-grater. This pulp was left for half-an-hour in a cool place covered up and was then squeezed through a clean
towel by the hands and afterwards by the meat press. The expressed fluid was collected in a sterilised flask plugged and allowed to stand over night. The reaction was then tested, and if not acid was left to stand until it became so. It was filtered through a fluted filter paper and the filtrate heated for half-an-hour in the autoclave at 100°C. The precipitated starch was then removed by filtration through a filter paper, and the filtrate having been measured, enough finely divided gelatine was added to make a ten per cent solution. The gelatine was dissolved by heating for one hour at 100°C, and the fluid filtered through a prepared hot-water funnel. This potato gelatine was collected in a sterile flask, and sterilised on three successive days in the autoclave at 100°C, for half-an-hour on each
occasion.

[NOTE: This potato gelatine was tested and its acidity was such that 10 c.c. required for exact neutralisation 3 c.c. of decinormal solution of caustic potash.]

V. Culture Tubes.

Were prepared by placing 10 c.c. of sterilised bouillon, or of gelatine, or of agar-agar, or of potato gelatine in a sterilised test tube or flask. These were re-plugged with sterile cotton wool and sterilised on three successive days for half-an-hour at 100°C.

VI. Parietti's Medium.

was prepared thus:—5 to 9 drops of carbolic acid solution were added to a test tube containing 10 c.c. of sterilised peptonised neutral bouillon. (The drops were run in from a sterilised pipette, 15 drops of
which measured one cubic centimetre.

The solution used was made as follows:

- Pure Carbolic Acid 5 grammes
- Pure Hydrochloric Acid 4 grammes
- Sterilised Distilled Water 100 grammes.

The tubes containing this acid bouillon were tested by placing in the incubator for 24 hours. At the end of this time if they remained clear they were laid aside as being suitable for use.

**VII Carbolised Gelatine.**

was prepared thus:

One gramme of pure carbolic acid was added to five hundred cubic centimetres of sterilised nutrient gelatine and ten cubic centimetres of this added to sterile plugged test tubes.
Method

For obtaining the Sludge.

The sludge which I used was obtained from the bed and sides of the channels through which the sewage passes at Craigentinny Irrigation Farm, near Edinburgh.

A large tablespoonful of the freshly collected sludge was placed in a sterilised flask capable of holding about 120 c.c. and the mouth replugged.

Experiments with the Sludge.

First with Ordinary Sludge.

To each flask prepared as above 3 c.c. of a bouillon culture of pure typhoid bacillus was added.

Each flask was labelled and placed in the incubator at 18°C.

In the first three experiments the flasks were left to incubate for twenty-four hours after which the contents were tested for the presence of the typhoid bacillus.
This was done in the following manner:

The contents of the flask were carefully emptied into a litre of distilled sterilised water and the whole well shaken. From this a few drops, never more than five, were added,

Firstly, to Parietti's medium and placed in the incubator at 30°C.

Secondly, to ordinary melted gelatine, which was poured into a Petri's capsule and incubated in a Foster's box at 19°C.

Thirdly, to ten cubic centimetres of gelatine containing five drops of Parietti's carbolic acid solution. This prepared gelatine was run into a Petri's capsule and incubated in a Foster's box at 19°C.

Fourthly, into Potato-gelatine and from each tube a plate was made and at 19°C, was incubated in a Foster's box.

Fifthly, into Carbolised gelatine and plates prepared and incubated at 19°C as above described.
The plates were carefully examined each day in order to detect any colony at all resembling those of typhoid. If such a colony appeared sub-cultures were made on potato hemi-cylinders, plates of gelatine, and of potato gelatine, agar-agar slopes, gelatine slopes, gelatine stabs and in Parietti's medium. Finally the organisms were carefully examined under the microscope.

Results: In the first three experiments the typhoid infected sludge was incubated at 18° C. for twenty-four hours. It was thereafter tested according to the above method and in no instance was the Typhoid bacillus to be found.

In the fourth the incubation was carried on at 18° C. for 7 hours and then tested by the above described method, with the result that no typhoid bacillus was found.
In the fifth experiment the period of incubating at 18° C, was reduced to 3 hours, and tested in a similar manner to the preceding four, but with this the result was negative.

In the sixth experiment the infected sludge was merely laid aside for half-an-hour and in this case the presence of pure typhoid bacilli was demonstrated.

Second with Sterile Sludge.

The sludge obtained in the manner already described was placed in the steam autoclave at 15 lbs extra pressure for three hours on two successive days. This prolonged period I found essential as shorter periods failed to ensure sterility.

To this sterile sludge 3 c.c. of the same bouillon culture of pure typhoid bacillus was added to each flask and
incubated at 18° C.

Experiments and Results.
Firstly, after incubating for 24 hours and testing in a similar manner to that described above in the case of ordinary sludge typhoid bacilli were found.
Secondly, after incubating for twenty-two days the presence of typhoid bacilli could still be demonstrated.

[Note: Strength of Typhoid Culture used in each case:—]

Experiment i Culture was 6 hours old.

" ii " 2 days old.

" iii " 4 "

" iv " 4 "

" v " 4 "

" vi " 4 "

For the sterile sludge the bouillon culture of the pure typhoid bacillus was six hours old in both series of experiments.
Conclusion

So far as my experiments go they seem to show that ordinary sludge does not form a suitable medium for the growth of the Bacillus Typhosus (Eberth-Gaffky). When intentionally infected with this organism, its presence could only be detected within the next half-hour after which time it seemed to disappear. In sterile sludge on the other hand the Bacillus Typhosus finds a medium in which it can exist for prolonged periods as I found it present as long as twenty-two days after infection.

The inhibitory influence on the growth of the micro-organism does not lie therefore in the chemical constitution of the sludge but in the presence of the other micro-organisms. This is a point which has also been demonstrated with regard to sewage by Parry, Laws and Andrewes.
From all this it naturally follows, and the recent experiments of Barry Laws and Andrews confirm the point, that the relationship of the Bacillus Typhosus to Sewage and Sludge is far from clear.

Investigations of epidemics have indeed been apparently traced to the contamination of water by typhoid infected sewage, but in the light of our present knowledge such a mode of spread of the fever seems difficult to explain. The Bacillus Typhosus (Eberth-Gaffky) if it gains access to sewage or sludge comes in contact with a medium in which it is rapidly destroyed. Even should it gain access to the drinking water it must be in considerable amount for even in that medium it rapidly disappears.

Those instances in which the bacilli have been found in suspected water must be received with caution — inasmuch as the method of Panizzi.
does not give such absolute proof of
the presence of the typhoid bacillus
as was originally stated.
Cassadebat found several organisms
which reacted to Parietti's medium in
the same way as the Bacillus Typhosus
does.
In my own testing of media I have
found that the Bacillus coli commune
and three other organisms obtained
from soil hitherto undescribed give
the same cloudiness with Parietti's
medium in the prescribed 24 hours.
The unequivocal presence there-
fore in water otherwise suspected
of spreading typhoid fever has scarcely
yet been absolutely demonstrated, and
so far as experimental bacteriology
is concerned the verdict with regard
to the water-carriage of typhoid fever
must be "Not Proven," assuming of
course that the Bacillus Typhosus
(Eberth-Gaffky) is the 'causa-causans'
of the disease.
In a practical research of this nature speculation must to a great extent be curbed. I cannot help however referring to the theory of typhoid spread originally advanced by Pettenkofer in which the "drying zone" of the earth was considered to be the factor of greatest importance. Inasmuch as typhoid bacilli have been found to live in soil for some months it is possible that the rise of the ground-water may cause a sudden invasion of the air by the bacillus typhosus with contamination of water and food stuffs. At any rate the theory is worth careful experimental investigation, in view of the unsatisfactory nature of our knowledge with regard to the spread of this most serious disease.
Appendix.

Owing to my inability to procure a copy of Parietti's paper, or the Rivista di igiene e sanità publica containing it, I was obliged to test the same to see if the following statement was correct:

Parietti adds to several tubes each containing 10 c.c. of neutral sterilised bouillon from 3 to 9 drops of hydrochloric acid phenol solution (4 grs hydrochloric acid, 5 grs carbolic acid to 100 grs of distilled water). The tubes are then placed in an incubating oven for 24 hours to ascertain whether they are still sterile after this addition.

If the bouillon remains clear from one to ten drops of the suspected water are added to each tube and they are returned to the incubating oven. If at the end of 24 hours the bouillon becomes clouded, this is due, according to Parietti, to the presence of the typhoid bacillus which is then to be obtained in pure cultures by the plate method.

(a) Stérenberg's Bacteriology (New York) 1892. p. 354
Testing the Method of Parietti.

1. I took several tubes each containing 10 c.c. of sterilised peptonised bouillon and added to these varying quantities of Parietti's medium, which had been prepared under antiseptic precautions, the distilled water having been previously sterilised at 15 lbs extra pressure in the autoclave, the flasks and pipettes also having been sterilised in the hot-air chamber at 170° C.

   These tubes were placed in the incubator for 24 hours to see if still sterile, and on the following day one-eyeful of a 3 days old pure bouillon culture of pure typhoid added to each. They were afterwards placed in the incubator at 30° C., and examined at the end of 24 and 48 hours.

<table>
<thead>
<tr>
<th>Quantity of Parietti in each tube</th>
<th>Result at end of 24 hours</th>
<th>Result at end of 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 5 drops</td>
<td>cloudiness</td>
<td>cloudiness</td>
</tr>
<tr>
<td>2 6 &quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>3 8 &quot;</td>
<td>cloudy but not so dense as 1 9 2</td>
<td>&quot;</td>
</tr>
<tr>
<td>4 10 &quot;</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>5 12 &quot;</td>
<td>nil</td>
<td>nil</td>
</tr>
</tbody>
</table>
With a larger quantity of the pure typhoid culture, than was used in the above experiments, say two or three cufifule, I was able to get a cloudiness in the 10 drop tubes but not in the twelve although they were allowed to stand for three days in the incubator.

The pipette used was of such bore that fifteen drops measured one cubic-centimetre. When more than ten drops of Parietti’s medium were added to ten cubic-centimètres of bouillon, a precipitate deposited at the bottom of the tube of a brownish colour.

II. With ordinary Sludge.

Here a cloudiness was produced in 24 hours with Parietti’s medium, the cause being Bacillus boli commune in this case.

III. With garden Soil.

A cloudiness was produced here again in Parietti’s medium at the end of 24 hours. Plates were made from this cloudy Parietti’s medium and three organisms
a description of which here is added, as no account can be found of them in any work.

Result: The Bacillus coli communis and the three bacilli, described later, obtained from garden soil, give the same turbidity with Parietti's medium as the typhoid bacillus in the same period.
Micro-organisms from Soil through Parietti's Medium.

A. Granular Colony.

Obtained from soil by cultivating in gelatine after passing through bouillon containing Parietti's hydrochloric acid phenol solution.

Morphology: - Short bacilli, varying from 2.5 μ to 3 μ in length and .8 μ broad, with rounded ends, occur singly, in pairs, and in chains.

Stains with the usual anilin colours, the central portion not stained so deeply as the margins.

Biological Characters: - An aerobic, slowly liquefying, non motile bacillus. Grows in the usual culture media at the room temperature. Upon gelatin plates forms white granular film colonies with irregular margins and thickened centres, later liquefaction takes place.
In gelatin stick cultures, growth along the line of puncture, also on the surface granular in appearance and with irregular margins. At the end of fourteen days liquefaction commences at the surface in a funnel-shaped depression.

Upon the surface of agar, a milkish gray smooth growth.

Upon potato, growth at first glossy, later becomes somewhat circular and depressed in centre. At the end of five days elevated and slightly flesh coloured which develops into an uneven elevated deep flesh coloured growth along the needle track (as seen in photographs taken on the 5th and 11th day.)
B. Opaque colony.

Obtained from soil after cultivating in gelatine after passing through bouillon containing Parrietti's hydrochloric acid phenol solution.

Morphology: Short bacilli with round ends, varying in length from 1.25 μ to 3.3 μ and .5 μ broad; occur singly in pairs and masses.

Stains readily with aniline methyl blue.

Biological characters: An aerobic, liquefying, non-motile bacillus. Grows well at 18°C. Upon gelatine plates an opaque regular margined glossy colony appears at the end of two days which later develops a transparent film ring around it. On the fourth day liquefaction has commenced.

In gelatin streak culture growth along needle track of very small spherical and distinct colonies. On the fourth day liquefaction begins near the surface by cupping and at the bottom of which cup there is a deposit of debris.
Gelatin streaks show no tendency of colonies to coalesce. Upon surface of agar the growth is abundant but motting characteristic.

On potato. at the end of thirty-six hours large patches of a skimmed-milk appearance elevated, with the potato surface moist. Colonies increase gradually in size and coalesce readily where two patches come together. and on the eleventh day we get a very typical creamy cauliflower excrescence appearance. (See photograph on 11th day)
C. Central Nuclear Colony.

Obtained from soil by cultivating in gelatin after passing through bouillon containing Pariettes hydrochloric acid phenol solution.

Morphology: a short bacillus with round ends, from 2.5 μ to 3 μ in length and 0.75 μ to 0.8 μ in breadth; occur singly, in pairs and chains.

Stains readily with the usual anilin colours.

Biological characters: An aerobic, slowly liquefying, non-motile, chromogenic bacillus. Grows readily at the room temperature in the usual culture media. On gelatine plates colonies with a central opaque nucleus, and concentric arrangement external to this nucleus, also border of bluish-gray tinge with an irregular dentate margin. In gelatin stick culture the surface growth is rapid but along the needle track very scanty. Liquefaction commenced on the 8th day at the surface.
On surface of agar a luxuriant smooth slightly pink growth.
On potato at first: dirty, moist, elevated colonies which later become flesh-coloured waxy, wrinkled and stain the potato substance (vide photographs made on 5th and 11th day.)