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Publication/Creation

[Place of publication not identified] : [publisher not identified], 1901.

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THE BACTERIOLOGICAL EXAMINATION OF THE
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DIFFERENTIAL EXPERIMENTS.

THE BACTERIOLOGICAL EXAMINATION OF THE RENAL
SECRETION IN CERTAIN OF THE ZYMOTIC DIS-
EASES, WITH SUBSIDIARY DIFFERENTIAL EX-
PERIMENTS.¹

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AT the outset of the investigation, it was desired to ascertain whether there is any elimination of pathogenic organisms by the kidney in certain infectious diseases, and whether, from a public health point of view, it is essential that the urine should, in these diseases, be systematically disinfected.

The presence of specific micro-organisms in the renal secretion, in cases of zymotic disease, has been described by many observers, more particularly in connection with enteric fever. The subject possesses not only scientific interest, but is of serious practical importance in relation to the spread of disease and the measures to be taken in prevention of infection arising from this source. Although disinfection of the fæces in enteric fever is recognised as essential and carefully attended to, disinfection of the urine, passed independently of the fæces, has not been the routine

¹ Abstract of Thesis, presented for the degree of D.Sc. in Public Health, and awarded the Gunning Victoria Jubilee Prize, 1901.

practice, and the question of its continuous necessity throughout the illness may still be regarded as *sub judice*.

The report deals with enteric fever, scarlet fever, and diphtheria, and is divided into four main divisions—(1) Historical consideration of the question of renal elimination of organisms; (2) zymotic bacteriuria; (3) relation of cases examined, methods and media employed, results obtained, and their comparison with prior research; (4) subsidiary differential experiments.

I. RENAL ELIMINATION.—The general question of elimination of organisms by the kidneys has been tested by experiment on animals, with several organisms, by many observers, who have arrived at different conclusions; some believing that bacterial elimination may be a physiological function of the kidney, and others that no such function exists, and that if any passage of bacteria through the kidney is allowed, it is in consequence of a pathological condition of that organ. The experiments and conclusions of Biedl and Kraus, Charrin and Ruffier, Trambusti and Maffucci, Enriquez, Klecki, Fütterer, Cornil and Brault, Kannenberg, Straus and Chamberland, Wyssokowitsch, Berlioz, Sherrington, Opitz and Metin, were quoted and compared.

Reviewing the general subject of renal elimination in the light of the work of these and other observers, there must be pointed out an important distinction between elimination of organisms by the kidney and the passage of organisms in the urine, whether they gain access to it through the renal tubules or otherwise. If the kidney did possess the function of eliminating living organisms from the blood, this power should be in active use throughout the course of any illness in which organisms are found in the blood, and there ought to be no difficulty in finding them in the urine, coincident at all times with their presence in the blood. In the majority of cases of infectious disease, however, specific organisms are not constantly present in the urine, and only occur in a very moderate percentage of cases.

The healthy kidney possesses no power of casting out living organisms, though it is able to remove the products of their action; and any escape of bacteria through the kidney is only accomplished when there is functional interference with the integrity of the renal epithelium, or some congestive or inflammatory condition.

Bacteria escaping by way of the urine are usually accompanied by some other abnormal constituent; it may be blood, it may be pus, it may be albumin. A kidney which will permit the passage of albumin may be believed, *primâ facie*, to be capable of allowing the passage of bacteria, and this would lead us to expect that in infectious diseases in which renal congestion and nephritis are prominent complications, bacteria would be more likely to be present in the urine than in those diseases in which such com-

plications are rare. Observations of typhoid bacilluria show that albumin and pus are common but not invariable accompaniments.

From the point of view of public health, it is unimportant whether specific organisms present in the urine entered it through the renal tissue or at a later stage. It is, however, important to decide whether, and in what zymotic diseases, the urine is voided in a condition to spread the disease, should the organism find a favourable environment. If in any particular disease bacteriuria is found to occur, continuously or at intervals, the prevention of infection by such an avenue becomes as much a necessity as in the case of channels earlier recognised.

II. ZYMOTIC BACTERIURIA.—The presence of specific bacteria in the urine in cases of plague and enteric fever is now an established fact, whilst in scarlet fever and diphtheria the difficulties of proof are greater, owing in the one case to some dubiety as to its specific organism, and in the other to the difficulties of cultivation.

Plague bacilluria may occur in the course of the disease or during convalescence. The bacilli are present in large numbers, and are easily recognisable, are always associated with coincident albuminuria, and persist into convalescence after recovery from other symptoms. The urine may be a pure culture of plague bacilli, but sometimes pus cocci are also present. Plague bacilli and staphylococci have been found post-mortem in the kidneys.

Typhoid bacilluria.—The presence of the *B. typhosus* of Eberth in the urine during enteric fever has been reported upon by a large number of observers, whose results differ by as much as 100 per cent. While some find it in every case with no difficulty, others, even after careful examination, have failed to identify it at all; and the general result is, that at the present time the bacillus is regarded as occurring in the urine in about 25 per cent. of all cases. The earlier observations were made at a time when the characters now known to differentiate the *B. typhosus* of Eberth from the *B. coli communis* of Escherich and its allies were unknown.

The experiments of Bouchard, Berlioz, Karlinski, Koujajeff, Neumann, Silvestrini, Borges, Blumer, Wright and Semple, Besson, Melchior, Flexner, Petruschky, Horton-Smith, Richardson, Carver, and Gwyn, were related, and their results tabulated for comparison. The important point in estimating the value of these researches, is the severity of the test to which the organism is subjected before acceptance as the *B. typhosus*. There are so many organisms which resemble it in many ways,—notably the *B. coli communis*, or rather its allies of atypical characteristics, and the organisms described by Houston as *B. typhosus simulans A, B, C, and D*, and the organism found by Remy in stools in cases of enteritis and gastro-intestinal catarrh,—that meagre

testing might easily result in the acceptance of one of these as the genuine bacillus. Horton-Smith's definition suggests the tests which should be applied, and is as follows:—"A bacillus which often showed long threads, which possessed high motility, which was provided with eight to twelve or more cilia, which, however long kept, did not produce gas in gelatin or dextrose-gelatin shake cultures, while growing well in the substance of those media; which, even *after* one month, did not produce indol in broth cultures, nor clot milk; which, however, did produce slight acidity in milk after twenty-four hours; and which, lastly, gave a positive reaction when tested with typhoid serum: then, and not till then, I think, one is justified in saying that it is really the *B. typhosus*."

With this I entirely agree, except that I would alter the one word *after* in relation to the indol reaction to the word *within*, since I believe that no fresh typhoid bacillus gives the indol reaction in broth culture within one month, but some typhoid bacilli certainly do so later. This will be more fully referred to in a note on indol reaction in the latter part of this report. Briefly summarised, it appears from the accounts of these observers that—(1) Typhoid bacilluria is a condition of the later stages of the disease, and, if the type be a mild one, occurs with or without albuminuria of slight amount, but that it is more common in severe types, and then usually occurs in the third and fourth weeks, associated with a considerable quantity of albumin. (2) Pyuria is apt to accompany the bacilluria in a large proportion of cases. (3) It occurs in about 25 per cent. of all cases of enteric fever. (4) It is more a bladder condition than a renal one, and is possibly curable by antiseptic injections, or the administration of antiseptic medicines. (5) The organism finds the urine a very suitable medium for growth, and when present it is found in almost pure culture and in enormous numbers. The only criticism of this summary which I desire to add has reference to the prevalence, which I consider over-estimated.

Bacteriuria in scarlet fever.—Since the specific organism of scarlet fever can hardly be said to be as yet generally recognised, there is not much to be recorded as to its presence in the urine; but up to this time Klein's *M. scarlatinae* or *Streptococcus scarlatinae*, which appears to be identical with the *Streptococcus conglomeratus* of Kürth, has most claim to recognition as the specific organism. It is necessary to note that this organism has not as yet been obtained in the urine of scarlet fever patients. Klein has published the results of his investigation of the urine in three cases during the stage of desquamation, and in none was a streptococcus found.

Bacilluria in diphtheria.—The *B. diphtheriae* (Klebs-Löffler) has been found in the kidney in fatal cases of diphtheria, as well as in inoculated animals. Wright found it in the latter four times

out of 151 examinations, and once in the former in fourteen cases; further, in conjunction with Stokes, he found it in six cases out of thirty-one. In another series of nine cases Stokes found it present in the kidney in four. Kütscher also records finding it once, and Kanthack and Stephens twice, out of three kidney examinations in twenty-six fatal cases. These observations would lead one to expect that *B. diphtheriæ* was not uncommon in the urine; but, so far, the records and one's own experience show that this is not so. Bujwid, indeed, records having found it in the urine in conjunction with the *B. tuberculosis* in a case of tuberculous kidney, but the symptoms were those of tubercle, and not of diphtheria, so there may have been accidental contamination.

III. EXPERIMENTAL.—

Methods.—The collection of specimens was at first confined to male patients, so as to avoid as far as possible exterior contamination. The catheter was seldom used. The meatus was carefully cleansed, and then washed with a weak solution of corrosive sublimate, and micturition was performed into a flask with a sufficiently wide mouth. This flask had been previously thoroughly washed, dried, and plugged with cotton-wool; over the plug was tied a piece of filter-paper, and the whole sterilised by a heat of 150° C. for one hour. At the bedside the plug and filter-paper being removed for as short a time as possible, the urine was passed direct into the flask, the first portion of the flow being received into one flask, and the latter portion into a second similar flask. The plugs and filter-paper were at once replaced, and the flasks removed to the laboratory. Both portions were examined, as a rule, though it was expected that the former would be more likely to contain urethral organisms, and sometimes only the second portion was examined bacteriologically, the first portion being tested as to reaction and albumin. Later in the investigation, specimens of the urine were occasionally taken from both male and female patients without these precautions, with the advantage of increasing the number of specimens without repeated trouble to the patients, and enabling serious cases to be investigated without disturbing them. A disadvantage was, that one found more organisms in the urine, and consequently there was more labour involved in the separation of impure cultures, and in isolating the different species of organisms present. The quantity of urine usually obtained for each specimen was about 150–250 c.c.

Laboratory procedure.—From the sterile flask the urine was transferred to a narrow glass cylinder, previously sterilised with strong sulphuric acid, and thoroughly washed with water, and then with sterile distilled water. Into this was passed a filter-bougie, either of the Pasteur-Chamberland or Nordtmeyer-Berkefeld pattern, which had been sterilised, and which was connected by sterilised rubber tubing with a filtering flask, which in its turn was connected with a safety flask, and that with a Giesler pump attached to the main water tap. The filtering flask was sterilised in the same way as the glass cylinder, if it was desired to obtain a sterile filtrate, but in the ordinary examination of urines this was not necessary. The mouth of the glass cylinder was covered with sterile filter-paper during filtration.

The urine having been filtered through the bougie, by means of the suction pump, the bougie was removed, and its exterior washed into a sterile beaker with 10 c.c. sterile distilled water, by means of a sterile brush. These washings thus contained all the organisms present in the specimen of urine. After mixing by shaking the whole washings, a portion, generally 0.6 to 1 c.c., was added, by means of a sterile pipette, to a tube of 10 c.c. of ordinary or phenolated broth. The broth was incubated for one night at 37° C., *i.e.* for about eighteen hours, and next day another tube of 10 c.c. of broth was inoculated from this, and incubated for one hour at 37° C. Plates were then prepared, and if of agar were incubated at 37° C., if of jelly at 24° C. All plates were kept from one to two weeks, unless liquefaction of gelatin rendered them useless before that time. Each kind of colony in the plates was examined microscopically, and a series of cultures in ordinary or special media made of each. From these the characters of the different organisms were ascertained, and they were identified as far as possible, and in all cases sufficiently to exclude known pathogenic species.

Incubation of the specimens of urine themselves for twenty-four to forty-eight hours, at 37° C. before cultivation, which in the hands of Wright proved so effectual, was frequently tried, but always with disappointing results, since invariably the urine was found at the end of that time to be swarming with organisms, many of which were cocci.

Plates were occasionally prepared direct from the urine, but on the whole the method given above proved most satisfactory, and rendered it unnecessary to try the method, substituted by Carver, of using cotton-wool as the filter.

Media.—The media employed were—(1) alkaline 1 per cent. peptone broth; (2) litmus broth; (3) phenolated broth (0.05 per cent.); (4) nutrient jelly (10 to 20 per cent.); (5) glucose jelly (2 per cent.); (6) jelly medicated with phenol (0.05 per cent.), formalin, the essential oils of peppermint, cloves, cinnamon, or eucalyptus, or rectified spirit; (7) Remy's jelly; (8) agar-agar; (9) agar-gelatin; (10) blood serum; (11) potato; (12) milk; (13) alkali-peptone bouillon (Peckham); (14) casein-peptone solution; (15) tyrosin solution. The only one of these whose preparation has not been published is—

Casein-peptone solution.—This medium is thus prepared. Into a flask put 10 grms. casein (commercial), add 250 c.c. of distilled water. Mix by shaking. Raise the temperature to 37° C. When this temperature is attained, render alkaline with solution of sodium bicarbonate (10 per cent.). To the contents of the flask add 2 grms. of Grüber's trypsin (Kühne), and shake thoroughly. Then keep for one hour at 37° C., shaking occasionally during this time. Test reaction and make alkaline as before. Keep for one hour longer at 37° C. After this time the mixture is boiled for three minutes. Then place the liquid in the autoclave for one hour, under pressure at 110° C. Cool and filter. Filtration is very slow when the reaction is alkaline. Next acidify the filtrate with dilute acetic acid, which throws down a precipitate. Filter again, and obtain a clear filtrate. To the clear fluid add 1.25 grms. of sodium chloride. Make the reaction slightly alkaline to turmeric paper. Make up quantity to 250 c.c. with distilled water. Tube and sterilise

in the autoclave, without pressure, for twenty minutes, on each of three successive days.

Note.—Before tubing, test a portion for indol and for peptone; it should be free from indol, and it should give with the biuret test positive indications of the presence of peptones. The solution usually contains nitrite.

(A) **Enteric fever.**—*Cases and specimens.*—I obtained the urine of forty-five cases of enteric fever in the Edinburgh and Leith hospitals, and examined 158 specimens, and in only one case have isolated the *B. typhosus*.

Widal reaction.—All forty-five cases gave a positive Widal reaction, and illustrated clinically different types of enteric fever.

Sex and age.—The forty-five cases were distributed almost equally between the sexes, twenty-four being males and twenty-one females. The ages ranged from 3 to 48 years, over 60 per cent. being between 10 and 30. The positive case was that of a female *æt.* 27.

Severity.—The cases examined were, on the whole, of a mild type, and only two deaths occurred in the forty-five, showing a lower case mortality than is customary in the disease. That the cases were a fair sample of the disease, as occurring in this district, may be supported by a consideration of the statistics of the Edinburgh and Leith hospitals, which show that, as a rule, the case mortality is not high.

Stages of disease.—The specimens were taken at all stages of the disease except in the very earliest days, and in time covered from the first to the thirteenth week of the disease. Most of the specimens were taken in the third, fourth, fifth, and sixth weeks, when one might expect to find bacilluria if it occur in any large percentage of cases.

Albuminuria.—Of the forty-five cases, eighteen showed albuminuria at one stage or another; but of these, six only contained mere traces in unimportant amounts and of short duration, ten were characterised by moderate amounts, and in only two were large quantities of albumin present.

Pyuria.—Pus was only observed in one case, and then was accompanied by the *B. typhosus* and *B. coli communis*.

Bacilluria.—Owing to the specimens being sometimes taken without strict bacteriological precautions, the urine was seldom sterile or contained a pure culture, but occasionally this happened, especially when such precautions were taken. The specific organism was only present in one case, but coliform bacilli were very common, and their varieties are discussed in a special note. A specimen which was taken post-mortem contained a pure culture of *B. coli communis*.

Media.—For enteric urines the media which proved most useful were phenolated broth, phenolated jelly, and Remy's gelatin medium. In the positive case the *B. typhosus* was recovered equally well in plates of phenol jelly and Remy's gelatin medium, but the latter is preferable where mixed cultures, and it may be liquefying organisms, are encountered.

The *B. typhosus* isolated from urine was submitted to all the tests which distinguish it from *B. coli communis*, and which are enumerated later, before it was accepted as undoubtedly Eberth's bacillus.

Methods.—With enteric urines, in addition to the general method formerly described, I tried other methods of treating the specimens, but

found none of them superior. The incubation of the urine at 37° C. before cultivation did not give satisfactory results even in those cases in which great care had been taken in collecting the specimen.

Direct cultivation from the urine itself, without filtration, occasionally gave good results, but only a small quantity of urine can be used in this way, and, if negative, such a test alone would not warrant the conclusion that there was no typhoid bacilluria. If the *B. typhosus* were present in pure culture, I believe direct cultivation would be good, but I have not met with such a condition. On the other hand, by filtering a large quantity of urine, one obtains all the organisms present, and in a fair sample of the washings can hardly fail to find the specific bacillus if it is present.

Case of typhoid bacilluria.—The patient was a female, æt. 27, and her case was a serious one, with delirium as a main feature. The urine contained a large quantity of albumin and some pus; the albuminuria commenced on the twenty-sixth day, and traces were present up to the sixtieth day. The temperature fell to normal on the forty-fifth day of the disease. The *B. typhosus* was present in the urine on the thirty-eighth, forty-first, fiftieth, and fifty-first days, at first in large numbers, and it was absent on the sixty-second day. It was not present in pure culture, and was accompanied by *B. coli communis* and sometimes cocci.

The urine at first was loaded with urates, and one was not able to recognise the "shimmer" described by Horton-Smith, or at that time the characteristic turbidity.

Examined microscopically, a drop of the urine showed pus cells and numerous organisms, both motile and non-motile, but no casts. The later specimens were turbid, and the urine was always acid notwithstanding the presence of pus.

Typhoid bacilluria.—Comparing my experience with that of others, it appears to me that there are two types of typhoid bacilluria.

1. The one is a condition occurring in severe cases of the fever, generally commencing during the height of the disease—that is to say, seldom in the first fortnight, frequently in the second fortnight, or later, associated always with albuminuria, often with pyuria, sometimes with hæmaturia, diminishing as the urine contains less albumin, and disappearing a few days or a week after the albumin. This type may merge into the second type, especially if cystitis has been set up, for this renders the disappearance of the organisms unlikely to occur so early as in more favourable circumstances. The case described above may be regarded as an instance of this first type.

2. The second type is characterised by its commencement in the later stages of the disease (it may be after the temperature has fallen to normal), by its persistence into and during convalescence, even for weeks, and it may be months, without causing severe symptoms, by its occurrence in comparatively mild cases as well as in those which have been severe, and by albuminuria not being an invariable accompaniment. There may be more or less

cystitis, but it is not constant. I have not met this type in these forty-five cases, and I am satisfied that it is not so common in Edinburgh as in some other places.

Prevalence.—At the present time, chiefly owing to the results of Richardson and Horton-Smith, typhoid bacilluria is regarded as occurring in 25 per cent. of all cases of enteric fever. I am convinced that this proportion is too large, at all events it is not found in this district in anything like so great a percentage; and there are now a considerable number of observers who have recorded a smaller percentage, notably Blumer and Petruschky. The explanation is probably to be found in the type of disease. I think if all the mild cases were examined as thoroughly as the grave ones, a considerably smaller ratio would be found to be the rule. Taking my own figures, the ratio would be only 2 to 3 per cent., but then, as I have pointed out, the cases were not severe, and this percentage is probably less than the actual ratio. If it be admitted that bacilluria results from the proliferation in the bladder of stray organisms which have passed through the kidney from the blood, and if bacilluria occur in 25 per cent. of cases, then the bacilli must be in the blood in more than 25 per cent. of cases, or if only present in that percentage they must escape every time. Now Kühnau's researches have shown that *B. typhosus* can only be found in the blood in about 25 per cent. of cases of enteric; and if we accept this as approximately true, it would appear that the kidneys eliminate or allow the passage of the organism every time, but this is a conclusion negatived by many experiments referred to in the section on renal elimination. To maintain the bacilluric percentage at 25 per cent., it is necessary to consider the possibility of organismal access to the bladder by another channel, namely, through the anterior rectal wall, and this has been suggested, I believe, by Blumer. I prefer, however, to consider that the organisms reach the bladder through the kidney, but in a smaller percentage of cases than 25 per cent.

Bacilluria and the public health.—It is from the second type of typhoid bacilluria that the health of the public has most to fear. Cases apparently recovered may still infect the community, and possibly the Long Orton epidemic reported by Walker may have been due to this cause. The fact that such a type of disease exists would suggest the necessity of a bacteriological examination of the urine in each case immediately before discharge from hospital or return to communal life. This is not the place to discuss the clinical treatment of bacilluria, but the most successful drug for internal administration appears to be urotropin; and in view of the very small amounts of formalin which by experiment I have found inhibitory to the *B. typhosus*, there is no difficulty in crediting the rapid beneficial effect of this compound.

Prophylaxis.—That the urine is indeed infective, and that the organisms are not cast out in a state incapable of doing harm, has

been definitely proved by a case related by Petruschky, in which the infection was conveyed by a drinking glass which had been used as a urinal. It is therefore imperatively necessary to disinfect any urine containing the bacillus, and for this purpose half its volume of 1-40 carbolic acid solution, or of 1 per cent. formalin solution, or one-twentieth of its volume of 1-2000 corrosive sublimate may be used. Intimate admixture of the disinfectant with the urine is necessary for half to one hour.

Sterilisation by heat would be preferable, since it avoids the risk of imperfect admixture, and it would not be difficult to devise an efficient apparatus for routine use in such cases in hospital.

(B) **Scarlet fever.**—I have examined by the same method the urine of sixteen cases of scarlet fever, fifty-one specimens being used for cultures, and I have found in seven cases, at one period or another, streptococci, of which a description is appended. Specimens taken with bacteriological precautions, as well as those not so taken, contained these streptococci.

Phenolated broth and jelly were not used in these cases. Of the sixteen cases ten were males and six females, and their ages ranged from 3 to 42 years.

Specimens were taken at all periods of the disease from the first week to the fifteenth week, but mostly during the first six weeks. Albuminuria was present in ten, and nephritis in five of the sixteen cases.

Bacteriuria.—Two varieties of streptococci were met with and described as *Streptococcus 1* and *Streptococcus 2*. Other organisms which were present do not require description, being common, non-pathogenic species, but the coliform bacilli are separately described.

Streptococcus 1.—*Morphology and cultural characters.*—In 1 per cent. peptone broth at 37° C.—In twenty-four hours there is slight general turbidity, and in a few days the growth is most marked at the bottom of the tube in small coherent masses: this character becomes more pronounced in a week or ten days. Examined in hanging-drop, the elements are seen to be single or in pairs, or in short chains of four to six elements in each. The chains are seldom straight, but appear incurved, and the end elements are sometimes larger, more spherical and refractile than the rest.

Indol reaction.—The organism gives a positive indol reaction in broth after several days, generally eight to ten.

On agar at 37° C.—Small grey dots coalescing to form a streak with a moist surface. The agar fluid shows some growth in mass, and this microscopically is the same as the broth culture.

In stab jelly at 24° C.—A delicate filmy sheath of slow growth, composed of fine grey colonies, forms along the whole course of the stab. At the deeper end there are isolated, rounded, small grey colonies, but over the surface it spreads as a greyish white growth. There is no liquefaction or gas formation.

In gelatin plate.—Small greyish white, non-liquefying colonies, circular in shape.

In glucose jelly at 24° C.—Gas is formed after several days, less often in twenty-four hours.

In milk at 37° C.—Coagulation with acid formation in forty-eight hours.

On blood serum.—As on agar.

In litmus broth.—Acid is produced in twenty-four hours.

Streptococcus 1 was present in the urine in two cases, and in both it was associated with albuminuria. It appeared late in the disease, namely, in the fourth and tenth weeks respectively, and was not present later when the albuminuria had ceased.

Streptococcus 2.—*Morphology and cultural characters.*—*In 1 per cent. peptone broth at 37° C.*—After twenty-four hours there is slight general turbidity throughout the tube, but the chief growth is in mass at the bottom, whence it can be shaken up in viscid threads and flocculent masses. After a few days there is marked general turbidity, with large deposit at the bottom of the tube resembling pus in urine. In ten days the broth is viscous and mucoid. With a power of 800 diameters, and examined in hanging-drop, the elements are recognised as cocci, either single or in pairs, but mainly in chains of short length, consisting of six to ten elements, or it may be up to twenty elements in each. The elements are of small size.

Indol reaction.—Broth cultures give a positive indol reaction in a week or ten days, but in casein-peptone solution the reaction is positive on the fourth day.

On agar at 37° C.—A flat, white-grey growth (not differentiated into separate colonies) with crenated edges and a moist glistening surface. There is some growth in mass in the expressed fluid at the bottom of the agar tube.

In gelatin plate.—In twenty-four hours the colonies have a pearly-grey opacity and a smooth, glistening, raised surface. If kept at room temperature for a fortnight, the plate shows both superficial and deep colonies. The superficial are white, raised, with very slight central depression, and resemble drops of wax. They have a glistening appearance, and an outline roughly circular. The larger and older colonies are sometimes concentrically ringed. The deep colonies are small, and are oval or circular in shape.

In stab jelly, growth occurs along the whole stab as a white or white-grey sheath, and towards the bottom of the stab isolated, rounded colonies are formed. There is no liquefaction, and there may be some gas production along the stab. On the surface of the jelly a white growth extends well over the surface, and has a more or less irregularly crenated margin.

In glucose jelly at 24° C. gas is produced generally within twenty-four hours.

In litmus broth at 37° C. acid is produced rapidly, the colour changing in twenty-four hours.

In milk at 39° C.—Coagulation and separation of whey in forty-eight hours, often in twenty-four hours. The whey is colourless and acid in reaction.

On blood serum the appearance resembles that on agar, but the growth is, if anything, more white and plentiful.

Streptococcus 2 was present in five cases, in only two of which was the urine albuminous.

Comparison and contrast with other organisms.—Referring to the identity of the organisms here described as Streptococcus 1 and 2, I was for some time in doubt as to whether either was the same as the *Streptococcus scarlatinae* of Klein (*Streptococcus conglomeratus* of Kurth). Owing to the courtesy of Dr. Klein (at whose request Dr. Gordon most kindly supplied me with a culture of Dr. Klein's organism, for which I am much indebted to him), I was enabled to compare the organisms and to satisfy myself that there were marked differences between them.

Contrasting Streptococcus 1 with Klein's organism, it resembles it in the acid production and coagulation of milk, but differs in producing gas and indol, and in the minor degree of coherency which it exhibits. The growth in broth is both more diffuse and profuse.

Streptococcus 2 is not at all like Klein's organism: the viscous growth in broth alone would serve to discriminate between them, even without the additional qualities of gas and indol production. My experience of the streptococci has served to convince me that Gordon is correct in stating "that chain formation is only an incident in the life history of a streptococcus."

Nothing is more easy than from microscopic examination of the unstained specimen alone to mistake the spindle and rod shaped elements of a streptococcus for a bacillus, or to look upon masses of cocci as possessing no relationship to an organism whose chief morphology is streptococcal. Still further to deceive the casual observer, streptococci show not infrequently large spherical elements which closely resemble in size and refraction, and sometimes also in staining properties, the spores of spore-bearing organisms, and may be, in fact, something of a similar nature, as has been suggested by Klein. These things render it extremely difficult to discriminate the true nature of the organism, which is only fully disclosed by a series of cultures, and by a careful observation of its behaviour in fluid media.

Streptococcus of Baginsky.—Lately Baginsky and Sömmerfeld have described a streptococcus which they found in forty-two fatal cases of scarlet fever to be present in the organs, the blood, and bone-marrow in every case, and which they also found in the throat during life.

Its morphology and cultural characteristics, as described by them, are very similar to those of Streptococcus 1, but I am not able to state absolutely that it is the same. The organisms appear to agree in the character of their growth on agar, the power of forming gas in glucose jelly, and of acid production with coagulation of milk, and in their microscopic characters.

(C) **Diphtheria.**—I have examined seventeen cases of diphtheria and forty-three specimens of urine, without in any case isolating the *B. diphtheriae* of Löffler. The media employed were somewhat different from those used in enteric and scarlet cases, agar and agar-gelatin being used for plates as well as nutrient jelly. No medicant was added either to the broth or plates.

Of the seventeen cases, three were males and fourteen females, the majority being under 10 years of age, and the specimens were almost

equally spread over the first three weeks of disease, only a few being taken in the fourth week. Albuminuria was present in five cases, and Löffler's bacillus was present in the throat in fourteen cases, either alone or accompanied by cocci. Antitoxine had been administered in all the cases in amounts varying from 3000 to 14,000 units.

Bacteriuria.—In two of the cases a bacillus, morphologically bearing some resemblance to *B. diphtheriae*, was present, but it was decolorised by Gram's method, and was not the bacillus of Löffler; other organisms met with were cocci, coli, and fluorescens, which do not require description, and a streptococcus, denominated Streptococcus 3, which occurred in three cases.

Streptococcus 3.—*Morphology and cultural characters.*—In 1 per cent. peptone broth.—A turbid growth, without viscosity, but containing coherent mass formations. The elements are small, often in pairs, and the chains short, with generally about six constituents. The broth is not rendered acid. The indol reaction is positive on the twelfth day, with or without the addition of nitrite as well as acid.

In gelatin plate at 24° C.—In twenty-four hours the colonies are growing as grey, cloudy, granular colonies, superficially resembling coli, and non-liquefying.

In stab jelly, a delicate growth of isolated grey small colonies along the stab, no gas or liquefaction.

In glucose jelly, shake culture at 24° C.—There is no gas formation, but the organism grows in the medium without liquefaction.

In milk at 37° C.—Coagulation occurs within a week.

On agar, a moist, whitish grey growth along the streak, not showing individual colonies, and not so copious as Streptococcus 2. In the expressed fluid, growth possesses the same microscopic characters as in broth.

IV. SUBSIDIARY DIFFERENTIAL EXPERIMENTS.—

On various points connected with the research, subsidiary experiments were conducted, the results of which are here briefly mentioned.

(A) *Sterility of normal urine.*—Control experiments were performed with the urine of healthy persons to test the methods used in the research, and gave satisfactory results. Such urine is more often sterile than that of the sick, possibly owing to the increased difficulty of collection, or to the presence of a larger number of organisms in the urethra in the latter.

(B) *Urine as a culture medium for B. typhosus.*—Sterile urine of healthy persons and of enteric fever patients was found to be a suitable culture medium for *B. typhosus*, though in the latter the organism moved with less of its characteristic rapid wriggle.

(C) *Varieties of B. coli communis.*—The typical form was by far the most common, but several atypical forms were met with; their deviation from type is sufficiently indicated in the Table.

Comparison between the varieties here enumerated and the comprehensive classification of varieties of *B. coli communis*, drawn up by Dr. Gordon, shows that some of these corresponded very closely.

Varieties of Bacillus coli communis.

	Naked-eye Appearance of Colonies in Gelatin Plate.		Microscopic Characters.			Agar.	Milk.	Glucose Jelly.	Indol Reaction in Broth Culture at 37° C.	
	Superficial.	Deep.	Gelatin Plate Colonies, Deep under Low Power.	In Hanging-Drop, High Power, Short Motile Bacillus.	Flagella, 1 to 4.					
1	+	+	+	+	+	+	+	+	+	Typical.
2	+	+	+	+	+	+	0	+	+	Atypical.
3	+	+	+	+	+	+	+	+	0	"
4	+	+	+	+	+	+	+	+	0	"
5	+	+	+	+	+	+	+	+	+	"
6	+	+	+	+	+	+	+	+	0	"
7	+	+	+	+	+	+	+	+	+	"
8	+	+	+	+	+	+	0	+	+	"
9	+	+	+	+	+	+	+	+	+	"
10	+	+	+	+	+	+	+	+	+	"
11	+	+	+	+	+	+	+	+	+	"
12	+	+	+	+	+	+	+	+	+	"

Blue-grey Colour, irregular Margin and Shape.

Khaki Colour, Circular or Oval.

Deep colonies, blue.

Deep colonies, blue.

Colonies have very irregular edges.

Circular.

Circular.

Gelatin Plate Colonies, Deep under Low Power.

In Hanging-Drop, High Power, Short Motile Bacillus.

Flagella, 1 to 4.

Copious Opaque Growth in 24 Hours, at 37° C.

Coagulation with Acid Production in 48 Hours.

Gas Formation in 24 Hours, at 24° C.

Positive test gives Pink Colour within 7 Days.

Coagulation with Acid Production in 48 Hours.

After 7 days.

After 5 days.

Alkaline reaction.

In 5 days.

In 3 days.

In 5 days.

Gas Formation in 24 Hours, at 24° C.

After 11th day.

After 8-11 days.

In 3 days.

Indol Reaction in Broth Culture at 37° C.

Positive test gives Pink Colour within 7 Days.

After 30 days.

Typical.

Atypical.

"

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(a)	No.	2	resembles	Gordon's	variety,	vi.	
(b)	"	4	"	"	"	iv.	
(c)	"	6	"	"	"	xiii.	
(d)	"	8	"	"	"	i.	} among the alkali producers.
(e)	"	10	"	"	"	xi.	
(f)	"	12	"	"	"	xiv.	

(D) *Tests used to distinguish B. typhosus and B. coli communis.*—Reliance was placed upon—(1) motility; (2) flagella; (3) gas formation; (4) coagulation of milk; (5) growth on potato; (6) indol reaction within certain limits; (7) rate of acid production in litmus broth; (8) Widal reaction, agglutination; (9) rate of growth in gelatin plate; (10) growth in semi-solid media.

Influence of B. coli communis on B. typhosus in mixed culture.—Numerous experiments showed that the recovery of the *B. typhosus* from the mixed culture depends on—(a) the vigour of the stock of typhoid organism used; (b) the time elapsed after inoculation of the *B. coli communis*; (c) the method used to separate the organisms.

(E) *Experiments with medicated media.*—The medicants used were phenol, formalin, sp. vin. rectific., and the essences of peppermint, eucalyptus, cloves, and cinnamon, and many experiments were conducted with varying proportions of the medicant. The conclusions arrived at were—(a) That 0.05 per cent. phenolated media would be useful in restraining undesirable organisms present in the urine, while allowing any typhoid and coliform bacilli to grow. (b) That formalin was too fatal to typhoid to be used in examining enteric urine, but that 0.0125 per cent. formalin jelly might aid in deciding whether a particular organism was typhoid or colon, since it is fatal to the former, while allowing the growth of the latter. (c) That the essential oils likewise would be of little use, since, except in the case of eucalyptus, small quantities sufficed to prevent the growth of typhoid, and all of them were less inimical to *B. coli communis* than to *B. typhosus*. (d) That cinnamon and cloves were much more powerful than peppermint and eucalyptus in restraining the growth of *B. coli communis*. (e) That rectified spirit alone does not prevent the growth of either typhoid or colon bacillus unless present in very large proportions, and this is especially the case as regards *B. coli communis*.

(F) *Note on indol reaction.*—The indol reaction has been much relied upon as a valuable distinction between *B. typhosus* and *B. coli communis*. Within certain limits this is still the case, but the reaction is not a fundamental distinction between the organisms. Typical *B. coli communis* gives the indol reaction in broth culture after incubation for five days or less at 37° C., but there are atypical varieties in which the reaction is delayed for varying periods up to a month, and there are some which do not give it at all. That the typhoid bacillus would ever give the indol reaction in broth was unknown to me, until I happened to apply the test to some old cultures, and found that it did so. On investigation, I found that Chantemesse had previously recorded the same observation, and that Peckham had found that *B. typhosus* will assume the indol function under special conditions, conditions which also favour the increase of indol production by the

B. coli communis, and which may in time cause the latter organism to lose its power of indol production.

The medium used by Peckham is termed by him "alkali-peptone bouillon," and in it seventeen stocks of *B. typhosus* all gave indol by the fourth generation, each generation representing three days' growth. Experiments were instituted to investigate the subject, and I found that—

1. In 1 per cent. peptone broth the *B. typhosus* will give the indol reaction after incubation at 37° C. for ten to twelve weeks.

2. If now this organism be inoculated into fresh broth, and incubated at 37° C., it will give the indol reaction in seven to ten days; or if it be plated on agar, and thence transferred to broth, the indol reaction may be obtained in the same time.

3. If the *B. typhosus* be cultivated anaerobically on agar for six weeks at 37° C., and if then it be inoculated into broth and incubated aërobically at 37° C. for twelve days or a fortnight, it will give the indol reaction.

The same bacillus cultivated anaerobically in broth gave the indol reaction in ten to twelve weeks, much the same time as in aërobic cultivation.

4. In alkali-peptone bouillon the *B. typhosus* produces indol. I did not obtain the reaction in the earlier generations, and did not discover the reason of the difference between Peckham's experiments and my own until I tested the alkali-peptone bouillon, and found that it contained either nitrite or some substance with a similar action. The addition of acid alone gave the indol reaction at the twelfth generation of *B. typhosus*. Probably indol had been produced in the earlier generations, in accordance with Peckham's results, but excess of nitrite had prevented the reaction.

The presence of nitrite in the bouillon before inoculation was proved by three tests, all positive in their results—(a) Indol + H₂SO₄; (b) metaphenylene-diamine test; (c) iodide of zinc and starch solution test.

5. The *B. typhosus* will in a suitable medium produce indol within a week, usually on the fourth or fifth day. I prepared a medium which I termed casein-peptone solution, the actual preparation of which has been detailed above, but is only tentative, since possibly it could be more simply prepared without loss of its value as a medium for testing indol production.

This solution I found also contained a small quantity of nitrite, which was not present in the original casein.

In casein-peptone solution, four stocks of *B. typhosus*, obtained from different sources, all gave the indol reaction by the fifth day. A most important point in connection with the indol reaction is the amount of nitrite solution required in the test. Any excess of nitrite prevents the reaction, so the solution used must not exceed 0·02 per cent., and of this 0·1 to 0·5 c.c. is sufficient to test 10 c.c. of broth or other medium.

It is well to remember that the medium may itself contain nitrite, and to abstain from using a medium in which this is in excess.

(g) *Tyrosin experiments*.—Neither *B. typhosus* nor *B. coli communis* produce indol when incubated in tyrosin solution (1 part of tyrosin to

2000 distilled water). The addition of tyrosin solution to broth does not intensify the indol reaction produced by *B. coli communis*, nor does it induce the early production of indol by the *B. typhosus*. It is unlikely, therefore, that tyrosin is a stage in the production of indol from peptone by these organisms.

CONCLUSION.—It is not necessary here to recapitulate the results of each section of the report; they have been summarised in their respective portions. It may be advisable, however, to emphasise one or two of the main facts.

1. That pathogenic organisms may be present in the urine in some zymotic diseases, notably plague and enteric fever, but the percentage of cases showing typhoid bacilluria is small.

2. That the spread of the infection of enteric fever by means of the urine must be prevented in the interests of the public health. Fortunately, the risk is not attached to every case, but the insidious nature of the bacilluria demands that no enteric fever patient should be discharged from supervision, until the absence of bacilluria has been proved. In those cases in which bacilluria has occurred during the course of the disease, its cessation must be proved by bacteriological examination of the urine, before the patient is allowed to resume ordinary life. It would be a counsel of perfection to recommend a similar examination in all cases; but so large a percentage of cases never have any bacilluria at all, that this course would entail much unproductive labour. By careful daily observation in every case of the appearance of the fresh urine, during the fortnight before the discharge of a patient, the physician should note any cloudiness or turbidity, or any sediment. These are valuable guides as to those urines in which further examination is desirable. The reaction of the urine is no guide, since a normally acid urine may contain the bacilli, and is more likely, indeed, to contain a pure culture of typhoid bacilli than an alkaline urine. The nature of sediments should be determined, and all urines observed to be cloudy should be examined bacteriologically. By this course I believe that typhoid bacilluria might be detected, and measures could then be taken for the prevention of infection by it.

3. The influence of other organisms on the *B. typhosus* in mixed cultures varies with the relative vigour of the respective stocks thus mingled. The typhoid organism is usually the less hardy, and as time goes on there is greater difficulty in isolating it.

4. That there are a large number of organisms, intermediate between the *B. typhosus* and the *B. coli communis*, approximating more or less closely to one or the other type. Their relationships require further elucidation.

5. No fundamental distinction exists between *B. coli communis* and *B. typhosus* in the matter of indol production. Both are indol-producing organisms in suitable media. The indol reaction

in 1 per cent. peptone broth is a valuable discriminative test between the two organisms, *only* when the typhoid organism is freshly cultivated, and the *B. coli communis* is typical.

6. That casein-peptone solution is the best medium for testing powers of indol production.

7. That Remy's jelly, alkaline-peptone bouillon, and casein-peptone solution, are useful additions to the list of media.

This research has been rendered possible by the kindness of the Senatus Academicus of the University of Edinburgh, in allowing me to work as a research student in the Public Health Laboratory of the University. I am very much indebted to Professor Hunter Stewart for placing at my disposal the resources of the laboratory, and for facilitating the work by much personal interest and advice of great value. Also I desire to thank most cordially, Dr. Ker, superintendent of the Edinburgh City Hospital, and Dr. Porter, late resident physician, Leith Public Health Hospital, for permission to collect and examine the specimens, and for furnishing me with details of the respective cases.

