

STUDIES ON THE SMEGMA BACILLUS

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PART I.—LITERATURE.

INTRODUCTION. Closely following the discovery of the tubercle bacillus by Koch, in 1882, other workers announced the finding of bacilli resembling the tubercle bacillus in staining and morphological characteristics. Several claim credit for this discovery—all working and reporting at about the same time—chief among whom are Alvarez, Czaplewski, Doutrelepon, Laseer, Matterstock, and Tavel. Only one year earlier, in 1884, Koch had suggested the idea and possibility of there being several other bacilli with staining properties similar to those of the tubercle bacillus.

SMEGMA BACILLUS. This newly discovered bacillus—the smegma bacillus or *Bacillus smegmatis*—came in apparent answer to this prophecy, and was doubly confusing because it resembled the tubercle bacillus not only in its chief and heretofore distinctive characteristic—its staining properties—but also in its morphology. This confusion and difficulty were likely to be encountered in bacteriological examinations of urine where mistaken diagnoses have probably resulted in many useless but serious operations.

We are indebted to Dr. M. P. Ravenel, director of the laboratory, for assistance and advice during the progress of this work and in the preparation of this paper.

The work divides itself into two main parts: (1) a study of the available literature, which is scarce as well as conflicting, and (2) the laboratory work.

OCCURRENCE. The smegma bacillus is found widely distributed over the surface of the human body. It appears in especially large numbers in any place where the normal skin secretions are allowed to collect, as about the genitalia, under the foreskin in males, and between the labia in the female, about the anus and the perineum of both, in the umbilicus, in the cerumen of the ear, about the teeth, etc. In this respect its occurrence is dependent upon the degree of cleanliness of the patient. It also appears in the urine—more abundantly in females—where, according to some writers, it may be found in 59 per cent. of the cases, while others report it as rarely present, especially in urine from males. It has also been

reported present in sputum and lung cavities in cases of gangrene of the lungs and fetid bronchitis. One case has been reported of an ovarian cyst in which bacilli very similar to if not identical with the smegma bacillus were found, which were thought to have been there by perforation from the rectum or by digital examination.

The occurrence about the genitalia and possibility of gaining entrance into the urine, where they might be mistaken for tubercle bacilli, resulting in a diagnosis of genito-urinary tuberculosis, forms the chief point of practical consideration in the study of the smegma bacillus. This source of contamination of urine, however, is not deep-seated, since no writer reports finding smegma bacilli in the bladder or the deeper portions of the urethra. On the other hand, several report unsuccessful attempts to find them there.

One investigator reports work done on animals in which he found the smegma bacillus about the genitalia of horses, dogs, cattle, guinea-pigs, and white rats. The cat and rabbit gave negative results. In smear preparations, several note the tendency of the bacilli to be found more abundantly in and about epithelial cells or other debris.

MORPHOLOGY. The difficulty and uncertainty of cultivation necessitate greater dependence on microscopic findings, but it is here that we find the close resemblance of the smegma bacillus to the tubercle bacillus, and morphology presents one of the two chief possibilities of confusion.

It varies widely in morphology, but usually is 3 to 5 μ long by $\frac{1}{4}$ to $\frac{3}{4}$ μ wide: a rather long, slender, often slightly curved bacillus or little rod with rounded ends. Its morphological variations seem no more limited to one dimension or property than to another. It may be about the same length as the tubercle bacillus as ordinarily found in human sputum—much shorter, much longer—or we may find some preparations with only straight bacilli, or others almost entirely composed of small curved rods, some thicker, some thinner. The short bacilli often appear like comma bacilli. Some authors claim the form and size are dependent on the kind of culture media used. One author suggests that the degree of curvature is apparently dependent on the position of the bacillus in the colony—those nearest the centre being most curved, while those farther out and composing filaments are straight. A wider variation from cultures is only to be expected from, and certainly would tend to strengthen the doubt in, the fact that the organism has not yet been cultivated, at least not so frequently and generally as claimed.

Dahms claims never to have found a "smegma bacillus showing in a typical way the peculiar sharp curve near one extremity which is so frequently observed in tubercle bacilli."

Many claim to have found so-called "spores," "spore bodies," "sporogenic bodies," etc., appearing at each end of the stained bacillus, taking a deeper stain and giving to short bacilli at least

the appearance of diplococci or of two cocci lying close to each other. Preparations from colonies on media producing rapid growth show evidence of manner of growth and development. Scattered among the bacilli throughout the field are to be found numerous short, oval segments or egg-shaped bodies of the same color as the bacilli, but quite separate and distinct from them. Also other bodies—little rods intermediate in size between the egg-shaped segments and the mature bacilli—are found and seem to be an intermediate stage of development. Some report finding granular bacilli on certain kinds of media, especially Löffler's blood serum, while others claim never to have seen granular forms. Hang-drop preparations show absence of independent motion.

STAINING. Staining is the second important point in which the smegma bacillus resembles the tubercle bacillus, and this is second in order of enumeration only, because in order of importance it really holds first place. Like the tubercle bacillus, the smegma bacillus belongs to the class of so-called "acid-fast," *i. e.*, it resists decolorization by acids after staining with carbol-fuchsin. Herein lies the real difficulty for the clinician, for the test long held for the tubercle bacillus was this property of acid-fastness. Then the discovery of the smegma bacillus introduced a source of error in that in certain cases, as for instance in the examination of urine for tubercle bacilli, the mere finding of red bacilli after decolorization, was not sufficient basis for diagnosis of genito-urinary tuberculosis, because it might be the smegma bacillus. Immediately several workers undertook the problem of devising a method whereby an accurate differential diagnosis could be made between these two similar organisms, and their attempts, especially those of the earlier workers, were to stain it under two conditions: in tissue and in smear preparations.

These efforts proved successful in quantity but of doubtful success in value, since scarcely any two workers have reached the same conclusions. Yet each has proposed and enthusiastically advocated some method quite different from all others, and one which he is sure will prove reliable in all cases at all times. As a rule, he has been equally enthusiastic and urgent in his disapproval of all other methods of differentiation by staining or otherwise. At the present time no absolutely reliable method of differentiation by staining properties has been found, and yet more than twenty different methods have been advocated by the originator of each. The cause of this wide variation in results is found primarily in the variable staining properties of the smegma bacillus, and possibly secondarily in the fact that no one observer has done sufficiently extensive work to prove the inaccuracies of his own method. More recent writers suggest the impossibility of a differential method of staining due to the variation in the staining reactions of the smegma bacillus and its close relationship to the tubercle bacillus.

The numerous methods thus far suggested have, in the main, agreed upon and been based on two points: (1) that the smegma bacillus stains with difficulty but is equally slow and difficult of decolorization, and (2) that it possesses both these properties to a less extent than the tubercle bacillus. Several observers have mentioned variation in staining reaction dependent on the nature of culture media or stage of subculturing. For instance, some claim the acid resistance to be greater when the organism is grown on drier culture media, and others claim each successive transfer or subculture shows a gradually decreasing degree of acid-fastness. A. Moeller, though, claims the smegma bacillus to be absolutely acid-alcohol-fast, not decolorized by twelve minutes' application of 3 per cent. HCl-alcohol and not diminished in this property after twenty-five generations.

Possibly the best idea of the various methods is obtainable from a summary and classification used by Drs. Young and Churchman. A brief form of their classification follows:

I. Methods Depending on Greater Avidity of Smegma Bacillus for the Stain.

(a) Method of Giacomi: Specimen in carbol-fuchsin heated barely to steaming; smegma bacilli deeply stained; Tubercle bacilli not at all or only faintly stained and easily decolorized.

II: Methods Depending on Decolorization without Alcohol.

(a) Method of Gabbett: Specimen heated in carbol-fuchsin, washed in water, decolorized, and counterstained with Gabbett's solution of methylene-blue, H_2SO_4 , and water.

(b) Method of Ziehl-Neelsen: Specimen heated in carbol-fuchsin, washed in water, decolorized by H_2SO_4 , and counterstained in aqueous methylene-blue.

(c) Method of Fränkel: HNO_3 replacing H_2SO_4 in (a).

(d) Anonymous method (recommended by Alvarez and Tavel): Glacial acetic acid replacing H_2SO_4 in (a).

(e) Method of Kühne: Carbol-fuchsin to stain, HNO_3 to decolorize, and picric acid to counterstain.

(f) Method of Weigert (modification of Gram's method): Stained and treated with KI solution as in Gram's method. Anilin oil to decolorize.

(g) Method of Giacomi: Heated in distilled water containing two to three drops of concentrated alcoholic fuchsin, wash in weak solution of perchloride of iron and then decolorize in concentrated solution of the same.

III. Methods Depending on Decolorization with Alcohol Alone.

(a) Method of Gram: Stain with aqueous solution of gentian violet, then with Gram's solution of KI, decolorize with absolute alcohol.

(b) Method of Weichselbaum: Stain in carbol-fuchsin, decolorize, and counterstain with concentrated methylene-blue in absolute alcohol.

(c) Method of Czaplewski: Carbol-fuchsin to stain, wash for five minutes in fluorescein methylene-blue, then for one-half to one minute in concentrated methylene-blue.

(d) Method of Pappenheim: Stain in carbol-fuchsin, then dip three to five times in solution consisting of absolute alcohol, corallin methylene-blue, and glycerin. Tubercle bacilli are stained red and smegma bacilli blue.

IV. *Methods Depending on Decolorization with Alcohol and Another Agent.*

(a) Method of Koch and Ehrlich: Stain for twenty-four hours in anilin water fuchsin (or gentian violet). Transfer to HNO_3 , one to four. Place in 60 per cent. alcohol for a few seconds, wash, and counterstain with methylene-blue (or vesuvin).

(b) Method of B. Fränkel: Stain with carbol-fuchsin, decolorize with a mixture of HNO_3 , alcohol, and methylene-blue.

(c) Method of Marzinowski: Stain in watery solution of carbol-fuchsin. Counterstain and decolorize with Löffler's methylene-blue.

(d) Method of Lustgarten: Stain twelve to twenty-four hours in concentrated alcoholic solution of gentian violet mixed with aqueous solution of anilin oil. Wash in absolute alcohol, ten seconds in 1.5 per cent. solution of potassium permanganate, then in concentrated aqueous solution of pure sulphurous acid (freshly made by action of H_2SO_4 on metallic copper). After disappearance of precipitate of manganese oxide, repeat permanganate, sulphurous acid, and water until decolorization is complete.

(e) Method of Alvarez and Tavel: Same as (d) except oxalic acid instead of sulphurous. Also counterstain with saponin, eosin, or picrocarmin.

(f) Method of Honsell: Carbol-fuchsin stain, wash, place in acid alcohol (3 per cent. HCl in absolute alcohol) ten minutes, wash, counterstain with alcoholic methylene-blue.

(g) Vesuvin and malachite green have also been advised (by Fränkel) and magenta and chrysoidin (by Gibbs).

V. *Methods Depending on Preliminary Treatment Aimed at Diminishing Resistance to Decolorization.*

(a) Method of Matterstock, Bitter, and Markuse: Preliminary solution of fatty substances by ether or chloroform. Stain as usual.

(b) Method of Gottstein: Heat specimen with KI in alcohol, wash in alcohol and water; the fatty substances are removed and the specimen is then stained as usual.

(c) Method of Bunge and Trautenroth (method for spores): Fix specimen and remove fat by absolute alcohol. Then treat with 5 per cent. chromic acid, stain with carbol-fuchsin, decolorize with dilute H_2SO_4 then with pure HNO_3 , counterstain with concentrated alcoholic methylene-blue.

CULTIVATION. Cultivation of the smegma bacillus is one of the most fruitful sources of confusion and difference of opinion among

various workers. Laser, in 1897, claimed to have cultivated it and Czaplewski, a little later, claimed to have secured the first pure cultures. Others followed quickly with similar claims, but more recent workers and the growing consensus of opinion seems to discredit these claims, believing that some other members of the acid-fast group instead of the smegma bacillus were cultivated.

The finding of these men and others who have claimed its cultivation was the result of accident, their purpose usually being to cultivate gonococci or to find a cause of syphilis.

The uncertainty about the reliability of their results is strongly substantiated by both the widely differing results and the ease with which some have obtained growth on almost any kind of culture media, as contrasted with the absolute inability of others to get any growth whatever. A brief summary of their claims, is here given:

The first cultures were made upon agar plates and slants which had been smeared with sterile human blood and incubated twenty-four hours to prove sterility before inoculation. Growth resulted in small colonies like diphtheria or streptococcus. Transplanted to blood-serum and glycerin-agar the growth continued in the same small almost dew drop-like colonies. Most investigators report a difference in cultivation between smegma bacilli and tubercle bacilli in that the smegma bacillus grows more rapidly—a few reporting several days required—but it is usually reported as growing within twenty-four hours to a few days.

The growth on different kinds of media is as follows:

Agar smeared with sterile human blood: numerous, small, diphtheria or streptococcus-like colonies showing mainly micrococci, but after several days are found a few, small, irregularly rounded, grayish-white colonies which prove to contain smegma bacilli.

Gelatin: No development. Some claim slight growth of rather short bacilli, often curved and knotted or with ends swollen in club-like swellings. Weber claims the growth to be composed of quite large bacilli.

Agar slants and plates: Sparse growth after several days at 37° C.

Peptone water and bouillon: Scarcely noticeable growth of a flocculent grayish color.

Glucose bouillon: Marked development in twenty-four hours. White sediment loosened in shreds on shaking. Moeller claims a dry pellicle-like growth floating on the surface of the bouillon, which sinks to the bottom in fragments on shaking.

Potatoes: No visible growth after three days, but rather long bacilli found on scraping the surface for a smear. Later scanty growth, yellowish layer, irregular shape, and indefinite outlines. Weber characterizes the bacillus grown on potato as short and almost coccus-like. He also claims better growth if the potato is alkaline in reaction.

Nutrose-serum agar (used for Czaplewski's first culture): Numerous, small, irregularly rounded colonies of rather long bacilli.

Glycerin agar: Twenty-four hours showed distinctly visible growth, a grayish, well-defined layer. Forty-eight hours showed thick, grayish-white, or yellow growth, margins sharply defined, indented and lobulated appearance, centre darker.

Löffler's serum: Twenty-four hours gave scarcely visible growth. Forty-eight hours, a yellowish-gray color. Variable size from mere specks to areas 2 mm. in diameter, which by confluence later formed a distinct layer. Bacilli often appear granular and as short rods.

Glycerin-milk agar (used and prepared by Neufeld): Claimed to reproduce more nearly the natural conditions of nourishment and to resemble smegma so closely that the bacilli grown on it are more like those observed in direct smear preparations, *i. e.*, some claim that a shorter, plumper bacillus grows on this medium.

Milk medium seems especially likely to produce a growth characterized by an abundance of pleomorphic forms. It is not coagulated, however, by the growth.

Weber reports the successful use of lanolin agar, claiming growth in sixteen out of eighteen cases showing smegma bacilli in one series examined. Stab cultures in agar and gelatin show limited extent of growth which is quite closely confined to the path of the needle. All agree upon an optimum temperature of 37° C.; no growth below 23° C.

The microscopic appearance of a colony of smegma bacilli (especially with the large flat colonies found on glycerin-agar) is wax-like, white or nearly so, dense, finely granular, and with sharply defined margins. The colonies are not always circular, yet never sharply angular, but their irregularities consist of rounded and lobulated extensions. The higher power of the microscope used on a stained impression smear from one of these colonies shows very well the arrangement of the smegma bacilli in the colony, often quite like tubercle bacilli. The bacilli may be in straight lines end to end, as portions of concentric circles, or branching from each other at various angles or forming long parallel lines.

PATHOGENICITY. On one point every investigator presents the same report, and that is the pathogenicity of the smegma bacillus. All agree in saying that it is a harmless, non-pathogenic organism, *i. e.*, a saprophyte. All pieces of work are practically a unit on this, that animal inoculation fails entirely to produce any serious results. Some claim, though, that a slight tubercle-like swelling or nodule is produced at the point of inoculation.

The one case reported by Dietrich of finding smegma bacilli in an ovarian cyst would seem to indicate the possibility at least of the organism possessing some pathogenicity. As mentioned above, the probable explanation of this case is the entrance of the bacilli by digital examination.

It might be added that the unity of opinion on the question of pathogenicity does not seem to verify the claims of cultural findings; for if others than the true smegma bacillus were really cultivated, they were all of the same class, harmless acid-fasts, and hence unable to give positive inoculation results.

PART II.—LABORATORY WORK.

The laboratory side of this paper, undertaken with a view of determining the prevalence of the smegma bacillus about the male genitalia and of verifying some of its cultural and staining properties, must from the nature of the object as thus stated divide itself into two main parts: The first consisted of collecting smegma from which smears were made to be stained and tabulating results of urinary examinations in this laboratory during the past two years. The second consisted in taking swabs or collecting larger amounts of smegma to be used for planting out on several kinds of culture media in an attempt to grow the smegma bacillus.

For this material, access was kindly granted to the male wards of the Wisconsin State Hospital for the Insane and the Dane County Asylum, and to Drs. Lorenz and Stebbins for kindness and coöperation, thanks are gladly extended.

Tabular form¹ will best show the results of each piece of work, preceded by a brief explanation of method or technique used, and followed by summary of results obtained. The first series will consist of a number of cases from which smear preparations were taken, stained, and studied. No complicated plan or procedure was required for this. The inmates were simply taken one at a time, the penis examined for presence of smegma in sufficient quantities for the purpose of this study, and then a number of smears were made on glass slides, banded together and numbered. Or if, as was true in many cases, the smegma found was the product of too long accumulation and therefore too thick and dry to be smeared out at once, a sufficiently large piece was removed and taken to the laboratory to be mixed with sufficient sterile water to make the required number of smear preparations. Then these, together with the rest, were fixed by heat and stained as follows: one in a borax solution of methylene-blue; one in carbol-fuchsin followed by decolorization with 25 per cent. H_2SO_4 until the slide presented the same appearance of decolorization as is usually presented by a slide of sputum stained and decolorized for tubercle bacilli, and then, after examination, counterstained in borax methylene blue (the oil being carefully removed with xylol before counterstaining); one in carbol-fuchsin followed by decolorization with acid alcohol

¹ Detailed tables omitted on account of length.

(5 per cent. HNO_3 , 70 per cent. of 95 per cent. alcohol, 25 per cent. H_2O) until the slide presented usual pale appearance; and one in carbol-fuchsin, followed by decolorization with Labarraque's solution until the slide had assumed a distinct brown color.

All carbol-fuchsin staining was done with Ziehl's carbol-fuchsin for twelve hours in a paraffin oven at 50°C . or twenty-four to thirty-six hours at room temperature.

No. of cases.	Stain.	Decolorizer.	Counterstain.	No. showing smegma bacilli.	No. not showing smegma bacilli.
126	Carbol-fuchsin	H_2SO_4	None	85	41
112	Carbol-fuchsin	H_2SO_4	Methylene-blue	19	107
196	Carbol-fuchsin	Acid alcohol	None	16	103
117	Carbol-fuchsin	Labarraque's solution	None	16	101

SUMMARY OF TABLE. Of 127 cases taken, practically all present the same picture when stained with methylene-blue. A number of different kinds of cocci and bacilli are present in abundance, the predominating organism always being mentioned first. Many of the bacilli are diphtheroid in appearance. The chief difference between any one slide and another is simply a matter of degree and proportion of each kind of organism, each having the same types.

Of 126 stained with carbol-fuchsin and decolorized with 25 per cent. H_2SO_4 , 41 cases, or 32.5 per cent., failed to show any red bacilli at all, while 85 cases, or 67.5 per cent. showed red bacilli in greater or less abundance as described under each case as to size, shape, etc.

Of the same 126 cases counterstained with methylene-blue, 85 of which had shown red bacilli before counterstaining, only 19 cases, or 22 per cent., continued to show them in greater or less numbers, *i. e.*, 77 per cent. of the cases showing red bacilli before counterstaining lost or had their red stain masked when counterstained.

Of 119 cases stained with carbol-fuchsin and decolorized with acid alcohol, 16 cases, or 13 per cent., showed a few or more red bacilli, usually very faint red or in larger masses of debris in the preparation which had not been so thoroughly subjected to the action of the decolorizer.

Of 117 cases stained with carbol-fuchsin and decolorized with Labarraque's solution, 16 cases, or 13 per cent., showed red bacilli in varying numbers and in varying stages of decolorization from faint red almost decolorized to dark red bacilli, as yet unmodified by the action of the decolorizer.

Since the material in the above series was secured from a class of men whose condition would mean habits of cleanliness not up to the average standard, a series of 20 cases from normal men was studied, somewhat as a control for results of the first series. The

subjects from whom these specimens were obtained were men about the laboratory, faculty, and students.

Material was collected as for the first series, save that only H_2SO_4 was used as a decolorizer, the purpose being merely to determine the presence or absence of smegma bacilli.

Of 20 cases studied in this second series, 13, or 65 per cent., showed the presence of smegma bacilli when stained with carbol-fuchsin and decolorized by 25 per cent. H_2SO_4 . This is to be compared with 67.5 per cent. in the first series.

Two cases, or 10 per cent. of all cases, still continued to show smegma bacilli even after counterstaining. This corresponds to 14 per cent. similarly obtained in the first series.

Methylene-blue used alone showed the same results as in the first series.

URINE. From an interest in the findings and conclusions of Young and Churchman that urine could not be safely collected unless by their method, which provides for previous urethral irrigation, and the conflicting statement of other writers that catheterization is sufficient, a study of the case records of this the State Laboratory of Hygiene has been made. This covers a period of the past two years, and includes all the cases of urine examined for tubercle bacilli, whether this examination was especially requested and indicated by history and suspicions of genito-urinary tuberculosis or was simply made as a part of the routine examination of urine.

The results of this study are tabulated below:

Findings.	To be examined for tubercle bacilli.	Routine examination.
Positive to tubercle bacilli	3	2
Negative to tubercle bacilli	10	18

This shows 22.5 per cent. of cases of suspected tuberculosis to show acid-fast and 10 per cent. of cases not suspected, or 14 per cent. of all cases examined for tubercle bacilli. These cases are taken as they came into the laboratory from all over the State, without special precaution or technique to avoid getting smegma bacilli into the urine, and probably few were collected by catheter.

Young and Churchman report 11 cases out of 24 collected similarly as positive, or 45 per cent., as compared with 14 per cent. found here, including more than one-third of the cases in which diagnoses of genito-urinary tuberculosis had already been made or suspected. This would tend to show less chance of the smegma bacilli getting into the urine than these workers suggest.

CULTIVATION. *Technique.* The second portion of the laboratory side of this study consists of a series of cultures from 42 cases, taken in different ways, treated differently, planted differently, and incubated differently in an effort to produce growth.

The material for the first 32 of these was collected from the penis by the usual sterile swab used for the taking of cultures and planted on various kinds of artificial culture media—meat-lactose-agar, human hemoglobin-agar, cat-blood agar, blood-serum, meat plain agar, and meat-glucose-agar. Cultures were incubated at 37° C. aëroically or anaëroically, as the case might be, until examined. Examination of smear preparations was made after varying periods of two days to two weeks, and with two staining methods. One preparation was stained with borax methylene-blue and the other with carbol-fuchsin, followed by 25 per cent. H_2SO_4 to decolorize, then examined, counter-stained with methylene-blue, and again examined.

The technique for the last ten cultures differed slightly from the first series. The antiformin method for isolation of tubercle bacilli was used on large quantities of smegma taken from several of the more abundant cases and the sediment planted out on Dorset's egg medium, three or four loopfuls each to the first two tubes and the remainder poured into the third.

RESULTS. Of the 42 cases studied, the first 32, or those planted as from ordinary swabbing, show but little difference save the 6 grown anaëroically, and these seemed to have slightly less abundant growths. In all cases, however, the growth was quite abundant, and upon examination proved to contain the same organisms, cocci and bacilli, of several kinds and in varying proportions. Very often bacilli were found which were so like diphtheria bacilli that a differentiation could be made only with difficulty, if at all. Many others, while not resembling the diphtheria bacillus so closely as to be called pseudodiphtheria, still were enough like to be called diphtheroid. These are probably the same organisms mentioned by several observers in their writings on the smegma bacillus.

Stained for acid-fasts, these same preparations failed to show any organisms which had resisted decolorization, only a few doubtful ones being found, and these were quickly proved negative on counterstaining.

The last 10 cases treated by a method calculated to eliminate the abundant growth of other bacilli and cocci failed to show any better results, save that the growth was less abundant.

CONCLUSIONS. From the foregoing we can see several conclusions quite clearly set forth for us, not least among which is the need for a thorough, comprehensive, dependable piece of work on this subject.

From the knowledge at hand it is evident that:

1. The smegma bacillus resembles the tubercle bacillus very closely in staining properties and in morphology.
2. No method of differentiation by staining is entirely adequate for all cases.
3. The successful cultivation of the true smegma bacillus is open to question.

4. Successful cultivation granted, the inherent difficulties by methods now available render this method of differentiation of little value in routine diagnosis.

5. The smegma bacillus is found in a large proportion of patients examined, but the widespread occurrence is quite dependent on the degree of cleanliness of the patient, together with the presence or length of foreskin in males.

6. By thorough cleaning of the genitalia, especially the meatus and by catheterization, urine to be examined for tubercle bacilli can be collected with a fair degree of certainty of freedom from smegma bacilli.

7. With the above precautions, the finding of acid-fast organisms after decolorization with acid-alcohol enables us to make a presumptive diagnosis of genito-urinary tuberculosis.

8. Final differentiation, however, can be made only by animal inoculation.

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