A PRELIMINARY REPORT ON ACID-RESISTING BACILLI, WITH SPECIAL REFERENCE TO THEIR OCCURRENCE IN THE LOWER ANIMALS.

BY D. MURRAY COWIE, M. D.,
First Assistant in Internal Medicine, University of Michigan, Ann Arbor, Michigan.
(From the Clinical and Hygienic Laboratories of the University of Michigan.)

At the suggestion of Professor Dock, I began four years ago to make some investigations on the occurrence of the smegma bacillus in man. While pursuing this work, which is not yet completed, it occurred to me that it might be not only of interest but of importance to know whether the smegma bacillus or bacilli resembling those of tuberculosis existed to any great extent in the lower animals. It is this point I wish to consider here.

Resistance to decolorization by strong acids (Säurefestigkeit), as was first shown by Ehrlich, is the most characteristic staining reaction of the tubercle bacillus and, at the date of its discovery, differentiated this bacillus from all other known bacteria except the bacillus of leprosy. Since this time, however, a number of other acid-resistant bacilli have been discovered, the list increasing with especial rapidity during the last few years. Soon after the discovery of the tubercle bacillus in 1882, G. Zahn noted the presence in non-tuberculous sputum of bacilli resembling the tubercle bacillus in staining reaction, and recently acid-resistant bacilli resembling tubercle bacilli have been found in the sputum in cases of pulmonary gangrene by A. Fraenkel, Pappenheim, and Rabenwitsch. Laabs and Möller have also recorded the presence of acid-resistant bacilli in non-tuberculous buccal secretion and sputum.

1 Read at the meeting of the Medical Society held in the Medical College, Ann Arbor, December 14, 1899.
3 Berliner klin. Wochenschr., 1898, pp. 346, 880.
4 Ibid., 1898, p. 309.
5 Deutsche med. Wochenschr., 1900, p. 297.
6 Inaug.-Diss., Freiburg i. Br. 1894.
7 Zeitschr. f. Hgy., 1899, xxxii, p. 211.
Acid-Resisting Bacilli in the Lower Animals

The prototype and longest known of these acid-resisting pseudo-tubercle bacilli is the smegma bacillus first described in 1885 by Alvarez and Tavel, and by Matterstock and Bitter, and obtained in artificial culture by Lasar and by Czaplewski. The occurrence of acid-resisting bacilli in the human intestine is mentioned by CrOmer, de Giacomi, and von Jaksch, so that there is proof that bacilli resembling in tinctorial properties and often more or less closely in morphology the tubercle bacillus may appear in the secretions from the human genito-urinary, intestinal and respiratory tracts. The acid-resisting bacillus cultivated by Czaplewski from a case of leprosy is probably not identical with the bacillus of leprosy. Dietrich reports an interesting observation of the presence of acid-resisting bacilli resembling the tubercle bacillus in a suppurating ovarian cyst which had ruptured into the intestine, their presence, together with the symptoms, leading to the erroneous diagnosis during life of tuberculous peritonitis.

An especial incentive to the search for acid-resisting bacilli has been the detection by Petri and by Rabinowitsch of such bacilli in butter and by A. Moeller of similar bacilli in timothy grass and dung. Since the first demonstration of tubercle bacilli in butter by Brusaferro in 1890, this hygienically important subject has been investigated by several writers with most discordant results, the percentages of samples of butter in which the tubercle bacillus was found varying from 0 to 100. Obermüller examined 14 samples of butter from a single source in Berlin, all of which he claims contained the tubercle bacillus. Rabinowitsch, who examined butter from a number of shops in Berlin and in Philadelphia, demonstrated the tubercle bacillus in all the samples from one of the largest Berlin houses, whereas all of the other samples were free from this bacillus. In a considerable proportion of the samples,

---

8 Progrès méd., 1885, 2, s., II, p. 195, and Arch. de phys. norm. et path., 1885, 3, s., vi, p. 303.
10 Virchow's Archiv, 1886, cv, p. 209.
12 Ibid, 1897, p. 1192.
17 Berliner klin. Wochenschr., 1899, p. 189.
18 Bauerngarten's Jahresbericht, 1890, vii, p. 271.
however, she found bacilli morphologically and tinctorially resembling
the tubercle bacillus. Butter containing these bacilli, injected into
the peritoneal cavity of guinea-pigs, produced nodules superficially like tu-
bercles and containing the bacilli, but distinguishable from true tubercles
by their histological characters. Injection of pure cultures of the ba-
cilli did not produce tubercles. Rabinowitsch says that Koch in 1896
recognized the occurrence in butter of acid-resisting bacilli resembling
the tubercle bacillus. Shortly before Rabinowitsch’s description of these
bacilli in butter, there appeared, as a comment upon Obermüller’s dis-
queting observations, a brief statement concerning the detection of these
pseudo-tubercle bacilli by Petri, who published in the following year a
fuller description in his study of the question of tubercle bacilli in
butter.

The publications of Rabinowitsch and of Petri have led to further
investigations concerning the presence of acid-resisting bacilli resemb-
ling the tubercle bacillus in butter, among which may be mentioned
those of Hormann and Morgenroth, Grassberger, Herbert, Weissen-
feld, Aecher, Coggi, and Korn. While the results vary as to the
frequency of these bacilli in butter, some indeed being negative, and
also to some extent as to their characters, it is established that bacilli
morphologically and tinctorially like tubercle bacilli have been repeat-
edly found in butter, and their occurrence should lead to great care in
the diagnosis of suspected tubercle bacilli in this material, as well as in
milk. Culturally these bacilli can be readily distinguished from the
tubercle bacillus, and the same distinction can usually be made without
much difficulty in their pathogenic properties.

Grassberger inoculated the peritoneal cavity of 20 guinea-pigs with
Vienna market butter and noticed in 10 of these characteristic anatomical
changes. Firm whitish yellow masses were found on the peritoneum.
These masses were made up of a delicate fibrinous frame-work with rem-
rants of butter and large numbers of bacilli within. The bacilli stained
red when treated with carbolic fuchsin and decolorized in the nitric acid

23 Hyg. Rundschau, 1898, viii, pp. 317; 1081.
24 Münch. med. Wochenschr., 1899, Nos. 11 and 12.
26 Berliner klin. Wochenschr., 1899, p. 1053.
Acid-Resisting Bacilli in the Lower Animals

solution. He obtained pure cultures of this so-called butter bacillus, but like Rabinowitsch he failed to produce lesions in guinea-pigs by their inoculation. If, however, he mixed the culture with sterilized butter or sterilized paraffin and inoculated the mixture into guinea-pigs, the animals died and on post-mortem examination showed the characteristic nodules described above. Sterilized butter or sterilized paraffin produced no lesions when inoculated. If preparations of this organism were left for 24 hours in alcohol and ether they no longer resisted the decolorizing solution. Grassberger found no genuine tubercle bacilli in the samples of butter examined. He further observed that if guinea-pigs were inoculated with Kretz's bacillus of timothy grass lesions similar to those produced by the butter bacillus occurred.

In her first publication on this subject Rabinowitsch stated that Capaldi in examining her specimens was impressed with the resemblance between these butter bacilli and acid-resisting bacilli which he had found in cow's dung. Severin had previously reported the existence of such bacilli in horse's dung and Ferran in cow's, horse's and human feces. Of especial interest and importance are the recent observations of Alfred Møller who has cultivated from vegetable fodder and from the dung of different animals bacilli which he designates respectively as the dung bacillus, the timothy bacillus or grass bacillus I, and the grass bacillus II. These bacilli of Møller are even more resistant to decolorization by acids than the tubercle bacillus, which they also resemble morphologically, but are distinguished from the latter by cultural characteristics. Especially noteworthy are Møller's statements concerning the production of nodules closely resembling genuine tubercles by inoculation into guinea-pigs of pure cultures of these bacilli. Møller speaks of confirmation or control of his results by Czaprowski, Kretz, and Lubarsch.

There can be no doubt that different species or varieties are represented among the various acid-resisting bacilli described by the investigators cited as "smegma bacilli," "butter bacilli," "dung bacilli," "timothy" or "grass bacilli," etc., but further work is needed to elucidate the differences or relationships between the members of this interesting group of bacteria.

31 Abst. in Centralbl. f. Bakter., 1897, xxii, p. 484.
As already stated, the special object of my investigation was to
determine to what extent acid-resisting bacilli are to be found on the
bodies of animals, a subject which has received hitherto but little at-
tention. The occurrence of such organisms in animal products was
also considered, but to a less extent. Inoculation and culture experi-
ments are under way and these results will be published later. In
my work I adopt the name acid-resisting bacillus in preference to
the too loosely used term smegma bacillus. It remains to be proved
whether there is any relationship of the smegma bacillus to similar
bacilli found on parts of the body other than the genitals, and to
some of those found in animal products. Moreover the question has
been raised as to whether the so-called smegma bacillus as it is seen in
man, is a distinct species or whether the term represents a group of
closely allied organisms.

The material for this investigation was obtained by scraping the
parts with a blunt instrument. At times, as in the case of soft smeg-
ma, the material was easily obtained, and in that case a small bit was
crushed between two cover glasses. In the case of dry epithelial
scales, the scrapings were rubbed up with a drop or two of sterile
water and with this suspension coverslip preparations were made and
allowed to dry in an incubator. The slips in all cases were fixed by
passing three times through the flame. They were all stained with
hot carbolie fuchsin, after which they were thoroughly treated with
fresh 25 per cent nitric acid solution, washed in water, and counter-
stained in Loefler's or aqueous methylene blue. From three to six
slips were thus prepared from each specimen and examined. The in-
struments used were sterilized before each examination. Fifty-five
animals and a few samples of milk and feces were examined.

Horse.—The smegma of the horse exists in large amount; 10 to
20 grammes, or more, may be obtained from the sheath at one time.
It is of a grayish-black color and is tolerably firm. The smegma of
the mare is quite soft, of a light gray color and is found in small
amount. The bacilli found in horse's smegma are for the most part
slender, clean-cut rods and resemble very much the tubercle bacil-
lus. Other forms may at times predominate, thus in the scrapings
Acid-Resisting Bacilli in the Lower Animals

from the vulva of one mare there was found on one slip a large number of capsulated bacilli which remained red after decolorizing in acetic acid. The smegma of one horse, two mares, one Shetland horse and one Shetland mare were examined, with positive results in all.

Dog.—The smegma of the dog, in the cases examined, was a creamy, semi-fluid, yellowish substance, existing in small amount. In this substance bacilli precisely resembling the tubercle bacillus may be found. Many beautiful beaded organisms were seen in some preparations. In the yellowish discharge so commonly seen on the penis of dogs similar bacilli were seen. The combination of these bacilli with the many leucocytes, mucus, and other microorganisms which abound, make a picture difficult to distinguish from that of tuberculous sputum. In this discharge, which apparently comes from the urethra, numbers of large phagocytes may be seen, but without bacilli. Four dogs were examined with positive results in three, negative in one.

Cow.—The cows used for this experiment were those of a dairy farm. There was no evidence of external disease of the teats or udders; aside from an occasional wart, they were to all appearances normal. These cows had not been subjected to the tuberculin test. The material for the examination was obtained by scraping the moistened teat or udder with a scalpel. The scrapings—dust, epithelial cells, etc.—were allowed to fall on bits of moistened filter paper placed in small tin boxes; coverslip preparations were made as already described.

It was not difficult to demonstrate acid-resisting bacilli in these scrapings. Generally speaking, these bacilli were much thicker than the tubercle bacillus. In some slips several different forms may be seen, as bent rods, rods with irregular outline, beaded forms, and club-shaped bacilli. In one specimen there were a number of slender bacilli, sometimes slightly bent, which stained a distinct but light pink, some isolated and scattered through the field, others in groups of five or more. These bacilli precisely resemble the tubercle bacillus. On account of the large amount of epithelium which takes the carbolic fuchsin deeply and resists decolorizing solutions, special
care was taken to expose these specimens for a considerably longer time than is necessary for the decolorization of sputum. Some slips were left in the acid solution until no further trace of red was visible to the naked eye, and still in these preparations red bacilli could be demonstrated. The bacilli were usually seen free from the epithelial cells. In every cow examined large numbers of yeast cells and spores which stained red were found. The habit so prevalent of lubricating the hands of the milker with the first strippings undoubtedly accounts for the invariable presence of yeast cells. The sugar in the milk favors the growth of these organisms.

In all, eight cows were examined for acid-resisting bacilli with positive results in five, negative in three. The so-called glair or vaginal mucus of one cow gave a negative result.

Eight preparations were made from the sediment obtained from a centrifugal separator. Each specimen showed many acid-resisting bacilli, some of which might easily be mistaken for the tubercle bacillus.

Guinea-pig.—The material for this examination was obtained from the glans penis, prepuce, vulva, anus, and from the mammæ of both male and female. Although the prepuce is quite long and completely covers the glans, yet the latter as a rule is very dry and only at times can a slight collection of a yellowish white material or smegma be seen. This substance has an odor similar to that of human smegma. The vulva which has no deep folds gave material which looked like slight epithelial desquamation mixed with dirt. All told, five male and five female guinea-pigs were examined. In all the males, acid-resisting bacilli were found on the genitals. On the mammæ of one and on the anus of another similar organisms were demonstrated. All the females but one gave positive results. The mammæ of two of these were examined with positive results in one. The organisms usually found were shorter and thicker than the tubercle bacillus. Occasionally longer and more slender forms were seen on the mammæ. Not infrequently large groups of red bacilli can be seen on a faded pink epithelial cell.

Rabbit.—The glans penis, prepuce, vulva, and perineal pouches were examined. The genital organs themselves were in all cases
very clean. The perineal pouches, situated on either side of the genitals, contain a large collection of a yellowish, soft, granular, oily material having a peculiar penetrating odor—the rabbit odor. This material microscopically is seen to be made up of numerous oily droplets, of squamous epithelial cells, which are more or less swollen, and of many kinds of bacteria. Notwithstanding the large amount of oily material in the perineal pouches, in the ten rabbits examined not a single acid-resisting bacillus was found. This fact is of interest in view of the common belief that fat is necessary for the staining reaction of the smegma bacillus. The fat in smegma, however, generally exists in a saponified form. The mammae of five rabbits were also examined but with negative results.

Cat.—In the cat, as in the rabbit, the genitals are not exposed, and are very clean, and it is with difficulty that a cover-glass smear can be made. A few bacteria which stain blue are all that can be seen. Two male cats were examined with negative results.

White Rat.—On the prepuce when retracted, and at times on the external surface near the meatus, there may be seen small yellowish semisolid lumps about the size of a common pin head. Microscopically this material contains swarms of very short, thick, acid-resisting bacilli. These may be seen in groups of one hundred or more, or scattered singly through the field. A simple examination of these acid-resisting bacilli shows that several varieties or species are present. Thus some resemble diplococci, others have a drumstick form, while others have a central, clostridium-like enlargement. Seven males were thus examined with positive results in five, negative in two. The female shows no special material or smegma, and it is only with the greatest persistence that any material is obtained. The vagina at times contains a mucous substance. Three females examined showed positive results in one, negative in two.

The feaces of the rabbit, guinea-pig, and white rat was examined with negative results.

Many are accustomed when speaking of the smegma bacillus, to think of it as a typical organism like the tubercle bacillus. This is not correct inasmuch as we have to deal with a group of bacilli which differ markedly among themselves. As in man, so in the lower ani-
D. Murray Cowie

mals, we find in the smegma various forms of microorganisms which in spite of comparatively long exposure to acid solutions retain their color, whereas the other bacteria with which they are associated decolorize readily. The extreme variation in the form of these acid-resisting bacilli clearly indicates a difference in species.

General Morphology.—The smegma of the lower animals very often contains slender, clean-cut, slightly bent rods which frequently show a distinctly beaded arrangement, and in this respect are not to be distinguished from the tubercle bacillus. On the other hand, smegma bacilli are met that are appreciably shorter or longer than the tubercle bacillus. Moreover, irregular forms can be observed, the bacilli instead of being straight may be bent in the middle; this bend may be scarcely perceptible (comma bacillus), or may be almost a right angle. At times the stain is taken up unevenly and as a result the bacilli show irregular borders.

The occurrence of acid-resisting diplococci or diplo-bacilli and of drumstick forms has been already referred to. Oval, yeast-like organisms, capsulated bacteria, short threads of from four to six cells, and spores are met which retain the stain the same as the ordinary smegma bacillus. The organisms found on parts of the body other than the genital organs, as on the teats of the cow, usually vary in form as much as those in smegma.

Decolorization.—In the resistance which the smegma bacillus and other forms referred to offer to decolorization by acids, these organisms resemble the tubercle and leprosy bacilli. At one time the tubercle bacillus was supposed to have a specially impermeable cell wall, but this view has gradually given way to the belief that the peculiar reaction is due more to the composition of the cell contents than to the cell wall proper. That the resistance of the smegma bacillus to decolorization is not inconsiderable is seen in the fact that specimens of smegma can be left in 25 per cent nitric acid solution for ten minutes without the least decolorizing effect. The same is true of the bacilli found in the scrapings from the cow. Invariably the specimens will resist exposure for three to five minutes to this acid solution, or to the same strength of sulphuric and of acetic acids. An exposure of ten seconds to absolute alcohol decolorizes the bacilli.
in the majority of cases, but at times a much longer exposure has practically little decolorizing effect.

As has been stated, some of the acid-resisting bacilli which are met among the lower animals resemble in form the tubercle bacillus. In the staining properties and especially in the resistance to decolorizing solutions this resemblance is rendered more striking. Nevertheless it would be a gross error to conclude that the tubercle bacillus was actually present. It is quite possible that the real tubercle bacillus may have been present, but its recognition by the only crucial tests—cultures and growth in the animal body—was not resorted to, for the reason that the immediate object in view was to demonstrate the presence of acid-resisting bacilli on the lower animals.

The presence of acid-resisting bacilli in milk or in butter may mislead and doubtless has misled those who depend entirely upon the recognition of the tubercle bacillus by its morphological and staining properties. Reports of the presence of tubercle bacilli in milk and in butter based on such observations are by no means few. The mere detection of such bacilli in milk or butter is now recognized as giving no support to the conclusion that they are tubercle bacilli. In order to identify such acid-resisting bacilli it is necessary to resort to cultures and animal experiments, but even here a certain amount of caution must be observed, not to mistake nodules produced by injection of butter and pseudo-tubercle bacilli for genuine tubercles.

CONCLUSIONS.

The results obtained in this study may be briefly summarized:

1. Acid-resisting bacilli are found in many of the lower animals, more especially the horse, cow, dog, guinea-pig and white rat. In the case of the rabbit and cat no such organisms were detected.

2. Many of these acid-resisting bacilli resemble the tubercle bacillus and the smegma bacillus of man.

3. The acid-resisting organisms are undoubtedly of different species and there is good reason to believe that the term smegma bacillus denotes not a definite species but rather a group of bacilli having common staining properties.