

NOTES ON THE GENUS NICOLAIERILLUS (B. TETANI)

STUDIES ON PATHOGENIC ANAEROBES VII

HILDA HEMPL HELLER

*From the George Williams Hooper Foundation for Medical Research, University of
California Medical School, San Francisco*

As one searches through the literature on the tetanus bacillus he is forcibly struck by its meagerness. The biology of the organism itself has been largely neglected because of the interest centering around the powerful toxin that it produces, and around the fascinating mechanism governing the distribution of the toxin in the nervous system. In the history of few diseases have individual case reports been so frequently detailed; in few diseases known to be infectious has the biology of the causative organism been so little studied. Yet the pathogenicity of the bacillus depends on its ability to live and multiply just as much as on its toxin production.

This paper gives a few notes on the behavior of certain tetanus strains that have found their way to this laboratory. The study of the tetanus organisms was not undertaken as other than a side line during the investigation of tissue invading pathogens. These notes make no pretense of being in any way exhaustive, and are intended simply to serve as an introduction to several papers by myself and other workers who have studied the same strains.

The anaerobic bacilli that cause tetanus have long been referred to one species under the name *Bacillus tetani*. In a recent classification¹ it was pointed out that such anaerobic bacilli as behaved similarly on ordinary mediums and had similar morphology should be placed in genera rather than in species because in such groups it is usually possible to demonstrate common types that differ consistently in minor characters and correspond to the general taxonomist's idea of species. Systematic bacteriology is so little developed that it is at present very difficult to find characters that are acceptable for the differentiation of strains. Especially is this true in the groups of anaerobic rods. One finds that a type of character that is apparently consistent in one genus is subject to variation in another and for each group various

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¹ Heller, H. H.: *Jour. Bacteriol.*, 1921, 6, p. 521.

characters must be studied before a logical classification can be formulated. Such study cannot be attempted for many genera by any one investigator. Certain points of interest bearing on the classification of the tetanus bacilli are deserving of publication.

The genus *Nicolaierillus* was defined ² as follows:

"Putrificoideae that in meat medium produce gas and various color changes: yellowish, pink, grey or mauve, dependent on the medium; the particles of meat are gradually suffused with a black pigment, and bleach at the top. The meat is softened but the particles do not greatly diminish in size. Do not attack sugars. Gram-negative (weak methyl violet) rods that form terminal spherical spores. Colonies in deep agar diverse. Common in soil, found in horse feces, may multiply in wounds, but do not normally invade tissue. Produce a characteristic neuro-toxin."

The known strains referable to this genus are probably all toxigenic because it is the toxigenic phenomenon that usually leads to their isolation. Other anaerobes of similar morphology have been isolated; they are nontoxigenic and are culturally referable to other genera. The organisms producing the neurotoxin that causes clinical tetanus are apparently all referable to the genus *Nicolaierillus*. Nevertheless, it is probably unwise to restrict the generic definition to such strains as are toxigenic; many times difficulty is experienced in obtaining toxin from known toxigenic strains, and the nature of toxin and the process of toxin formation are too little understood for us to use the production of a poison as a critical generic character.

The Tetanus Committee, under the chairmanship of Sir David Bruce, determined, in 1916, to investigate the cause of the occurrence of cases of tetanus that proved refractory to tetanus antitoxin. Tulloch undertook a serologic investigation of the strains of *B. tetani* obtainable from laboratories and isolable from septic wounds. In three reports ³ he published his results. He succeeded in demonstrating that the agglutination reaction separated the group into four distinct types. The toxins produced by these types were apparently identical. Agressins were produced that were relatively specific to each type and were apparently unfiltrable or highly labile.

COLONY FORMATION OF NICOLAIERILLUS STRAINS—MUTATION

A search for descriptions of tetanus colonies in deep agar is not very illuminating. Kitasato ⁴ pictures a deep colony, apparently in

² Heller, H. H.: *Ibid.*, 1922, 7, Jan.

³ J. Roy. Army Med. Corps, 1917, 29, p. 631; *Proc. Roy. Soc., B*, 1919, 90, p. 145 and p. 529.

⁴ *Ztschr. f. Hyg. u. Infektionskrankh.*, 1889, 7, p. 225.

gelatine, that is a very dense symmetrical sphere with encircling fine short radiations. He states that old colonies consist of thread-like masses. Kitasato is credited with being the first to isolate the tetanus bacillus in pure culture. This he probably did, but his critique of the purity of a tetanus culture was not reliable, for the cut accompanying the description of a pure culture shows two typical sporulating rods of undeniable sporogenes type. I have never found any such subterminal oval sporulating organism in a pure tetanus culture.

Apparently the work of Sanchez-Toledo and Veillon⁵ was careful. They describe agar colonies, as well as gelatine colonies, finding the agar colonies the less characteristic. The "clouds" are less transparent, and the "needles" less fine. Their pictures of deep gelatine colonies may have been of pure cultures and may not. The cuts (lithographs) leave some room for doubt as to whether a slender oval endsporulating organism may not have been present, but the sporogenes type is apparently absent.

Fränkel and Pfeiffer,⁶ in 1892, depicted a dilution shake of tetanus colonies in deep gelatine, and this has been extensively copied in textbooks. Apparently two forms were present. Were the culture one of another anaerobe than the tetanus bacillus, one would be justified in concluding that a mixed culture had been studied. But colonies of a mutated tetanus strain may well have been the subject of the photograph.

Sanfelice⁷ (p. 356) states that gelatine colonies are not characteristic, but that the deep agar ones may be distinguished from those of other anaerobes by the fineness of their filaments; he pictures two sorts of deep agar colonies (Figs. 41 and 42).

Righi⁸ found a fluffy colony with a dense nucleus characteristic for tetanus organisms, in spite of the serious contamination of his cultures. Grixoni⁹ recognized the same type.

Von Hibler's¹⁰ critique of the purity of tetanus cultures was exceedingly at fault. Strain 14 (pl. II, fig. 15) shows only clostridial forms with oval spores, and strain 15 (fig. 16) shows two thirds of the bacilli of sporogenes type and one third of tetanus type. Von Hibler tells us of the behavior of strain 11, that was long grown on glycogen-

⁵ Arch. de méd. expér., 1890, 2, p. 709.

⁶ Mikrogr. Atlas d. Bakterienkunde, 1890, plates 27-41.

⁷ Ztschr. f. Hyg. u. Infektionskrankh., 1893, 14, p. 339.

⁸ Riforma med., 1894, 3, p. 651.

⁹ Ibid., p. 698.

¹⁰ Ueber di path. Anaeroben, 1908.

containing serum medium. Many bacilli thereafter "lost" the power of liquefying gelatine, but regained it after growing six days in the muscles of a living guinea-pig. Von Hibler makes many interesting statements on atypical colonies, on injuring of the bacterial protoplasm by growing the organisms on unfavorable mediums, etc. Some of his observations may refer to real mutations, but in view of his astounding ignorance of the morphology of tetanus bacilli his notes cannot be considered of value. It is of interest that von Hibler's critique of the purity of non-proteolytic anaerobic cultures was, in the main, fair, while the distinctions between one proteolytic type and another were not duly appreciated. Von Hibler states that deep tetanus colonies in gelatine are spherical with radiations, and a concentric space of liquefaction develops within them. In agar deep colonies are "zerschlissen" or of rough contour. A cut (pl. 3, fig. 6) shows an irregular woolly colony. Atypical ones pictured (pl. 4, figs. 2, 3 and 4) are lenticular with woolly projections.

McIntosh¹¹ states that "Shake colonies are best obtained in glucose agar; in the medium the colonies in twenty-four hours are relatively large, and at the end of forty-eight hours may measure 2-3 mm. in diameter. The growth is delicate and forms a light, cloudy mass consisting of very delicate intermingled filaments, spreading out from a small central nucleus." McIntosh pictures two colonies that do not look alike. The Medical Research Committee¹² give a similar account of tetanus colonies in deep agar.

Textbooks quote Kitasato's original description almost verbatim. These descriptions of deep and surface gelatine colonies are not readily applicable to our present day technic that employs agar almost exclusively. Yet one modern textbook after another gives us solely observations dating back to 1889. Von Lingelsheim,¹³ in an elaborate compilation, apparently wonders whether a deep agar tetanus colony may be distinctive in morphology, but leaves the matter for the future to decide.

While anaerobes of most groups or genera make colonies of somewhat similar structure, the strains of this genus form colonies of exceedingly diverse types. A series of these is illustrated by the photographs accompanying this article. Of interest is the extreme variation noticed in the size of the colonies produced by the different strains. The

¹¹ Med. Research Committee, Special Report Series 12, 1917.

¹² *Ibid.*, 39, 1919.

¹³ Kolle u. Wassermann: *Handbuch d. path. Mikroorg.*, 1912, 4, p. 737.

range in size of colonies of 24 hours is from invisible spots 0.02 mm. in diameter to huge fluffs 3 mm. in diameter. Form also is diverse. The slow-growing types are apparently nonmotile and form colonies of fundamentally lenticular contour. These lenticular colonies are complex and often form peculiar lengthened structures (plate 1). Such shapes are unusual in other groups of anaerobes. The large colonies owe their size to higher motility and to greater powers of utilization of their substratum as food.

The colony forms illustrated may be loosely divided into several types. These types should probably not be termed species. A relationship is not necessarily implied between the various strains that form each type of colony. A similar utilization of nutriment and a similar motility are all that connect the strains that form colonies resembling one another. Thus strains that agglutinate differently may make colonies of the same type, although the agglutination reaction is probably much more fundamentally specific than is the shape of the colonies.

Neither do the differences between these colonies represent, in the majority of cases, what is usually termed variation. When the variations for a given strain are once understood, it is found that mutations occur that in turn breed true, and may be sharply distinguished from variations. The colony form becomes the chief expression of any mutation that may take place in the metabolism of the organism. De Vries¹⁴ employed specific and not subspecific names for what he supposed to be the products of single mutations in the genus *Oenothera*. These later proved to be recombinations representing sums of single mutations that had occurred previously, any one of which would not deserve specific differentiation. In other groups such as the insects, whose mutations have been well studied, a trinomial nomenclature is resorted to, and the specific name of the original wild type has been retained, while a Latin or vernacular name has been applied to the mutant, which is termed a biotype, the name subspecies being reserved for groups that are delimited by their geographic distribution. Biotype, then, is a name that may be used for these elementary species that differ from one another and breed true, but we should not term two similar colony forms of different history the same biotype, as one might do with two strains of similar higher forms developed from two different types of parents, for the reason that the colony character is not so well understood as are the characters of higher plants and animals, is not so significant or so truly an essential part of the organism as are

¹⁴ Mutation Theory, 1909, 1.

the leaves or stems of complex forms, and there is more chance for a colony type to represent various elementary species than in the case of more elaborately detailed characters. But we should expect that different strains and species of our organisms, like those of more complex forms, should frequently mutate independently in the same fashion.

A description of some of the mutations of *Nicolaierillus* strains will be presented in a future paper. They are frequent enough to explain the wide range of colony form found in the genus. The bearing of this phenomenon on the taxonomy of the genus is probably nil. Other workers in this laboratory that are using the same strains may be able to confirm the results of Tulloch or to assign certain colony types to various other groups of their own; in other words, to attach some chemical significance to the change in colony form. It is premature even to select a "type species" for the genus. Till further study is made, type 1 of Tulloch, identifiable only by an agglutinating serum, may be taken for a so-called "type species" with the name "*N. tetani*." A chemical or morphologic character rather than a serologic one is of course preferable for the subdivision of a group.

THE ISOLATION OF NICOLAIERILLUS STRAINS

Many complaints have been made of the difficulty encountered in isolating these organisms. This is due to several factors. In mixed cultures the organisms are recognized only when they form spores—a comparatively late period—and their early active multiplication phase in the vegetative form is not taken advantage of. In many mediums they are somewhat overgrown by other proteolytic organisms. It is extremely difficult to get tetanus bacilli to grow on the surface of an agar plate that is free from blood. In deep agar the colonies of different strains are so unlike that no widely representative "type" can be recognized. The strains that form minute colonies are perforce incapable of easy isolation from gross mixtures. Of interest is the fact that Righi accepted as typical of tetanus bacilli colonies with a dense center and loose radiations, while a collection of cultures sent me by Dr. Duval of New Orleans that were probably isolated by one worker, all made large opaque woolly colonies. Tulloch devised a selective medium for *B. tetani* by means of which Robertson was able to isolate the organism from wound cultures by the Barber technic. McCoy and Bengtson¹⁵ isolated a series of strains which they were

¹⁵ Am. J. Publ. Health, 1919, 9, p. 427.

so kind as to furnish this laboratory. Their isolations were made as follows: Fermentation tubes were filled with freshly boiled veal infusion, inoculated with suspected material, and incubated at 37 C. until spherical spores were observable in the cultures. Such a fermentation tube was then heated at 70 degrees for 30 minutes and material from it was inoculated into deep agar shakes of veal infusion agar, and the colonies were fished after 3 days' incubation. I have not tried this method in its details, but give it here because it is so simple. When tetanus strains have become contaminated in the laboratory I have had little or no difficulty in isolating the types of active growth by deep colony procedure from liver peptone agar. For some time I was baffled by mutating colonies, and I believe that many supposed contaminations by other anaerobes were cases of mutation. Meat medium should be avoided as a substratum on which to propagate contaminated tetanus strains as it encourages too many other forms. When dealing with badly overgrown cultures or with material from wounds, feces or soil, such a medium as Tulloch's is to be recommended, followed by a deep-colony procedure. The only element that will then make the isolation of the bacilli difficult is the worker's ignorance of the colony form of the particular strain that he desires to isolate. He must, in most cases, fish more colonies than he would were he dealing with a genus of uniform colony formation. The illustrations will help somewhat in recognizing various sorts of tetanus colonies. In case a strain is present that forms minute colonies, one may try other agar substrata, or Barber's technic,¹⁶ or one may use many dilution tubes and incubate for 3 or 4 days until the minute colonies in one or other of the tubes are large enough to be fished readily. Tetanus colonies grow with fair speed at room temperature, and in some cases it may be found advantageous to incubate at a low temperature. For fishing crowded colonies the binocular is of great assistance.

Robertson (per. com.) notes that tetanus cultures become more readily contaminated than do those of other anaerobes. I formerly noted the same phenomenon, and believe it to be due to the fact that she and I usually reincubated tetanus cultures in anaerobe jars to observe spore formation. Long incubation in anaerobe jars frequently causes contamination, as does also the exhaustion of meat tubes containing gas, in which the medium is allowed to boil up onto the cotton plugs. I find that incubation of meat medium cultures of tetanus bacilli in air with or without vaseline allows characteristic development of the cultures.

¹⁶ Phil. J. Sc., 1914, 9, p. 307.

THE BEHAVIOR OF NICOLAIERILLUS STRAINS IN MEAT MEDIUM

Statements concerning the proteolytic activity of the tetanus bacilli vary considerably. There is almost entire unanimity that gelatine is liquefied. Von Hibler¹⁰ and Zeissler,¹⁷ on the one hand, ascribe to tetanus organisms an intense proteolytic activity. Von Hibler worked with badly mixed cultures. He states, however, (p. 95) that many strains of tetanus bacilli do not blacken brain deeply. Zeissler goes so far as to state that brain is blackened intensely and that the odor varies between a pungent odor, and a very bad one, and that milk is peptonized in from 4 to 14 days. His photograph apparently shows a culture contaminated by oval-end-sporulating organisms. On the other hand, the Medical Research Committee¹² state: "Meat medium: Pink color or no change in the medium according to the samples of meat; softening of the consistency of the meat. The odor is characteristic but not putrefactive. Milk: Poor growth; no change in medium. Coagulated serum: Little or no liquefaction." Adamson¹⁸ found the organism mildly proteolytic.

My experience would lead me to emphasize the proteolytic action of the organism a little more than do the members of the Committee. Perhaps they worked with a peptone-free meat medium that did not afford so much nutriment for the organism as that employed in this laboratory. But such descriptions as that of Zeissler cannot be construed as applying to pure cultures of tetanus bacilli, for these are among the less active proteolytic anaerobes. The Committee is certainly correct in stating that the color change in meat medium varies with the batch of medium. A series of pure tetanus strains inoculated and incubated uniformly on one batch of medium behave with striking similarity—those that form minute colonies on agar cause changes in the medium with less rapidity than do those that form large colonies. Meat medium is made in this laboratory of one part of coarsely ground beef heart with two parts of distilled water. Five hundred cc of Martin's peptic-digest broth is added to 3 liters of meat medium. The reaction is P_H 7.2. For a long time it was thought that the behavior of tetanus bacilli on this medium was exceedingly uniform and characteristic. Later other assistants made the medium and separate batches were found to give very different results. All batches with an initial reaction in the neighborhood of P_H 7.2 gave evidences of moderate proteolysis inside of a few days and showed slight softening

¹⁷ Ztschr. f. Infektionskrankh. d. Haustiere, 1920, 21, p. 1.

¹⁸ J. Path. & Bacteriol., 1920, 23, p. 241.

of the medium, and never, even on long incubation enough softening to cause more than a slight settling of the meat particles. In odor they were much the same, and the morphologic aspect of the bacilli was much the same. But the color changes were different in the various batches of medium.

The most usual type of color change noted was an initial yellowing of the meat, followed shortly by the appearance of a pinkish shade that changes later to a light terra cotta, rarely to a dark terra cotta. A black pigment is usually noted suffusing the particles nearest the surface and a few of the morsels lower down. If the particles be finely ground, the top layer later bleaches in a few days to pale yellow. If the incubation is in hydrogen a diaphanous black ring appears at the surface of the liquid, but disappears on exposure to the air. Some batches of medium show the initial yellowish stage protracted, and the pink color does not become intense. The black pigment is then very tardy in making its appearance, or it may be absent. In other batches neither pink nor yellow appear. The meat becomes gray and apparently allows good growth of the bacilli. Black pigment may be present. Another batch that apparently afforded excellent growth early gave a mauve color suffused with black pigment. A lot of meat medium that titrated P_H 6.6 sometimes failed to give any growth with tetanus bacilli, but when it did the organisms did not change its appearance. A collection of meat medium tetanus cultures that was three months old showed considerable variation in reddening and blackening. This is consistent with the ready mutation observed with some strains on protein medium. Cultures a year old that have been left at room temperature in air are not uncharacteristic in appearance. If the column of meat is high, it will be noted that all sharp edges of the particles of meat have disappeared, but that the particles have not settled greatly in the tubes. Their basic color may still be red, but this color is almost coated by a thin black film. The top layer of meat a quarter of an inch thick is almost white, the boundary between this layer and the blackened meat below being sharp if the meat has been finely ground.

Gas production is abundant early and becomes less after a few days.

Most authors agree that the odor of tetanus cultures is characteristic and not like that of other proteolytic anaerobes. A young meat culture is faintly and not unpleasantly proteolytic and no butyric acid or sourish smell is noticeable. After 10 days the odor at the mouth of the tube is of hydrogen sulphide only, that of meat smeared on a slide is

pungent and different from that of most proteolytic anaerobes. Most people would term it unpleasant. At 3 months the odor is rank, pungent and almost overpowering.

Bacilli are moderately abundant in 24-hour meat medium cultures whether incubated in air, under vaseline or in hydrogen. They are most abundant in 48-hour cultures and do not drop off in numbers abruptly after this time as do so many anaerobes. This is the most marked distinction to be noted between the tetanus bacilli and the nonproteolytic *Macintoshillus* bacilli (*Bacillus tetanomorphus*^{10,11}) when stained cultures are observed. Vegetative rods are quite characteristic in their morphology. Their thickness is very uniform, rarely far from 0.8:1.0 micron. The length may vary from 1 micron to very long filamentous rods. The usual length is from 2-8 microns and filaments and long rods and attached bacilli are rare. The rods are nearly always straight and the ends neatly rounded. They are uniformly and consistently gram-negative when treated with a weak solution of methyl violet, and are gram-positive after treatment with a strong violet stain, such as Sterling's. The gram-negative bacilli are remarkably clear in their staining with the carbol-fuchsin counter-stain, and there are no pale forms and no bacilli showing irregularities of protoplasm in cultures under 5 days of age. The morphology of the nonsporulating tetanus bacillus, at first insignificant and hard to distinguish from that of other organisms, becomes to the experienced eye characteristic and definite, and contaminations of tetanus cultures are usually readily noticed. The anaerobic tetanomorphous bacillus of McIntosh and doubtless some other organisms are, however, not definitely to be distinguished by their morphology alone.

Spore formation may commence within forty-eight hours but usually begins on the third or fourth day and is active for a long period; occasionally it fails to appear. Spores vary considerably as to the time of their formation and as to their abundance in the different strains. Spores are invariably spherical and orgonts¹⁰ (p. 4 or 389) show an oval end only in the very early stages. Swelling of the rods takes place apparently only in the portion where spore formation occurs. Spores remain in the rod for a long period. Orgonts are not abundant, sporulating rods are frequent, and only after 10 days or so does one note numerous free spores. The majority of spores are, in most strains, strictly terminal, but occasionally a strain is found that repeatedly forms some spores centrally. A tip of protein from the rod is

¹⁰ Heller, H. H.: J. Infect. Dis., 1920, 27, p. 385.

occasionally noted on the distal end of the spore, giving the rod an appearance superficially like that of a sporulating sporogenes or botulinus organism. But the wall of the spherical tetanus spore is thinner than in those species, and the tip of protein more rounded, the sides of the bacillus are more nearly parallel. I have rarely observed two spores in the same rod. The bacterial wall about the spore is thin, and the spore wall is thin; refractility is low. Sometimes the spores themselves retain the methyl violet of the Gram stain to some extent, remaining transparent the while. The spores of *Macintoshillus* do the same; both types occasionally show young spores thickly covered by the mother cell, and these may stain gram-negatively like the mother cell or they may retain the stain. *Macintoshillus* bacilli form more spores during the first two days than *Nicolaierillus* bacilli. Colonies of the former organisms are pictured in this article.

NICOLAIERILLUS STRAINS ON OTHER PROTEIN MEDIUMS

The behavior of tetanus strains on protein mediums other than meat has been so well treated by Adamson¹⁸ that extensive experiments have not been tried. The following notes were made on the behavior of five pure strains that were incubated in hydrogen at 37 C.

Indol broth gave good growth and after five days of incubation a negative test for indol.

The bacilli grew well on brain medium, producing at first a little gas and abundant rods. After four weeks the medium was not blackened, nor did it show any sign of a greenish or grayish tinge. The lower portion was pink as it had been before inoculation. The odor was faint and ester like, in no way repellent and putrefactive.

The organisms apparently refused to grow on alkaline egg.

Deep coagulated beef serum furnished active growth, and at first gas. Spores were formed early and abundantly. The medium soon showed a gray-greenish color, and at the end of a month was suffused with a slightly greenish leaden tinge and was somewhat softened but not liquefied. The odor was faintly proteolytic.

Although there was multiplication on deep Loeffler's serum, the bacilli did not produce a greenish color. The odor was musty.

In deep Dorset's egg gas was formed early and a slight softening of the medium took place. Later lead-colored spots appeared with a general pale suffusion by the lead color. The odor was faintly of hydrogen sulphide.

Brom-cresol milk was clotted by a rennet like enzyme of slow action. The clot appeared during the first week, and at the end of four weeks most of the casein had settled to a somewhat rubber-like clot at the bottom of the tubes. This clot was compact and could be torn in pieces that disintegrated in invisible particles. The mass behaved much like a compactly settled lump of white lead in paint. The supernatant liquid was not clear, but was clearer than normal milk, and alkaline. Peptonization, if present, was certainly not visible to the eye. The odor was musty.

SUMMARY

Certain differences may be found between *Nicolaierillus* strains on careful search. It is premature to apply these differences to specific differentiation.

Tetanus strains show remarkable powers of mutation on complex protein substrata. These powers are easily observed in colony formation, but do not affect grossly the picture of proteolytic action on meat medium.

The morphologic and staining reactions of the bacilli are very characteristic and permit the accustomed worker to recognize them readily and to spot contaminations with ease. The behavior of pure cultures on meat medium is fairly characteristic and is different from that of most common contaminating anaerobes. Meat medium is probably the best protein medium for the differentiation of tetanus strains from other anaerobic organisms. All strains observed were noticeably proteolytic and none were intensely so.

Such strains as form large colonies are easily isolated by deep colony procedure.

APPENDIX. LIST OF TETANUS STRAINS STUDIED

From the Hygienic Laboratory, Washington, D. C.

Agar stabs. May, 1917.

THL = "Tetanus No. 1 Hyg. Lab."

November, 1918.

TA = "Tetanus Ag. Dept."

TB = "Tetanus 2 NY Dept. June, 1919."

TC = "Tetanus Courtplaster."

TD = "Tetanus P. D. Co."

TE = "Tetanus 089."

TG = "Tetanus from gelatine."

TM = "Tetanus Mulford."

TN = "Tetanus N. Y. Board of Health."

TO = "Tetanus OT 026580."

TP = "Tetanus Smith's from pease."

TR = "Tetanus OTK 02."

TT = "Tetanus Tulloch type I."

TU = "Tetanus U. Chicago."

June, 1919.

TVP = "Tetanus from vaccine virus point."

From Miss Muriel Robertson, Lister Institute, London.

TMcC = "Tetanus of MacConkey" London, Nov., 1916. Meat culture.

November, 1918.

TUSA = "B. tetani USA II" Tulloch's type I. Egg broth.

T220 = "B. tetani R 220 L" Tulloch's type III. Meat culture.

T67 = "B. tetani T 67 V" Tulloch's type II. Egg broth.

From Dr. K. F. Meyer, San Francisco.

VT = "Tetanus from vaccination case." A small boy who had been vaccinated played in a stable and developed tetanus and died. Two types were cultivated from the culture made from the vaccine pustule.

From Parke, Davis and Company, Detroit.

T 087 = "Tetanus 087" Agar stab.

From the Gilliland Laboratories.

TL = "B. tetani" Agar stab.

From Dr. Duval, New Orleans. Agar stabs.

T 2 A = "B. tetani 204A." Isolated from wound.

T 2 B = "B. tetani 204B." Isolated from soil.

T 2 C = "B. tetani 204C." Isolated from wound.

T 2 E = "B. tetani 204E." Isolated at necropsy.

T 2 F = "B. tetani 204F." Isolated from wound.

From the Cutter Laboratory, Berkeley.

T 4 = "B. tetani 4." Agar stab.

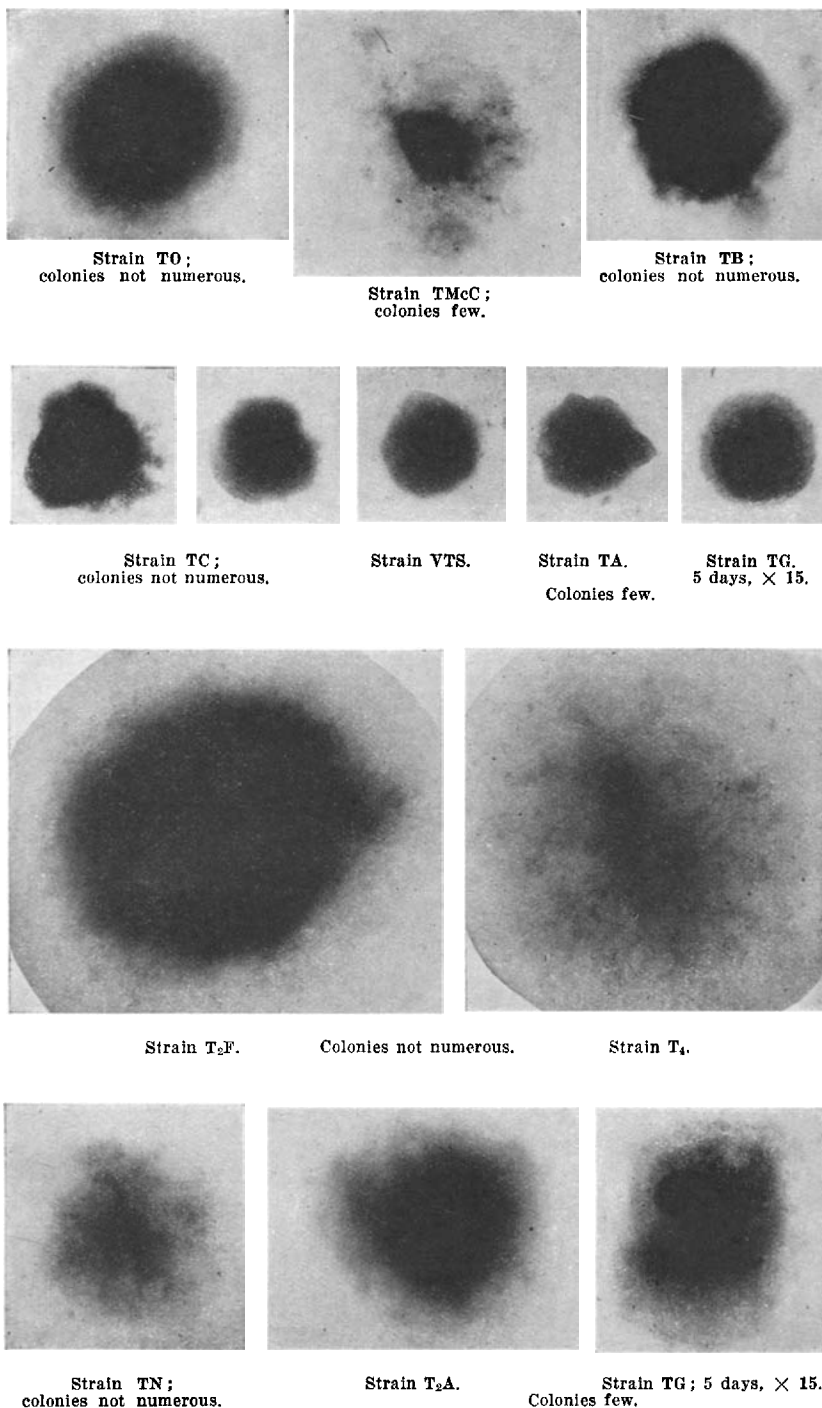
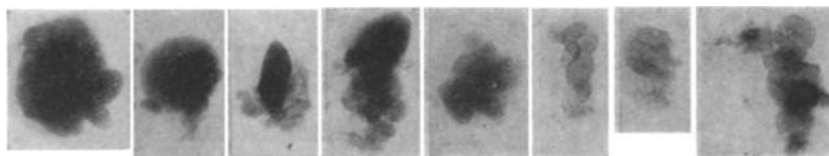
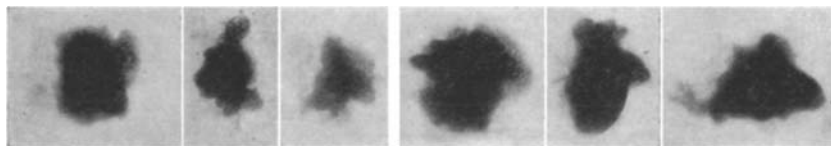


Fig. 1.—Various types of *Nicolaierillus* (Tetanus) colonies. Unless otherwise indicated, these photographs represent colonies that were incubated for 24 hours at 37 C. in deep liver peptone agar and were enlarged 50 diameters.



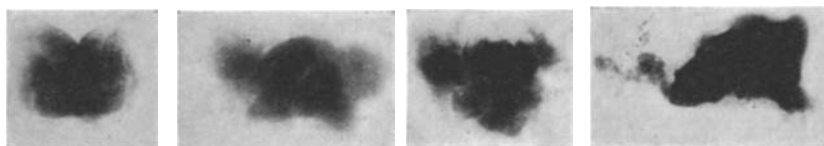
Strain TE; colonies few.

Colonies moderately abundant.



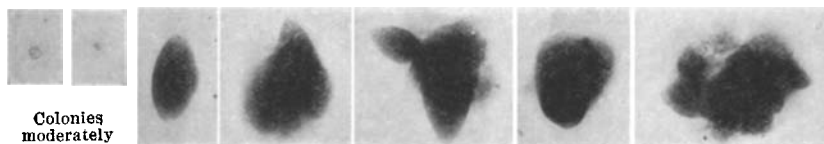
Strain TU; colonies not numerous.

Colonies few.



Strain TE; 48 hours' incubation; colonies moderately abundant.

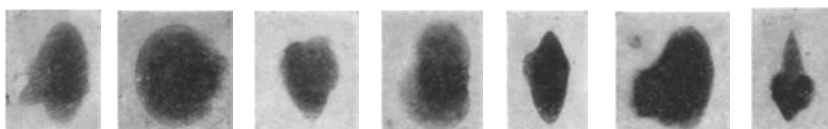
Strain TU



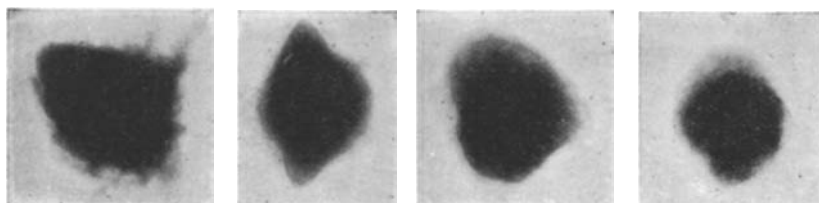
Colonies moderately abundant.
Strain TD.

Four days' incubation; colonies few.

NICOLAIAERILLUS (TETANUS) COLONIES



Macintoshillus tetanomorphus, type strain, PT; colonies abundant.



Strain 297.

Strain 221.

Colonies moderately abundant.

Fig 2.—Macintoshillus (*B. tetanomorphus*) colonies.