VARIABILITY IN MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF CLOSTRIDIUM HISTOLYTICUM (WEINBERG AND SEGUIN)

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INTRODUCTION

Weinberg and Seguin reported, in 1916, the isolation of a newly-discovered species of anaerobic spore-bearing bacillus from several cases of gas-gangrene infected war wounds.

A pure culture of this micro-organism, when injected into the muscles of common laboratory animals such as guinea pigs, mice or rats, was found to cause a considerable lysis and liquefaction of the muscle around the injected area in less than 24 hours, the lysis usually continuing until the death of the animal. This organism, because of its particularly pronounced ability to attack living tissue, a property possessed by no other sporulating anaerobes, was named "*Bacillus histolyticus*" by its discoverers.

Although the first strains were obtained from war wounds only, in later years, several investigators succeeded in isolating other strains from many other sources such as soil, sewage and the faeces of healthy persons, thus showing that the organism is much more widely distributed than was originally supposed.

In its biochemical and morphological properties, *Clostridium histolyticum* is closely related to *Clostridium sporogenes*, the most common representative of this genus. Both are strongly proteolytic: when inoculated in milk, a rapid digestion occurs, the medium becoming translucent and finally quite clear; gelatin is rapidly liquefied, usually within 24 hours; abundant growth occurs in most media rich in proteins, whether or not sugars are present.

While the majority of investigators agree upon the carbohydrate fermentation by *C. sporogenes*, much undesirable confusion exists in the literature regarding the fermentation ability of *C. histolyticum*.

The original paper of Weinberg and Seguin (1916) gives only a limited number of the biochemical properties of the new species with no data on sugar fermentation, except the statement that no gas is formed in agar-shake cultures containing sugars. Henry (1917), investigating one of the original strains isolated by Weinberg, reported this strain able to ferment glucose, laevulose and maltose from among the sugars tested.

Results similar to those of Henry were reported by McIntosh and Fildes (1917) for other strains: fermentation was weak, but after 7 days' incubation was distinct with glucose, maltose and starch. Later, the same investigators (1919) reported the fermentation of glucose, laevulose and maltose. It should be noted that if these observations are correct this behaviour towards carbohydrates is the same as that shown by *C. sporogenes*.

Hall (1922), and Reddish and Rettger (1924), found that *C. histolyticum* fermented the same sugars as did *C. sporogenes*. A year later, however, Hall (1923) reported that *C. histolyticum* failed to ferment sugars. Since a reasonable explanation for these anomalies was not evident, the possibility of an unknown contaminant in the culture of the earlier experiments was suggested.

Kendall, Day and Walker (1922), Kahn (1924) and also Torrey (1925) deny the sugar fermenting ability of *C. histolyticum*.

Thus, it is seen that highly contradictory results have been reported by the investigators studying this micro-organism. That this contradiction still persists is evident from the fact that Bergey in the 1934 edition of his "Manual of Determinative Bacteriology" still arranges *C. histolyticum* under the sugar-fermenting anaerobes, whereas Spray (1936) in his "Tentative Key to the Sporulating Anaerobes" lists the same bacteria among the nonsaccharolytic representatives.

Since sugar fermentation is usually considered as one of the important criteria for the identification and classification of the

sporulating anaerobes, the deplorability of such an anomaly need hardly be stressed.

The writer's attention was drawn to the contradictory data as regards fermentation while working on the digestion of tumor tissue by representatives of this group. It was found that one of the seven available strains of *C. histolyticum*, a strain obtained from the American Type Culture Collection and tested immediately upon receipt, fermented glucose with the formation of acid and gas, whereas, all other strains gave negative results. For the special study of tumor digestion, all of the strains were carried on a stock culture medium containing a sterilized transplantable rat tumor tissue suspension (1:5) in a solution of 0.1 per cent K_2HPO_4, 0.05 per cent $MgSO_4$ and 0.01 per cent $FeSO_4$ in tap water.

During a period of four months following the receipt of the several strains of this organism, each strain was transplanted several times on the special tumor tissue medium. The sugar-fermenting ability was then tested again, and it was found that all of the strains gave faint but decidedly positive results. Since great care had been taken to avoid contamination, by testing each tube thoroughly before transplanting into it, it appeared worth while to investigate this phenomenon more closely. As a matter of fact there are two possibilities, which can cause the cultures of *C. histolyticum* to ferment carbohydrates, although they were decidedly negative when received. Though transfers had been made very carefully, nevertheless contamination with a sugar-fermenting anaerobe (fermentation persists after pasteurization) is possible and has to be considered. On the other hand the possibility that we are dealing with a form of variation in which sugar fermenting variants of *C. histolyticum* arise from the ordinary non-sugar-fermenting strains need not be rejected at once.

Bacteriologists have long since been reconciled to the idea that there occur variations in single species of micro-organisms, in biochemical behaviour as well as in morphology and colony form. In this regard we may refer to the classic example of Massini's *Bacillus coli-mutabile*, a bacterium which failed to ferment lactose, but the colonies of which upon continued incuba-

tion, formed small papillae composed of bacteria which are able to ferment lactose, a property which could be transmitted by subculturing from these papillae on new plates, and in which also the new character appeared to be permanent.

That this behaviour of *B. coli-mutabile* is by no means a bacteriological curiosity was proved when, a few years later, more examples could be added. So, variants of *Eberthella typhosa* are described which are able to ferment dulcitol or rhamnose and which were obtained from strains unable to ferment these substrates (Penfold 1910, Müller 1911); *Salmonella paratyphi* behaves similarly towards raffinose (Müller 1911). Variants are also known of *S. paratyphi* which are unable to produce any gas during the fermentation of certain carbohydrates; under certain conditions, however, this lost property can be regained and the variant may be transformed into a true *S. paratyphi* (Pot and Tasman 1932). From cultures of *Corynebacterium diphtheriae* and also from *Shigella paradysenteriae* (Sonne), variants are obtained which are unable to ferment certain carbohydrates and other modifications which strongly ferment these sugars (Goodman 1908, Hobby 1935, Chinn 1936, Sears and Schoolnik 1936).

Though this summary certainly is incomplete it may show that variability in sugar fermentation, especially among the pathogenic micro-organisms, is not a rare phenomenon at all, and that therefore the possibility of a spontaneous variation, leading to sugar-fermenting variants need not be excluded.

It has long since been proved that variation in colony form (rough-smooth variation) also occurs frequently in the group of anaerobic spore-bearing micro-organisms, and special attention has been paid in this regard to *Clostridium welchii* and *Clostridium tetani* (Buchaly 1930, Condrea 1930, Orr *et al.* 1933, Stevens 1935). In agreement with what has been found for most of the other bacteria this rough-smooth variation usually does not involve any change in biochemical behaviour towards sugars.

It is generally accepted that there is no direct correlation between variation in colonial type and variation in metabolism. That such a correlation sometimes occurs however has been shown by Colef (1935) for *Clostridium oedematiens*, a bacterium

belonging to the same group as *Clostridium histolyticum*. This investigator succeeded in obtaining colonial variants from characteristic hairy colonies of *C. oedematiens*; these variants had a pronounced tendency to become smooth, and they were non-pathogenic. Contrary to the typical strains of *C. oedematiens*, however, these variants were able to ferment glycerol.

The results of the present study show a similar, though still further-reaching phenomenon with cultures of *C. histolyticum*; it is shown that fermentation of sugars need not necessarily be ascribed to contamination of the *C. histolyticum* cultures but that it can be due to a similar variability, which makes itself manifest not only in a profound change in biochemical properties of the organism, but also simultaneously in the morphology of the colony.

BACTERIOLOGICAL INVESTIGATION

It seemed advisable, first, to make a comparative study of transplants from strains which had been found to ferment sugars and the original non-sugar-fermenting strains, using the tumor-tissue suspension medium.

Peptone agar plates, streaked from a tube culture of each strain incubated for two days at 37°C. in anaerobic jars, showed only minor differences in colony form, which did not justify the conclusion that either contamination or variation had occurred. All of the colonies were practically round and small, and were transparent to opaque.

A different result was obtained, however, when the smears were made on liver-veal agar, a medium advocated by Spray for the cultivation of anaerobes. The growth on this medium was abundant, even after only 24 hours. A marked difference was now evident between the plates obtained from the original pure cultures, and their transplants on tumor tissue suspension. The original pure cultures all gave perfectly round colonies resembling those of streptococci, 1 to 2 mm. in diameter, opaque, and with a decolorized zone around each colony on the dark brown culture medium. The transplants on tumor-tissue suspension showed, for the greater part, similar colonies. However, a small portion

of the colonies had irregular margins, some even having short thick shoots protruding from the periphery. With a few colonies, this was so pronounced that the agar around the colony was covered with a network of filaments.

Of importance is the fact that between these two extremes, the regular round and the threadlike colonies, all kinds of intermediate stages were observed. As all the plates had many absolutely isolated colonies, no difficulties were experienced in transplanting single colonies to subsequent liver-veal agar plates. On transplanting a regular round colony there was obtained without difficulty, with some strains at least, a pure culture of the round colony form, identical with those obtained directly from the original non-sugar-fermenting culture.

Some strains, however, were extremely difficult to purify. Every time, even though a well isolated regular colony was taken, intermediates and even threadlike colonies appeared again after replating. Sometimes six successive transplants on liver-veal agar plates were necessary to eliminate both the intermediate and the threadlike forms.

The same procedure was necessary when attempts were made to obtain a pure culture of the threadlike colonies. No difficulty was experienced with some strains; by taking one of the most filamentous and well isolated colonies, a pure culture was obtained after one or two successive transplants. For some strains, however, not less than 12 successive transplants of single isolated colonies were necessary to eliminate the round and the intermediate colonies. This great number of transplants, necessary to obtain pure cultures of the regular as well as of the threadlike colonies is rather suspicious and indicates that we are not dealing with a simple contamination, which could have been removed easily with one or two transplants of single isolated colonies. It is highly improbable that every time, up to more than ten plates, a mixed colony is transferred. In this regard it has to be borne in mind that the modern technique, using McIntosh and Fildes anaerobic jars and a suitable culture medium (Difco-liver-veal agar) makes the work with the sporulating anaerobes scarcely more complicated than the work with most of the

aerobic bacteria. Practically without exception every plate contained many very well isolated colonies of 1 to 2 mm. in diameter.[1]

BIOCHEMICAL AND PATHOGENIC DIFFERENCES OF THE TWO TYPES

After obtaining pure culture plates of each strain of *C. histolyticum* from the regular as well as from the filamentous colony form, one colony from each plate was used for the inoculation of a fermentation medium, containing glucose, as well as for one without glucose. After two days incubation, the round colony form, from all of the 7 strains used, had not formed any acid or gas from the glucose, when compared with the blank without glucose. On the other hand all of the threadlike variants had formed considerable amounts of gas and acid, compared with the glucose-free blanks.

There is, thus, no doubt that the appearance of the fermenting ability after several transplants in tumor tissue suspension was due to the presence of organisms which gave rise to the threadlike colonies.

In this regard, attention may be called to the fact that Henry and also McIntosh and Fildes, who observed a sugar-fermenting ability of *C. histolyticum*, describe its colony form as delicate and flat, with crenated or irregular edges. Photographs given show that undoubtedly these investigators were dealing with colony forms intermediate between the perfectly round and the filamentous colony forms. When the sugar-fermenting ability of a number of such intermediate forms was tested during the present investigation, it was found that all were active, and that there was a certain correlation between the colony form and the amount of acid and gas produced from glucose. The more that

[1] After pouring the liver-veal agar plates, they were allowed to cool slowly. This prevented syneresis and usually the surface was dry after gelatination, so that the plate could be used immediately. Three one-inch-broad, parallel smears with a bent platinum needle were made over the surface, thus giving a continued dilution of deposited bacteria and leaving a space between the smears, which was of great value for detection of any contamination, as growth was allowed only on the surface touched with the needle. The dish was placed upside down in the jar with a piece of filter paper, wetted with three drops of glycerol in the cover; this gave the surface the right degree of moisture, but prevented creeping.

differentiation had proceeded towards the filamentous form, the more acid and gas were produced from glucose.

There is no doubt that the non-sugar-fermenting strains correspond closest to the original description of *C. histolyticum* given by Weinberg and Seguin. Since their identification is based mainly on their specific action on living muscle tissue, it was decided to test the pathogenicity of both the above types of colonies. On injecting 0.5 cc. of an 18-hour brain-medium culture intramuscularly into the shaved leg of a guinea pig, it was seen that the regular colony type caused the specific lysis of muscle tissue, as described by Weinberg and Seguin and Combiesco (1923) and others. The animals usually died within 48 hours after inoculation with any of the strains used. The filamentous colony form, on the other hand, was found to be relatively harmless. Sometimes a local oedema appeared after 24 hours without causing appreciable damage to the tissue, disappearing gradually afterwards. When injected, however, into necrotic tissue such as the inside of a large transplantable rat tumor, the whole tumor was usually liquefied within 48 hours with the production of a relatively large quantity of a hemorrhagic fluid with an offensive odor.

Another difference between the two types was the reaction in nutrient gelatin to which a strip of iron had been added. According to Spray (1936), *C. histolyticum* gives in this medium, 1 to 2 days after inoculation, a beautiful wine-red color, specific for this species, though the nature of the reaction is still unknown. In line with this, it was found that all tubes, inoculated with pure cultures of the regular colony form, gave this reaction, whereas those inoculated with the filamentous colonies did not. Intermediate forms gave intermediate reactions, more or less faint red, the color usually disappearing later. The color appeared only when growth occurred under semi-anaerobic conditions. By excluding all traces of oxygen, in an anaerobic jar, no red color appeared, even when, after growth had ceased, the tubes were placed in the air. Thus, it seems that traces of oxygen are necessary for the appearance of this reaction.

Finally, another difference between the biochemical properties of the regular and the filamentous colony types was found in

their conduct towards nutrient lead acetate agar. This medium was not blackened in 6 days by the regular colony type, whereas the filamentous form gave a smoky brown precipitate of lead sulfide in the same period of time.

IDENTITY OF THE FILAMENTOUS VARIANT WITH C. SPOROGENES

The foregoing facts undoubtedly indicate that the filamentous variants of *C. histolyticum* can be identified as *C. sporogenes*.

Inoculated in iron-milk, an inactive gaseous fermentation occurred, accompanied by a rapid digestion of the casein. Usually, in 48 hours, the medium was strongly blackened. Tyrosin was sometimes formed, though much less than that given by the original strains. Nutrient lead acetate medium was slightly blackened, but the color was not so dense as that usually given by *C. sporogenes*. Formation of indol was not observed during growth; however, a few strains (not all) gave, on reaction with a 5 per cent alcoholic vanillin solution and concentrated HCl, a positive "vanillin violet" test, a reaction also given by *C. sporogenes*. Gelatin was rapidly liquefied by the threadlike forms. Glucose and maltose were fermented, whereas lactose, sucrose and salicin were not. Threadlike colonies, similar to those obtained from the *C. histolyticum* strains, were also obtained from one authentic strain of *C. sporogenes* which was available. A still more convincing proof of the close relationship between the filamentous variants of *C. histolyticum* and *C. sporogenes* was the fairly good agglutination of these variants with serum of a rabbit which had been immunized against one strain of *C. sporogenes* which had been obtained from the American Type Culture Collection.[2]

METHODS FOR OBTAINING THE SUGAR-FERMENTING, SPOROGENES-LIKE VARIANTS OF C. HISTOLYTICUM

It must be borne in mind that *C. sporogenes* is probably the most common anaerobic contaminant of laboratory cultures, and

[2] More details about these agglutination reactions and other serological evidences of the close relationship of *C. histolyticum* and *C. sporogenes* will be given in a separate article by L. Smith to whom the author is much indebted for his interest in the problem.

great care must be taken that all possibility of contamination with this species be excluded. The gradual change, and the intermediate forms given are, however, sufficient proof that the filamentous colony form ultimately obtained is not due to contamination.

For the same reason, methods were sought which would give reproducible results with a minimum of manipulation. One of the methods devised is as follows:

A single isolated colony from a pure culture plate of *C. histolyticum* is inoculated into Difco-nutrient gelatin solution (pH = 7.3) to which a strip of stovepipe iron is added.[3] Before inoculation, sterility is definitely established by incubation for at least 4 days at 37°C. in an anaerobic jar.

No seal is required, since the tubes must be incubated under semi-anaerobic conditions. Usually, within 16 hours after inoculation growth occurs, a wine-red color gradually developing which reaches its maximum intensity in 2 to 3 days.

When growth has been well established, and in less than 24 hours after inoculation, a transplant is made into a new medium regardless of whether color has appeared or not. This is repeated daily, thus enabling the bacteria to multiply for a considerable time under the most favorable conditions.

Gradually the growth becomes more abundant than in the initial tubes and the final wine-red color becomes paler. Ultimately the color fails to appear even after 3 days incubation, or else it appears on the first day, only to disappear on the following day.

When smears are made in this stage on liver-veal agar from tubes in the early stages of growth (at the time that a transplant to the new medium is made) colonies which are no longer perfectly round and which are sometimes more transparent than the others gradually appear (figs. 9-12). Furthermore, colonies with one or two thick, short shoots, also develop although, except on close examination under a dissection microscope, they do not appear markedly different from the others (figs. 2, 7 and 8).

[3] More details as to the preparation of this medium may be found in the publication of Spray (1936) cited above.

These irregular outlines are the first signs of differentiation, and such colonies appear as soon as the red color no longer reaches its maximum intensity. Usually, when such a single separated irregular colony is replated on liver-veal agar, more than 90 per cent perfectly regular, round colonies appear. Of the remainder, nearly all appear like the original mother colony, while a few are more pronounced in their irregularity. By repeated selection and replating of one of the most irregular colonies on five or six successive plates, a pure culture of the threadlike variant is obtained.

Actually, the threadlike variants may be obtained more easily if the daily transplants in the iron-gelatin medium are continued beyond the appearance of the first signs of differentiation. Usually when the transplants in this medium are continued, the differentiation becomes more pronounced. Even though no red color is observed in a number of successive transplants there is always still a majority of the original regular colonies to be seen in the smear obtained from the final tube of such a series. In order to obtain a pure culture of the hairy colonies selective transplants on solid medium were always necessary. It was observed sometimes that the red color reappeared and the irregular colony forms disappeared again, when making subcultures every two or three days instead of within 24 hours.

When, to a pure culture of *C. histolyticum*, was added artificially an initial, very slight contamination of *C. sporogenes*, and a similar procedure was followed as with the pure culture, even the first transplants gave a great number of very hairy colonies and no intermediates by making smears on liver-veal agar. Moreover, the red color was still undiminished. This striking difference from a pure culture series makes it improbable that a contamination is responsible for the effect described.

The gelatin medium was by no means the only medium on which differentiation was obtained. This medium has the advantage, however, that the progress of the dissociation can be followed macroscopically. However, similar results may be obtained (usually even after a smaller number of transplants) by an analogous procedure in milk or in brain medium, and especially

in tumor-tissue suspension. It is probable that this differentiation could occur in any medium which is rich in suitable proteins. It is not even necessary to use a liquid medium. Continued transplants on liver-veal agar of single, separated colonies (if possible the most irregular ones) lead to the same result, though usually more transplants are required. All strains of *C. histolyticum* are much more resistant toward variation when the transplants are made on media containing only a restricted amount of peptones. The better the nutrient conditions, the more the variation proceeds in the direction of the sugar fermenting variants. The poorer the medium, the more the original properties of *C. histolyticum* are maintained.

Even on protein-rich media, such as liver-veal agar, it was observed that areas in the smear with a high population had less pronounced irregular colony shape, whereas those at the margins or decidedly isolated colonies, having the best nutrient conditions, were the most markedly irregular.

For the same reason it also seems advisable to use as a stock medium for *C. histolyticum* a 1 per cent peptone solution, solidified by the addition of 0.5 per cent agar, instead of the usual stock media, brain or Robertson medium.

The gradual change from the perfectly round and regular colony form of *C. histolyticum* to the hairy colonies, identical with those of *C. sporogenes*, is shown for one strain in figures 1 to 6.

This variation has been accomplished on liver-veal agar plates by replating each time the most irregular single, separated colony. In 9 transplants, the whole transformation was completed from the original *C. histolyticum* to a variant practically identical with *C. sporogenes*.

EXPERIMENTS TO OBTAIN THE ORIGINAL STRAIN FROM THE VARIANTS

Practically no difficulties in transforming variant forms into the round colony type were encountered when starting with an intermediate colony form, giving a faint though decided sugar fermentation. When a single colony from such a plate was replated on new solid media, there appeared usually, even on

the first transplant, perfectly regular colonies, besides many irregular ones. When one of these round colonies was used for a succeeding plate all intermediate forms gradually disappeared after several transplants, although sometimes 5 to 10 transplants of a single regular colony from successive agar plates were necessary. The farther variation had proceeded, the more difficult it was to obtain the original non-sugar-fermenting strain.

Consistent results were not obtained on liver-veal agar with the extremely filamentous colonies, although on several occasions a marked regression was observed after continued selection.

In general the best results were obtained by cultivation on poor media, as for example, 1 per cent or even less bacto-peptone agar, thinly poured. On this medium the colonies remained very small, but with the help of a dissection microscope, it was possible to transplant single separate colonies to new media of the same composition. Usually a considerable number of such plates was necessary to obtain regular round colonies.

It was observed that the sporogenes-like variants of *C. histolyticum* are like the original strain in that they are not strictly obligate anaerobes. On meat infusion agar, they form very minute colonies, scarcely visible, under aerobic conditions. It was possible to make several successive transplants.

DISCUSSION

Hitherto, we have always considered the filamentous sporogenes-like colony forms, obtained from pure cultures of *C. histolyticum*, as "variants" of the latter. It is questionable, however, whether it is not more reasonable to consider *C. histolyticum* as the variant, originating genetically from *C. sporogenes*. Indeed, variants are usually obtained as the result of a prolonged continuance of conditions *unfavorable* for normal growth (presence of phage, chemicals, antiserum, etc.) whereas growth under optimal conditions generally favors stabilization. In this respect it may be remembered that the better the nutrient conditions the more the *C. histolyticum* tends to give the sugar fermenting, filamentous sporogenes-like colonies. On the other hand, cultivation on poor media of the latter colony form tends to change

them back again to the original streptococcuslike colony form of *C. histolyticum.*

This does not mean of course, that every strain of *C. sporogenes* can be changed into a pathogenic, non-saccharolytic strain of *C. histolyticum.* However, on the other hand, it seems that although the conclusion here made is based on experiments with only seven different strains, every strain of *C. histolyticum* may be induced to undergo a change, which ultimately leads to a variant identical with *C. sporogenes.*

The different strains of *C. histolyticum* used undoubtedly showed different resistance, but using suitable methods, this resistance could be broken. It is practically broken as soon as irregular colonies appear on liver-veal agar plates, e.g., as represented in figures 7 to 12. Selection on this solid medium always leads gradually to the hairy colony form of *C. sporogenes.*

It is not intended to propose that the designation of the species *C. histolyticum* be changed to one such as *C. sporogenes, var. histolyticus.* Under usual conditions, the biochemical properties of *C. histolyticum* are markedly different from those of *C. sporogenes,* and, in addition, are stable enough to justify the collection of all nonsaccharolytic, pathogenic strains, with regular, streptococcuslike colonies, and giving a wine-red color in nutrient iron-gelatin, in the species *C. histolyticum.*

On the other hand, it must be borne in mind that, where variation can occur in morphology as well as in colony form or in biochemical behaviour, the three most important foundations on which classification of micro-organisms is based, the possibility is open that related species, which hitherto have been considered as distinctly different from each other, are nothing else than stabilized variants of the same species.

Bacillus undulatus and *Bacillus mycoides* (den Dooren de Jong, 1933) are one example, *C. histolyticum* and *C. sporogenes* are another and there are evidences that more will be found in the near future.

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SUMMARY

1. *Clostridium histolyticum* on liver-veal agar grows in perfectly round, streptococcuslike colonies, is nonsaccharolytic, thoroughly pathogenic, and produces a wine-red color when inoculated into nutrient iron-gelatin.

2. Repeated early transplants on media rich in suitable proteins, allowing multiplication under the most favorable conditions for a considerable time, lead to the appearance of colonies with irregular shape.

3. Continued selection of the most irregular colonies on successive liver-veal agar plates, leads gradually to pure cultures of a filamentous colony form.

4. Gradual change in colony form corresponds with gradual change in biochemical properties: the appearance of sugar-fermenting ability and H_2S formation, and the loss in pathogenicity and the ability to form the wine-red color in iron-nutrient gelatin.

5. The pure culture of these hairy variants is practically identical, biochemically, as well as in colony form, with *Clostridium sporogenes.*

6. To obtain the original strain of *Clostridium histolyticum* from the filamentous variants, continued selection on poor media was found to give the best results: this transformation could not always be obtained.

7. From the results of this study it would appear that there is a genetic relation between *Clostridium sporogenes* and *Clostridium histolyticum.*

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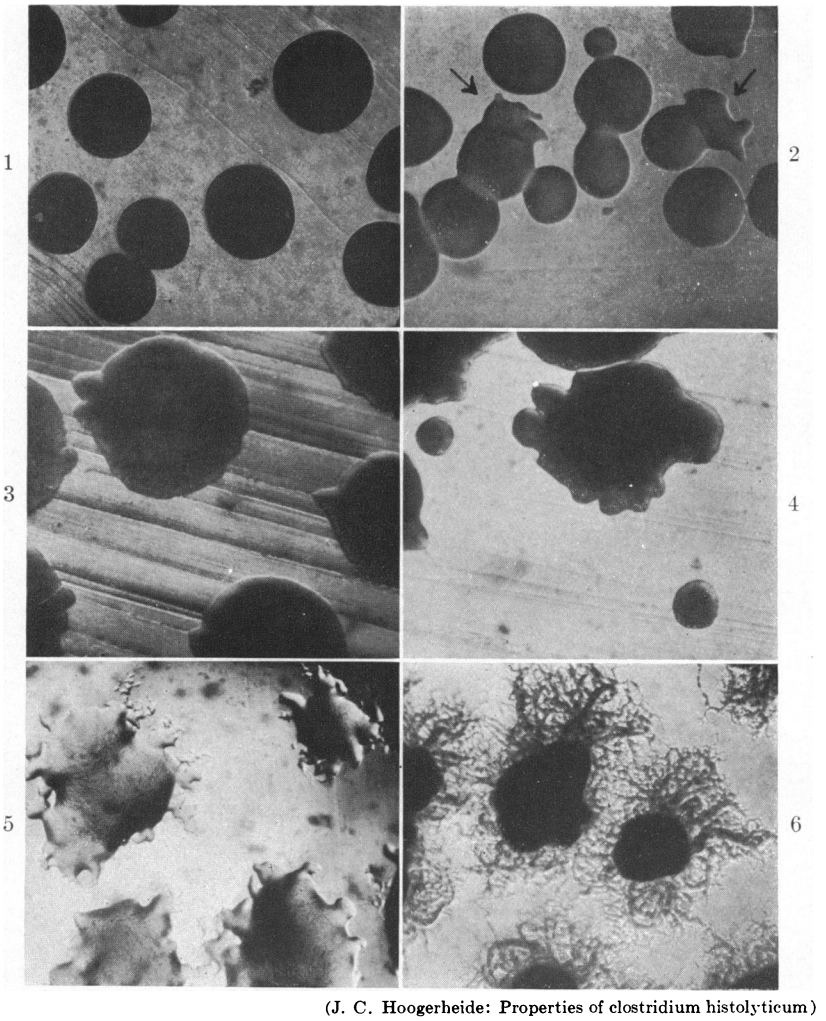
PLATES

PLATE 1

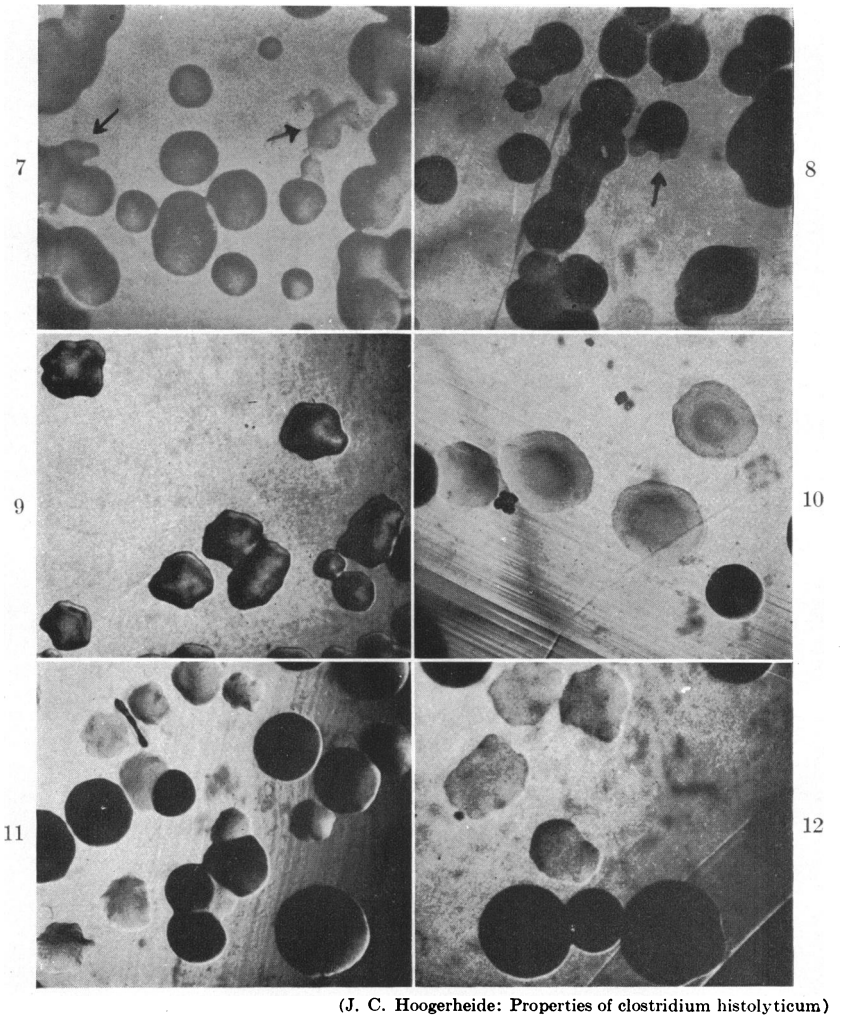
Figures 1 to 6 show some phases of the gradual change from the round, streptococcus like colonies of *C. histolyticum* to the hairy ones of a variant, identical with *C. sporogenes*. A series of daily transplants of single, separated colonies was made on liver-veal agar plates. Figure 2 shows the appearance of the first signs of differentiation.



(J. C. Hoogerheide: Properties of clostridium histolyticum)

PLATE 2

Figures 7 to 12 give the results of smears on liver-veal-agar from tubes with brain medium, iron-gelatin medium, milk and rat tumor suspension; media in which by daily transplants the first signs of differentiation occurred.



(J. C. Hoogerheide: Properties of clostridium histolyticum)