THE DECAPSULATION PHENOMENON OF *KLEBSIELLA PNEUMONIAE*

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The rapid withering away of typically mucoid colonies of some strains of *Klebsiella* was first observed by Beham (1912) and later termed "suicide" colony formation by Collins (1924). Hadley (1927) in his review of microbial dissociation postulated that this phenomenon may result from the presence of a lytic factor or an unusual mode of dissociation. Goslings (1935) examined ten strains of *Klebsiella rhinoscleromatis* which produced "suicide" colonies. Lytic factors, which he believed to be bacteriophage, were present in filtrates from four of these ten strains.

The studies to be described were undertaken to characterize the sequence of events during the so-called "suicide" phenomenon and to reinvestigate the role of bacteriophage as the responsible agent.

MATERIALS AND METHODS

Two strains of *Klebsiella pneumoniae*, C-2 and X-4, which manifest the "suicide" phenomenon, were chosen for the present investigations. The biochemical and serological characteristics of these strains have been previously described (Pickett and Cabelli, 1953). Unless otherwise indicated, the organisms were cultured and maintained on a medium containing 2 per cent agar (Difco) and 1 per cent peptone (Difco) in distilled water. All cultures were incubated at 37 C. This medium was found to be adequate for the production of fully capsulated organisms.

To test for the presence of bacteriophage, peptone agar cultures of strains X_4 and C_2 were incubated for 48 hr at 37 C. At this time, when the colonies were withered away, the growth from each culture was taken up in 5.0 ml of physiological saline and filtered through sintered glass or Seitz filters. Two methods for determining the presence of lytic factors in the filtrates were used. In the first, spread plates of a variety of strains of *K. pneumoniae* were prepared and the activity of each filtrate determined by spotting drops of the materials on these plates. In the second method (Adams, 1950), 0.1 ml of each of the suspensions of viable organisms and 0.1 ml of each culture filtrate were plated together and spread with sterile glass elbows. In this manner, each filtrate was tested against each organism. A control in which physiological saline was substituted for the filtrates was also performed. All cultures were incubated and examined for the presence of plaques or "suicide" colonies.

Total viable counts were done to compare the numbers of organisms in colonies before and after the "suicide" phenomenon had occurred. All the colonies selected for counting were of uniform size, i.e., 3 mm in diameter. With a spatula each colony along with some of the underlying agar was removed to a tube containing 10.0 ml of physiological saline. Standard methods for the determination of viable counts were then used with each of the suspensions.

Capsule stains were prepared using the negative staining technique of Jasmin (1945).

To prepare the immunizing antigens, plate cultures of mucoid and "suicide" organisms were harvested in 20.0 ml of 0.5 per cent phenolized saline. The suspensions were incubated at 37 C for 24 hr, tested for the presence of viable organisms, and concentrated to one-seventh of their original volume. Four intravenous injections of 0.5 ml of each of the prepared antigens were administered to young albino rabbits. Four days after the final injections the rabbits were bled and the sera harvested.

For the preparation of the precipitating antigens, suspensions of mucoid and "suicide" organisms were turbidimetrically standardized with saline to 26 per cent transmission using a Coleman 14 spectrophotometer at 450 mμ. This was done so that the capsular material extracted would be obtained from approximately the same number of mucoid and "suicide" organisms. These precipitinogens were prepared by centrifuging equal amounts of each suspension and

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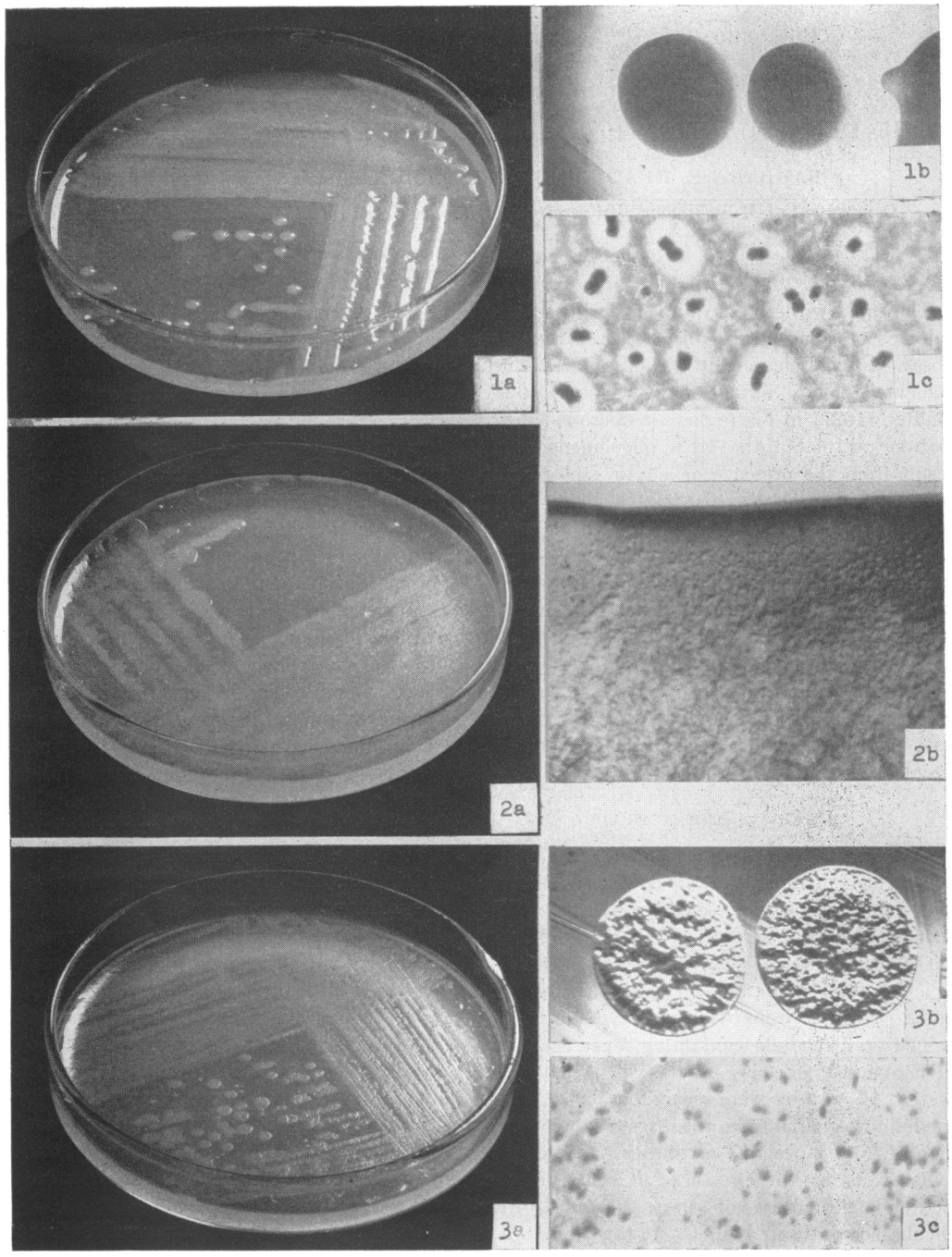

Figures 1–3.

Figure 1a. Peptone agar plate of *Klebsiella pneumoniae* strain C-2 before decapsulation; 8 hr incubation.

Figure 1b. Individual colony from 1a as seen by transmitted light. 5 ×.

Figure 1c. Capsule stain of organisms before decapsulation. 3,500 ×.

Figure 2a. Peptone agar plate of *Klebsiella pneumoniae* strain C-2 during decapsulation. Decapsulation most pronounced in the confluent areas of growth.

Figure 2b. The edge of a confluent area of growth from a peptone-agar plate (2a) as seen by transmitted light. The first sign of decapsulation can be seen as a pitting of the surface of the colony. 5 ×.

Figure 3a. Peptone-agar plate of *Klebsiella pneumoniae* strain C-2 after decapsulation. 14 hr incubation.

Figure 3b. Individual colony from (3a) as seen by transmitted light. 5 ×.

Figure 3c. Capsule stain of organisms after decapsulation. 3,500 ×.

utilizing the supernatants. Using the interphase precipitation method, varying dilutions of each precipitinogen were tested with both antisera. The reactions were read after 15 min at room temperature.

The cell suspensions used as the agglutinogens were prepared and standardized as described above. These antigens were used immediately after preparation to minimize the elution of the capsular material. Using standard agglutination techniques, each antigen was tested against both the antimucoid and anti-"suicide" sera. The agglutination tubes were incubated at 56 C for 1 hr and then centrifuged for 3 min before the titers were read. When the cells remained clumped after shaking, the reaction was recorded as positive.

RESULTS

When strains exhibiting the "suicide" characteristic were subcultured on the peptone agar medium, they initially developed into fully capsulated, mucoid, convex colonies typical of *Klebsiella pneumoniae* (figures 1a and 1b). However, within 12 to 48 hr (depending upon the strain used), the colonies lost their mucoid consistency, flattened out, and took on a ground glass appearance (figures 3a and 3b). The first sign of this phenomenon could be observed by the transmitted light of a dissecting microscope as a pitting in the surface of the colony (figure 2b). All the mucoid colonies of a strain capable of "suicide" exhibited this phenomenon; however, it appeared first in the confluent areas of growth, later in the less crowded areas, and finally in the most isolated colonies (figure 2a). When either mucoid or "suicide" colonies (as late as 10 days after they had withered away) were subcultured, all the cells gave rise to typical mucoid colonies which in turn exhibited this change.

Attempts to isolate from "suicide" strains reproducible particles (bacteriophage) which would then produce clear plaques on plates of the same or any of fifteen other normal strains of *K. pneumoniae* tested were unsuccessful. However, the filtrates prepared from "suicide" organisms decreased the length of time required for "suicide" formation in strain X-4.

The results of the total viable counts of mucoid as compared to "suicide" colonies are presented in table 1. It can be seen that in strains C-2 and X-4 there was no significant decrease in the number of viable organisms as the colonies progressed from the mucoid to the "suicide" state.

Capsule stains performed on cells from colonies in various stages of "suicide" showed that as the

TABLE 1

Total viable counts of colonies before and after decapsulation

Strain	State	Total Viable Count per Colony		Average
		Expt. 1	Expt. 2	
C_2	M	7.2×10^8	5.2×10^8	6.2×10^8
C_2	Dc	4.9×10^8	5.9×10^8	5.4×10^8
X_4	M	7.5×10^6	9.2×10^6	8.3×10^6
X_4	Dc	12.2×10^6	12.6×10^6	12.4×10^6

All colonies 3 mm in diameter.
M, mucoid; Dc, decapsulate ("suicide").

TABLE 2

Reciprocal precipitin tests with organisms before and after decapsulation

Antisera	Precipitinogen	Antigen Dilutions 1:								
		1	2	4	8	16	32	64	128	Control
C_2	C_2	+	+	+	+	+	+	+	+	–
C_2	C_2 Dc	+	+	–	–	–	–	–	–	–
C_2 Dc	C_2	+	–	–	–	–	–	–	–	–
C_2 Dc	C_2 Dc	–	–	–	–	–	–	–	–	–

Dc, decapsulate.
+ = Precipitate. – = No precipitate.

TABLE 3

Reciprocal agglutination tests with organisms before and after decapsulation

Agglutinogen	Antisera	Antisera Dilutions 1:									
		10	20	40	80	160	320	640	1280	2560	Control
C_2 Dc	C_2 Dc	+	+	+	+	+	+	+	+	±	–
C_2	C_2 Dc	±	–	–	–	–	–	–	–	–	–
C_2 Dc	C_2	+	+	+	+	+	+	+	+	+	–
C_2	C_2	+[1]	+[1]	±[1]	–	–	–	–	–	–	–

Dc, decapsulate.
[1] Flocculant agglutination.
+ = Agglutination.
– = No agglutination.

TABLE 4

The effect of various carbon sources on decapsulation

Media	Approximate Time in Hours when Decapsulation Appeared											
	12	24	36	48	60	72	84	96	108	120	132	144
1% Peptone (basal medium)	+											
2% Peptone	+											
4% Peptone	−	−	+									
1% Casein hydrolyzate	−	−	+									
4% Casein hydrolyzate	−	−	−	−	−	−	−	−	−	−	+	
Synthetic medium + 1% glucose	−	−	−	−	−	−	−	−	−	−	−	+
Basal medium + 1% glucose	−	−	+									
Basal medium + 1% galactose	−	−	−	−	+							
Basal medium + 1% fructose	−	−	−	−	+							
Basal medium + 1% lactose	−	−	−	−	+							
Basal medium + 1% arabinose	−	−	+									
Basal medium + 1% citrate	+											
Basal medium + 1% malate	−	−	+									
Basal medium + 1% pyruvate	−	−	+									
Basal medium + 1% succinate	−	−	−	−	+							
Basal medium + 1% acetate	−	−	−	−	−	−	+					

+, Decapsulation in confluent areas of growth; −, mucoid growth.

colonies flattened out, there was a coincident decrease in the amount of capsular material surrounding the organisms (figure 1c and 3c). Since there was no decrease in cell numbers but rather a loss of capsular material, the term "decapsulate" seems more appropriate than "suicide."

It was suspected from the results of the capsule stains and total viable counts that the decapsulation phenomenon was due to a loss of the capsular material. To confirm this, immunological tests were performed. The results as recorded in table 2 indicate that the supernatants from mucoid organisms contained a considerably greater amount of capsular precipitinogen than the supernatants from decapsulate (Dc) organisms. The results of the reciprocal agglutination tests can be seen in table 3. A comparison of the agglutination and precipitation titers of the anti-Dc serum with both antigens revealed that this serum contained very few antibodies against the soluble capsular material but contained relatively large quantities of antibodies against the underlying somatic antigens of these organisms. The agglutination of decapsulate organisms by antiserum prepared against the capsulated cells was probably due to antibodies produced against layers underlying the capsule. These antigens were probably exposed during immunization. A granular type of agglutination was observed with Dc cells; whereas, the mucoid cells gave a flocculent agglutination.

When either casein hydrolyzate (Nutritional Biochemical Co.) or a synthetic medium (Cabelli, 1955) containing NH_4NO_3 as a nitrogen source and glucose as a carbon source were substituted for peptone in the basal medium, a significant delay in the formation of Dc colonies was noted (table 4). Furthermore, the interval of time necessary for decapsulation to appear in the casein hydrolyzate media was markedly shortened by the addition of one per cent peptone. All the mono- and disaccharides tested, when incorporated into the peptone agar, were found to delay the phenomenon. With the exception of citrate, the tricarboxylic acid cycle intermediates tested produced the same effect (table 4).

DISCUSSION

On the basis of Goslings' (1935) description of "suicide", it would appear that the phenomenon he investigated and that described in these studies are the same. In both his studies and those described herein, a loss of capsule was observed coincident with the withering away of

the colonies. However, in these experiments we were unable to confirm his conclusion that bacteriophage was the responsible agent.

From the results of the capsule stains, the immunological data, the total viable counts and the bacteriophage determinations, it is concluded that the so-called "suicide" colonies are in effect colonies which have undergone "decapsulation." The process of decapsulation appears to be due to an active resorption of the capsular polysaccharide.

It would appear that a nonreproducible inhibitor, which was found in filtrates from Dc cultures grown in peptone medium and which considerably hastened decapsulation of other cultures exhibiting this trait, is involved in the mechanisms of this phenomenon. This information takes on even more importance when coupled with the fact that when organisms are grown on a medium in which peptone is omitted, such as casein hydrolyzate or glucose synthetic media, decapsulation is considerably delayed.

The inhibitory effect of peptone is observed to be reversed by the presence of an available carbohydrate source. Even when a nonpeptone containing medium is utilized, the decapsulation appears to occur only when all the available carbohydrates are utilized. Thus, the depletion of carbohydrates or the production of an inhibitor from peptone in a noncarbohydrate containing medium results in an active resorption of the capsular material. In view of this evidence, postulations about the effect of this inhibitor on the energy metabolism of the cells appear to be justified. Investigations are at present in progress concerning the nature and action of this inhibitor.

SUMMARY

The sequence of events coincident with the formation of so-called "suicide" colonies is described. Attempts to isolate bacteriophage from filtrates of such cultures were unsuccessful. Viable counts indicated that the numbers of organisms in colonies before and after this phenomenon occurred were essentially the same. Capsule stains and immunological data demonstrated that there was a loss of specifically precipitable capsular material coincident with the withering away of these colonies. Therefore, the term decapsulation (Dc colonies) is proposed since it describes this phenomenon. The elimination of peptone from the medium or the addition of monosaccharides, disaccharides, or tricarboxylic acid cycle intermediates was able to delay decapsulation.

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