A STUDY OF BACILLUS NECROPHORUS OBTAINED FROM COWS

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Bacillus necrophorus is an important organism in animal disease since it produces severe septic processes in a number of domestic animals. The organism is widely distributed and is responsible for considerable losses. It may invade almost any tissue and is associated with various necrotic foci, as in calf diphtheria, necrotic ulcers of the intestine in hog cholera, foot-rot of sheep and cattle, grease heel or necrotic scratches of horses, lip and leg ulceration of sheep, gangrenous dermatitis of horses, metastatic necrosis of liver and lungs of cattle and swine, necrotic stomatitis of calves, lambs and pigs.

A few human cases of infection with a similar organism have been reported. These are referred to by Harris and Brown (1927) who isolated from cases of puerperal infection in women cultures closely resembling *B. necrophorus* and designated by them *Act. pseudo-necrophorus*.

Because of the wide distribution of necrophorus bacilli and the fact that they produce disease in such a variety of animals it seemed worth while to find out as much as possible about strains from different sources. This work has been started with a group of 10 cultures isolated from cows, studies of which are reported in this paper. They include 7 strains isolated from liver abscesses, 1 from a spleen abscess, 1 from a lung, and the last from an abscess in the omentum.

Isolations were made in two ways, either directly through cultures or by passage through a rabbit. If a stained preparation of the original material showed a predominance of necrophorus

bacilli, then the isolation was made directly through cultures. Since the organism grows well on ordinary media plus a little serum, to isolate a pure culture a suspension of the original material in bouillon was inoculated into serum agar shake cultures. Colonies appeared usually in two days. The column of agar was then forced out of the tube into a sterile petri dish by inserting a capillary pipette to the bottom of the tube and exerting pressure on the bulb. Individual colonies could then be picked from the agar and inoculated into serum bouillon for further study.

If the original material showed other bacteria present in any considerable numbers, then a suspension of this material in bouillon was inoculated subcutaneously into a rabbit. Usually the contaminating bacteria disappeared or decreased in numbers while *B. necrophorus* multiplied and produced an infection. From the necrotic tissue or serous fluid or often from the heart blood of the rabbit pure cultures were obtained. However, to establish a pure strain the rabbit material was inoculated into serum agar shake cultures and separate colonies picked from these tubes as described. In the study of these pure cultures anaerobic conditions were maintained by using a vaseline seal on the tubes of media.

The experimental work with these strains consists of (*a*) morphological studies including tests for the existence of spores by staining methods and by testing the resistance of cultures to heat, drying and exposure to air; (*b*) cultural reactions in various media and fermentation results; (*c*) certain serological reactions including agglutination and agglutinin absorption studies; and finally (4) some observations on the pathogenicity of the cultures.

MORPHOLOGY

Morphologically the present series of bovine strains agree in general with the descriptions of *B. necrophorus* in the literature (see bibliography). *B. necrophorus* is a plemorphic organism varying from coccoid forms to filaments 100μ long. All the present strains show these variable forms. One strain differs somewhat from the others by the appearance of the short rod forms sometimes joined together in chains. Young cultures

show chiefly long rods and filaments while older cultures contain mostly shorter rod forms of varying length. The rods are non-motile and negative to Gram's stain. Some elements are evenly stained but often distinctly beaded forms are seen. Sometimes a rod or filament shows a swollen or thickened area appearing either as a short round swelling near the center of the rod or as an elongated thickening toward one end. Beaded forms are characteristic and are clearly shown in preparations stained with carbol fuchsin. Such filaments show clear or faintly stained spots alternating with deeply stained portions. The unstained areas and those which stain may be of equal size although variation in size is not uncommon. Mohler and Morse (1904) have described in detail these various types of beaded forms.

The general appearance of the clear, unstained spots in the rods suggests spores. The question of spore formation is important since this organism is a strict anaerobe and the presence of spores would readily explain its survival outside the body for long periods and thus help to explain its frequency in animal infections. However, all descriptions in the literature state that *B. necrophorus* cultures contain no spores. Most authors mention the occurrence of beaded forms as characteristic for this organism but do not connect this appearance with spores. Mohler and Morse, however, mention that the unstained spaces in the filaments were first thought to be spores but that spore staining methods did not change their appearance.

In the present study the unstained areas, although they suggested spores in general appearance, were considered to differ from true spores on account of the variation in their size and arrangement in the rods or filaments. It was also found that they failed to stain by methods employed for staining spores. Another point indicating lack of agreement between spores and the unstained spaces in the filaments is the fact that the rods of the older cultures, where one would expect to find spores, show fewer beaded forms than the filaments of the young cultures. Mohler and Morse also stated that the longer rods and threads particularly exhibited the beaded appearance.

In order to determine the ability of the unstained spaces in the

filaments to behave as spores, cultures were tested for resistance to exposure to air, drying and heat. The first experiments were made to test the effect of exposure to air at room temperature. Two strains were tested at the same time, and both a three days' and a two weeks' old culture were used. The serum bouillon cultures were poured into petri dishes so as to form thin layers. Subcultures into serum bouillon were made at the start and at various intervals. The effect of this exposure to air is given in table 1.

Later, another series of tests was made with older cultures, since if spores are formed one would expect to find them in such

TABLE 1

Resistance of B. necrophorus cultures to exposure to air

Cultures poured into petri dishes in thin layers and kept at room temperature.

TIME OF EXPOSURE	3 DAYS' SERUM BOUILLON CULTURE OF STRAINS		2 WEEKS' SERUM BOUILLON CULTURE OF STRAINS	
	D	E	D	E
Control (unexposed)	+* (24 hours)	+ (24 hours)	+ (2 days)	+ (2 days)
hours				
4	+ (24 hours)	+ (2 days)	+ (3 days)	−
8	+ (5 days)	+ (4 days)	+ (5 days)	−
11	+ (5 days)	+ (6 days)		
15			−	−
18			−	−
24	−	+ (5 days)	−	−
28	−	−	−	−

* + = growth; − = no growth.

cultures. The results of the test with 9 different strains of three weeks' old serum bouillon cultures are given in table 2.

The results are variable. For instance, no growth occurred in subcultures from Strain D after two hours' exposure to air, from H after four hours, from I after five hours, or from A, C, and E after seven hours. Strains B, F, and G still developed growth after seven hours' exposure to air but only after prolonged periods of incubation of six to nine days; evidently a good many of the original forms had been killed. A third test with a twenty-four

TABLE 2

Resistance of B. necrophorus cultures to exposure to air

Cultures poured into petri dishes in thin layers and kept at room temperature

TIME OF EXPOSURE	3 WEEKS' OLD SERUM BOUILLON CULTURES OF STRAINS								
	A	B	C	D	E	F	G	H	I
Control (unexposed)	+ (2 days)	+ 2 (days)	+ (2 days)	+ (1day)	+ (3 days)	+ (3 days)	+ (2 days)	+ (3 days)	+ (1 day)
hrs.									
1	+ (3 days)	+ (3 days)	+ (3 days)	+ (5 days)	+ (3 days)	+ (5 days)	+ (3 days)	+ (3 days)	+ (1 day)
2	+ (9 days)	−	+ (14 days)	−	+ (12 days)	+ (11 days)	+ (5 days)	+ (5 days)	+ (4 days)
3	−	−	−	−	−	+ (5 days)	+ (9 days)	+ (6 days)	−
4	−	−	−	−	+ (11 days)	+ (5 days)	+ (5 days)	−	+ (6 days)
5	+ (6 days)	+ (6 days)	+	−	+ (6 days)	+ (5 days)	+ (14 days)	−	−
7	−	+ (6 days)	−	−	−	+ (6 days)	+ (9 days)	−	−

TABLE 3

Resistance of B. necrophorus cultures to drying

Two to three drops culture in bottom of sterile tube, left to dry at room temperature. Tested for resistance by adding fresh serum bouillon, sealing with vaseline and incubating.

TIME OF DRYING	24-HOUR SERUM BOUILLON CULTURE OF STRAINS				6-DAY SERUM BOUILLON CULTURE OF STRAINS		
	A	B	C	D	A	B	C
Control (not dried)	+ (24 hours)	+ (24 hours)	+ (24 hours)	+ (24 hours)	+ (24 hours)	+ (24 hours)	+ (24 hours)
days							
1	−	+ (3 days)	+ (5 days)	+ (3 days)	−	−	−
2	−	+ (3 days)	−	−	−	−	−
4	−	−	−	−	−	−	−
5	−	−	−	−	−	−	−

hour growth of 4 strains (A, B, C, D) showed in all cases resistance to exposure to air for 11 hours, but, the longer the culture was exposed to air, the later growth appeared in the subcultures. The controls inoculated with unexposed culture and tubes from the two hours' exposure developed growth in one to two days, but after eight and eleven hours' exposure to air growth in subcultures did not appear until after six to seven days. Comparison between young and older cultures shows in every case that the older culture was apparently less resistant to exposure to air than younger ones. It is clear, that the organism does not behave as a true spore-bearer, and it is also apparent that air has a distinctly toxic effect.

The effect of drying at room temperature was next considered. The details are given in the second group of experiments. Both twenty-four-hour and six-day serum bouillon cultures were employed for the tests. The results are given in table 3.

The strains showed some variation in degree of resistance. Among the twenty-four-hour cultures, A showed no resistance to drying, while B, C, and D survived the first day. C and D were killed the second day while B remained alive, but this strain gave no growth in tests on the fourth and the fifth day. The six-day cultures did not resist even one day of drying. Again, the older cultures showed less resistance than the younger ones. A twenty-four-hour growth of these strains was also mixed with sand and spread out in petri dishes to dry. The control tubes inoculated with freshly mixed culture and sand gave positive growth, but after one and two days of drying a generous inoculation of the sand into fresh media gave no growth.

Since spores are not readily destroyed by heat it seemed desirable to test the effect of certain temperatures on cultures which had the characteristic beaded appearance. The third experiment deals with the effect of heat. The tests were made in two ways. First a serum bouillon culture under a vaseline seal was heated in a water bath at 60°C. and at intervals culture was withdrawn and inoculated into fresh serum bouillon. Five strains (A, B, C, D, J) were tested. The unheated controls all grew in subcultures. Four cultures grew after exposure at 60°C. for one minute, but

all failed to grow after heating at 60°C. for three minutes. When the test was made by inoculating fresh bouillon tubes, sealing with vaseline, then heating and at intervals withdrawing them and incubating, the results were similar to those obtained by the first method.

This apparent sensitiveness of these cultures to heat, drying and exposure to air indicates that the clear unstained areas seen in the rods and filaments are not true spores. The slight resistance of *B. necrophorus* to exposure to air was noted by Hagan (1924) who showed that exposure to air of a fluid culture in a thin layer caused considerable lag in growth on subculture into cooked meat media, but, if the subinoculations were made into bouillon, growth seldom occurred after thirty minutes' exposure to air. On the other hand, Albrecht (1928) refers to a report by Fr. Meyer who found necrose bacilli resistant to heat and drying. He stated that heating at 95°C. for a short time did not necessarily kill the bacilli and that they would remain virulent when dried for eighteen weeks. Albrecht also mentions a report by Hasenkamp stating that necrophorus bacilli are sensitive to heat and drying. Kelser (1927) found that strains obtained from horses were destroyed immediately at 100°C. and in twenty minutes at 65°C.

CULTURAL CHARACTERS

The cultural studies of the strains show in general a uniform group with slight variations. All strains are strict anaerobes. In serum agar shake cultures they always grow 1 to 2 cm. below the surface. The colonies are round or biconvex, smooth, even and compact. The appearance of the colonies differs from the descriptions of others in the literature. Most observers describe the colonies as woolly or fuzzy. Kelser (1927) compares the colonies in agar shake cultures to small tufts of cotton. In the present work, however, all the strains show smooth, even, compact colonies in agar shake cultures and magnification fails to change their appearance. In serum bouillon all grow chiefly as a sediment, usually of soft particles or masses gradually settling together. In plain bouillon the majority of strains show more clouding and a fine sediment, with the exception of two cultures

TABLE 4

Fermentation reactions

In digest bouillon + 1 per cent of sugar. Original pH 7.2

STRAIN	GLUCOSE		LACTOSE		SUCROSE		MALTOSE		MANNITOL		PLAIN BOUILLON CONTROL	
	pH	Gas	pH	Gas	pH	Gas	pH	Gas	pH	Gas	pH	Gas
		cm.						*cm.*				
A	5.8	+7.7	6.4	– (bubble)	6.8	– (bubble)	5.9	+6.0	6.6	– (bubble)	7.0	– (bubble)
B	6.0	+3.7	6.6	– (bubble)	6.6	– (bubble)	6.0	+4.2	6.6	– (bubble)	7.0	– (bubble)
C	5.9	+3.5	6.6	– (bubble)	6.6	– (bubble)	5.9	+8.3	6.6	– (bubble)	7.0	– (bubble)
D	6.0	large bubble	6.6	– (bubble)	6.6	– (bubble)	6.0	+3.0	6.6	– (bubble)	7.0	– (bubble)
E	5.8	+10.5	6.6	– (bubble)	6.6	– (bubble)	6.0	+6.7	6.6	– (bubble)	7.0	– (bubble)
F	6.0	+14.5	6.6	– (bubble)	6.8	– (bubble)	6.0	+6.0	6.8	– (bubble)	7.0	– (bubble)
G	5.8	+9.5	6.6	– (bubble)	6.4	– (bubble)	5.9	+8.7	6.4	– (bubble)	6.9	– (bubble)
H	5.8	+9.9	6.4	– (bubble)	6.4	– (bubble)	5.9	+5.2	6.4	– (bubble)	6.9	– (bubble)
I	5.8	+3.5	6.4	– (bubble)	6.6	– (bubble)	5.9	+6.1	6.6	– (bubble)		
J	5.9	+4.0	6.6	– (bubble)	6.6	– (bubble)	5.9	+8.4	6.6	– (bubble)		

(B and I) which always grow chiefly as suspended particles and sediment. These two cultures also agglutinate spontaneously with complete clumping in normal salt solution. The growth in digest bouillon seems slightly heavier than in ordinary veal infusion broth and the addition of glucose increases the growth. All cultures produce indol, hemolyze blood corpuscles, coagulate milk, and do not liquefy gelatin. Two strains (A and B) were inoculated into a series of fermented bouillon tubes of different pH values. The results in these cases indicated that the limiting H-ion concentrations within which growth could occur were pH 6.0 to 8.4. Gas is produced in most media, in serum bouillon, serum agar shake cultures, and to a considerable degree in cooked meat media. Sometimes a bubble of gas is formed in plain infusion bouillon and a large bubble always appears in plain digest bouillon. Two strains which have been tested for H_2O_2 formation when exposed to air gave positive results.

These bovine strains as judged by fermentation reactions in various carbohydrates form a fairly uniform group. The complete seriesof tests were made in digest bouillon to which sufficient carbohydrate had been added to make a 1 per cent solution. A few strains were also tested in fermented bouillon. The fermentation results are given in table 4.

Glucose and maltose were fermented with both acid and gas production with the exception of Strain D which formed very little gas in glucose. The pH in all the other sugar tubes was slightly decreased and no more gas production occurred than that observed when plain bouillon tubes were inoculated. The comparison of digest and fermented bouillon showed practically no difference in the final pH values but there was little gas production in the tubes of fermented bouillon + glucose.

SEROLOGICAL REACTIONS

The group, then, appeared from a morphological and cultural point of view as relatively homogeneous and, as will be shown later, the strains possessed certain pathogenic properties in common. It seemed of interest to determine whether or not the group was a compact one immunologically as judged by common

TABLE 5

Agglutination of B. necrophorus strains with 4 different antisera

STRAIN	DILUTIONS OF SERUM										0.1 PER CENT SALT SOLUTION + CULTURE CONTROL
	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	
						B. necrophorus A antiserum					
A	C	C	C	C	C	C	++++	+++	++	+	−
B	++++	++++	+++	++	+	+	+	+	+	+	+
C	C−	C	C	C	C	C	++++	+++	++	±	−
D	C	C	C	C	C	C	+++	++	+	±	−
E	C	C	C	C	C	C−	++++	++	+	±	−
F	C−	C−	C−	C−	C−	++++	+++	++	+	±	−
G	C	C	C	++++	++++	++++	+++	+++	++	+	±
H	++++	++++	++++	++++	++++	+++	+++	++	++	+	+
J	++++	++++	++++	++++	+++	++	+	+	±	−	−
						B. necrophorus B antiserum					
A	+++	++	+	+	+	+	+	+	+	+	+
B	C	C	++++	++++	++++	++++	++++	+++	++	+	+
C	−	−	−	−	−	−	−	−	−	−	−
D	++	+	−	−	−	−	−	−	−	−	−
E	−	−	−	−	−	−	−	−	−	−	−
F	+++	+	+	±	−	−	−	−	−	−	−
G	+++	++	++	++	+	+	+	+	+	+	+
H	+	+	+	+	+	+	+	±	±	±	±
J	±	−	−	−	−	−	−	−	−	−	−

B. necrophorus F antiserum

	A	B	C	D	E	F	G	H	J
A	C	C	C	C	C	C	C	C	+
B	++++	++	++	++	++	++	++	++	++
C	−	−	−	−	−	−	−	−	−
D	++	+	+	+	+	+	+	+	+
E	−	−	−	−	−	−	−	−	−
F	C	C	C	C	C	C	C	C	C
G	+++	+++	+++	+++	+++	+++	+++	+++	+++
H	+++	+++	+++	+++	+++	+++	+++	+++	+++
J	++	++	++	++	+	+	+	+	+

B. necrophorus J antiserum

	A	B	C	D	E	F	G	H	J
A	++	++	+	+	+	+	+	+	−
B	C	C	C	C	C	C	C	C	+
C	++	+	−	−	−	−	−	−	−
D	+++	++	−	−	−	−	−	−	−
E	++	++	−	−	−	−	−	−	−
F	++	+	−	−	−	−	−	−	−
G	+++	++	−	−	−	−	−	−	−
H	++	+	−	−	−	−	−	−	−
J	C	C	C	C	C	C	C	C	+

agglutination affinities. With this in view a number of rabbits were immunized with various cultures. In most cases the treatment consisted of a series of injections given subcutaneously and intraperitoneally with a whole bouillon culture three days to a week old, which had been killed either by heating at 60°C. for one-half hour or by exposing to air for at least one day. The injections were made at intervals of three to five days and the doses increased gradually from 0.5 to 2 cc. Bleedings were made seven to eleven days after the last injection. Antiserum A used in the tests recorded in table 5 was obtained from a rabbit treated only with intravenous injections of culture filtrate from bouillon or of cooked meat media cultures nine to nineteen days old. Other lots of A serum from rabbits treated in the usual way with subcutaneous and intraperitoneal injections of killed whole bouillon cultures behaved in a similar manner. The other three sera (B, F, J) were also from rabbits injected subcutaneously and intraperitoneally with killed whole bouillon cultures. The sera prepared with the four different strains were tested for agglutinins against all the cultures. The results are given in table 5. Since some strains agglutinate spontaneously in normal (0.85 per cent) salt solution, the final dilutions were made in 0.1 per cent salt solution and a glucose digest bouillon growth was used as antigen.

The A antiserum agglutinates all strains at significant titers except Culture B. B antiserum, however, is relatively specific for B, while F antiserum agglutinates to a certain extent 7 of the strains and fails completely to agglutinate 2 cultures (C and E). The J serum agglutinates strongly only one culture (B) besides the homologous strain. It was noted that J and B, which agglutinate most poorly with A antiserum, agglutinate best with J antiserum, while others agglutinating well with A are not greatly affected by J.

According to the agglutination results, A seems to be an important strain since it is the only one among those used for producing antisera which stimulated the production of agglutinins for all strains. This result suggests that A antiserum possesses distinct agglutinins for all cultures. To test this, a complete series of agglutinin absorption tests were made between this antiserum and

each culture. Agglutinin absorption tests were also carried out with the other sera and a certain number of strains to see what other relationships could be established. The absorption was made in 1:10 dilutions of the serum in 0.2 per cent salt solution. Sediment obtained from centrifuging glucose digest bouillon cultures was added to the serum dilutions and the mixture incubated for 2 hours and then refrigerated. The next day, after centrifuging, agglutination tests were made with the absorbed serum. Some results of the absorption experiments with each serum are found in table 6.

The results of the complete absorption series with A antiserum indicate that A is a complex strain antigenically. It is apparently able to produce agglutinins for each strain, which in most cases can be removed only by absorption with A or with the particular strain under consideration. For instance, absorption by A takes out agglutinins for all strains. Strain G is apparently the next most complex culture antigenically, since absorption with this culture reduces to a considerable extent agglutinins for all strains except A and F and these are probably slightly reduced. Strains D, F, H, and J each remove from A antiserum chiefly agglutinins for themselves and leave agglutinins reacting with the other strains, although absorption by D causes a very slight reduction of agglutinins for a number of strains and the agglutinins for J are often decreased somewhat by absorption with other cultures. Strains C and E behave alike, in that each absorbs agglutinins for the other as well as for itself, and both leave agglutinins reacting with all other strains. This similar behavior of C and E agrees with their similarity in failing to agglutinate in the B and F antisera (table 5). Altogether these absorption results indicate that the power of the A antiserum to agglutinate all strains is apparently not due to some common group agglutinin, since, in general, the different strains fail to remove agglutinin for each other. On the other hand they suggest that the A antiserum possesses distinct agglutinins for each strain, since absorption by one heterologous strain removes chiefly agglutinins reacting with that particular strain and does not take out agglutinins for other heterologous strains except in special instances.

TABLE 6

Some results of absorption of sera by homologous and heterologous strains

ABSORBED BY	TESTED BY	DILUTIONS OF SERUM										0.1 PER CENT SALT SOLUTION CULTURE CONTROL
		1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:2,560	1:5,120	1:10,240	
B. necrophorus A antiserum												
B	B	+	+	+	+	+	+	+	+	+	+	+
	A	C	C	C	C	C	C	+++	++	+	±	−
D	D	++++	++++	++	+	−	−	−	−	−	−	−
	A	++++	C	C	C	C	C	+++	++	+	±	−
F	F	++	+	±	±	±	±	±	±	±	±	−
	A	++++	C	C	C	C	++++	++	+	+	±	−
J	J	++	++	+	+	±	−	−	−	−	−	−
	A	++++	C	C	C	C	C	+++	++	+	+	−
A	A	++++	+++	++	++	+	−	−	−	−	−	−
B. necrophorus B antiserum												
A	A	−	−	−	−	−	−	−	−	−	−	−
	B	++++	++++	+++	+++	+++	+++	++	+	+	+	+
D	D	−	−	−	−	−	−	−	−	−	−	−
	B	++++	++++	++++	++++	++++	++++	++++	+++	++	+	+
F	F	−	−	−	−	−	−	−	−	−	−	−
	B	++++	++++	++++	++++	++++	++++	++++	+++	++	+	+
J	J	−	−	−	−	−	−	−	−	−	−	−
	B	++++	++++	++++	++++	++++	++++	++++	+++	++	+	−
B	B	+	+	+	+	+	+	+	±	±	±	±

B. necrophorus F antiserum

A	A	−	−	−	−	−	−	−	−	−	−	−
	F	C	C	C	++++	++	+	±	−	−	−	−
B	B	+	+	+	+	+	±	±	±	±	±	±
	F	++++	++++	++++	++++	++++	+++	++	+	+	±	−
D	D	±	±	−	−	−	−	−	−	−	−	−
	F	C	C	C	++++	++++	+++	++	+	+	±	−
J	J	+	−	−	−	−	−	−	−	−	−	−
	F	++++	++++	++++	++++	++++	+++	++	+	+	±	−
F	F	+	+	+	±	+	+	+	−	−	−	−

B. necrophorus J antiserum

A	A	−	−	−	−	−	−	−	−	−	−	−
	J	+++	+++	+++	+++	++	+	−	−	−	−	−
B	B	++++	+++	+	+	+	+	+	+	+	+	+
	J	C	C	++++	++++	+++	+	−	−	−	−	−
F	F	−	−	−	−	−	−	−	−	−	−	−
	J	C	C−	++++	++++	++	±					
J	J	−	−	−	−	−	−	−	−	−	−	−

In the case of the other sera the absorption tests, as far as carried out, show that absorption by the homologous culture removes specific agglutinins, while absorption by heterologous strains, although removing agglutinins for the absorbing strain, fails to absorb the specific agglutinins (table 6). Thus, at least in the case of the four strains used to produce these antisera, each one shows strong specific agglutinin production. Also, by the absorption experiments, A was found to possess complex antigenic properties.

PATHOGENICITY OF B. NECROPHORUS FOR LABORATORY ANIMALS

These bovine strains of *B. necrophorus* are pathogenic for rabbits. One cubic centimeter of a bouillon culture injected subcutaneously usually produces a spreading necrosis of the subcutaneous tissues. The area becomes edematous and necrotic and the rabbits die in four to ten days. Strain B differs from the others in not causing death but only producing an abscess at the point of inoculation which heals after a number of weeks. Strain A which has been kept in the laboratory for about three and one-half years is still as virulent as when first isolated. This strain has been tested on mice and guinea pigs. A subcutaneous inoculation of 0.1 or 0.2 cc. of a bouillon culture into mice caused a spreading necrosis and the mice died in eleven to twenty-five days. The culture had less effect on guinea pigs, at most producing an abscess which healed after two to three weeks.

In addition to living cultures, culture filtrates have been tested. In mice, 1 cc. of filtrate injected intraperitoneally produced illness and sometimes death. In rabbits, 4 cc. of filtrate injected intravenously produced illness and frequently death within a few hours (11 out of 17 cases). If the rabbits survived they showed considerable loss in weight within twenty-four hours, for instance, from 70 to as much as 235 grams or from 5 to 12 per cent of their body weight, and sometimes the loss in weight continued for two or three days following the administration. Smaller doses of filtrate (2 cc.), intravenously injected, regularly made the rabbits sick for a number of hours and caused a definite loss in weight. Since similar doses of bouillon had little or no effect on rabbits,

this action of culture filtrates is apparently due to some toxic substance from the culture.

DISCUSSION

This bovine group of necrophorus cultures show similar morphological appearances. The characteristic beaded forms occurring in all strains are due to unstained areas in the filaments which give the appearance of spores. However, these are not considered to be true spores since they are not affected by spore stains and the cultures containing them are readily killed by heat, drying and exposure to air. Furthermore, these beaded forms are more numerous in young cultures than in old ones and the clear areas vary in size and arrangement in the rods. Thus the unstained areas differ from spores in every respect except their failure to stain. There is no good reason why they may not be regarded as vacuoles occurring in the rapidly growing filaments.

These bovine strains in general represent a uniform group in regard to their behavior in various culture media and in fermentation reactions. As to pathogenicity, the strains exhibit similar properties with the exception of one which is much less virulent than the others.

When considered from an immunological standpoint, sharp differences appear. Although Culture A produced agglutinins reacting with all strains (table 5) yet absorption of the serum with heterologous strains failed to remove the agglutinins reacting with Strain A (table 6); and, moreover, each heterologous strain in general absorbed only agglutinins for itself and left agglutinins reacting with other cultures, indicating that A is a complex strain antigenically which apparently produces a number of distinct agglutinins. The three sera produced by immunization with other cultures showed further differences by agglutinating individual strains to varying degrees. All antisera behave alike, however, in retaining the homologous agglutinins after absorption with heterologous strains. Apparently each strain shows an homologous specificity in agglutinin production and may or may not develop agglutinins reacting with other strains.

SUMMARY

Ten strains of *B. necrophorus* isolated from cows have been described. They are similar in morphology, in most cultural reactions, and in pathogenicity for rabbits with the exception of one strain. When compared serologically by agglutination reactions certain differences were readily apparent, since immune sera prepared with four different strains allowed varying degrees of cross agglutination and apparently no cross absorption of specific agglutinins. Each immunizing strain apparently produces specific agglutinins since they are not absorbed by any other culture. Also, as found in the case of the A culture, a strain may stimulate the production of distinct heterologous agglutinins. The fluffy type of colonies usually found by other workers has not been observed in these cultures, which in all cases form smooth, even, compact colonies in serum agar shake cultures.

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