SOME OBSERVATIONS ON VARIATIONS OF BRUCELLA ABORTUS

PRELIMINARY REPORT

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On growing Brucella abortus strains in 10 per cent immune serum broth it was found that after several passages, if the cultures were plated, two different types of colonies appeared. After forty-eight hours' incubation at 37° C. growth occurred on the surface of the agar plate as small, round, convex, coarsely, granular, opaque colonies. After five or six days the colonies grew much larger and became more granular and brownish-yellow in color. Another type of colony which was usually smaller and was round, convex, translucent and finely granular appeared after ninety-six hours' incubation. These colonies when very young looked colorless and finely granular through transmitted light, when they grew older they were pale straw in color. It was observed that the second type of colony usually grew in the deep layers of the agar, and when they grew close to the layer of the glass of the Petri plate, they looked decidedly blue or bluish-grey through transmitted light.

Observing these colonies closely through a long series of pour plates we found that these translucent colonies as they grew older gave rise to coarse granules, within the colony; that eventually tended to coalesce toward the center and form small bodies. On aging, one to five of these small bodies were formed in the center of the colony resembling papillae or daughter cells. These bodies were usually elliptical in shape, dark brown and opaque in contrast to the rest of the colony which were pale and translucent. When such colonies were cultivated in mass, aerobically, only the daughter cells yielded growth, the translucent, glassy areas disappearing and giving rise to colonies of the opaque type.

As we had observed that the translucent type of colony would usually develop in the deep layers of the agar plate, we tried to grow them under partial anaerobic conditions. We used the Prodigiosus method. A deep agar plate was poured. When the agar was dry, a slit was made in the center with a sterile spatulla. The plate was then incubated to prove sterility. The next day one side of the

plate was inoculated with a culture of Prodigiosus; the other side of the plate was seeded with the translucent or bluish-grey colony and the Petri plate was carefully sealed with plasticine to avoid the entrance of air. Colonies developed very slowly taking from three to ten days. They grew round and flat and looked irregular. As the colony grew older it appeared ragged and became pigmented. In about thirty to forty-five days large areas of a brown pigment-like substance were seen all through the colony but specially near the periphery. These areas gradually became round and formed daughter colonies of the opaque type. If the colony was allowed to grow indefinitely the translucent part of it would disintegrate, leaving the opaque daughter cell which in turn would gradually develop into an adult colony. If the colonies were planted again in the Prodigiosus plate prominent opaque, coarsely granular colonies with irregular surfaces and outlines appeared. The colonies looked firm in appearance and were adherent to the agar. They developed in from 72 to 96 hours and on aging began to show a peculiar erosive phenomenon in the center which finally destroyed most of the colony. When the destroyed areas were subcultured no growth occurred. However, if these opaque colonies were planted aerobically no erosive phenomenon occurred and the colony developed normally.

These opaque colonies, taken from immune broth, may be grown at low tensions of atmospheric oxygen.

Strains of Brucella abortus that had been passed repeatedly through immune serum broth were plated and plates incubated for 72 hours. Then these strains were grown at 75 per cent atmospheric oxygen tension; at the end of five days the atmospheric oxygen tension was decreased to 50 per cent and in another five days to 25 per cent and kept at this tension throughout the experiment. In from 25 to 30 days the colonies began to show papillae, especially in the periphery. These papillae gradually formed daughter colonies which were clear and translucent, resembling the clear translucent colonies previously described. Unfortunately we have been unable, up to the present to grow these daughter cells. It may be a question of CO_2 tension or the necessity of some special media.

Morphological and Cultural Characteristics.—The translucent colonies when stained showed small gram-negative bacilli and a great number of coccoid forms. The opaque type showed many rods and some plump cocci. There was no greatly appreciable difference between individual cells of the different colonies.

Subcultures from the translucent type of colony will form a homogenous suspension of uniform turbidity when emulsified in normal

salt solution. The opaque type gave a clumpy suspension with a tendency to precipitate to the bottom rapidly.

Both types agreed with Brucella abortus as to morphology, staining reaction and carbohydrate fermentation.

SEROLOGICAL REACTIONS

The results of immunization with the different types of colonies and agglutination of their antisera have been very variable. By direct agglutination they seem to fall into two groups; however, this apparent transition we have been unable to demonstrate with certainty. Between these two main types there are probably many subtypes, each representing one stage in the process of transition. This may account for the numerous subdivisions that have been made on serological grounds by previous workers.

AGGLUTINATION OF CLEAR AND OPAQUE COLONY ANTIGEN BY OPAQUE COLONY ANTISERUM

Antiserum	Antigen	Dilution 1-40	1-80	1-160	1-320	1-640	1-1280	1-2560
456	C. A.	2 +	+ −	−	−	−	−	−
	O. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +
483	C. A.	4 +	4 +	2 +	+ −	−	−	−
	O. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +
P. R. I.	C. A.	4 +	2 +	−	−	−	−	−
	O. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +

AGGLUTINATION OF CLEAR AND OPAQUE COLONY ANTIGEN BY CLEAR COLONY ANTISERUM

Antiserum	Antigen	Dilution 1-40	1-80	1-160	1-320	1-640	1-1280	1-2560
456	C. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +
	O. A.	4 +	4 +	4 +	+ −	−	−	−
483	C. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +
	O. A.	4 +	3 +	−	−	−	−	−
P. R. I.	C. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +
	O. A.	4 +	4 +	2 +	−	−	−	−
P. R. II	C. A.	4 +	4 +	4 +	4 +	4 +	4 +	4 +
	O. A.	4 +	4 +	2 +	.....	.....	.....	.....

DISCUSSION

From this brief report it can be readily seen that this work is incomplete. These experiments have been carried out mostly with four strains which had been isolated for some time and it would be desirable to repeat them with several additional freshly isolated ones. However, we are of the opinion that the evidence obtained is strongly

suggestive of dissociation. The same phenomenon, but less obvious, has been observed in cultures that were not passed through immune broth, but were submitted to the partial atmospheric oxygen conditions alone. So it would appear that the immune serum is not necessary for the changes to take place, but that it enhances the production of variant forms decidedly.

Bang and Strebolt(1) found that there were two optima on the partial pressure of oxygen; one somewhat less than the partial pressure of atmospheric oxygen and the other partial pressure of nearly pure oxygen (90 per cent). A tube culture of serum gelatin agar incubated in pure oxygen or under ordinary air at a pressure of 5 atmospheres gave rise to two zones of colonies; one near the surface of the medium and the other near the bottom of the tube, between them the medium remained clear. Holth obtained growth equally successful with increased oxygen tensions and Presiz(2) cultivated the organisms from the tissues on agar surfaces in sealed tubes to which oxygen had been previously added. It is generally accepted that Brucella will not grow under anaerobic conditions; however, Horrocks(3) mentions the fact that the anaerobic growth of Brucella melitensis is feebler than the aerobic, and Smith(4) has grown abortus in atmospheres of 100 per cent CO_2, 10 per cent CO_2 and 90 per cent nitrogen, and 10 per cent CO_2 and 90 per cent hydrogen, although he admits some traces of oxygen may have been present in all these gases. In our experiments the organisms grew well in 25 per cent atmospheric oxygen tension after they were accustomed to that environment and gave rise to the changes described.

Henry(5) on making studies with Traum on the causes of discrepancies encountered in testing cattle sera for Brucella abortus agglutinins observed that different lots of antigen from certain strains gave varied reactions with the same sera. He concluded that the antigen varied and in trying to determine the characters of this variation observed two distinct types of colonies; one which he describes as moist, clear and only slightly granular showing a blue-green florescence and another opaque, very definitely granular and growing more rapidly than the clear type.

Plastridge and McAlpine(6) observed that when B. abortus was cultured in liver broth for eight to fifteen days a distinct pellicle was seen, later slimy sediment appeared which collected on the bottom and which could be drawn out in threads. This new form they called the mucoid form. They found further that this form was encapsulated and that it differed antigenically from the normal abortus colony.

In our experiments there seems to be a definite relation between the changes recorded and the difference in gas tensions used.

SUMMARY

No conclusions will be attempted on this preliminary report; however, we think that the evidence presented is strongly suggestive of dissociation and that different gas tension may be one of the incitant causes to dissociation in the Brucella group of organisms.

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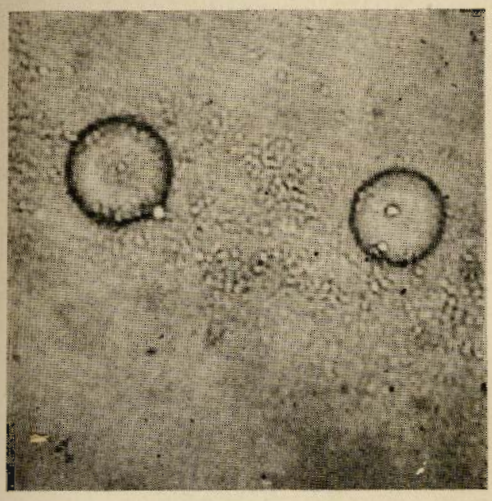
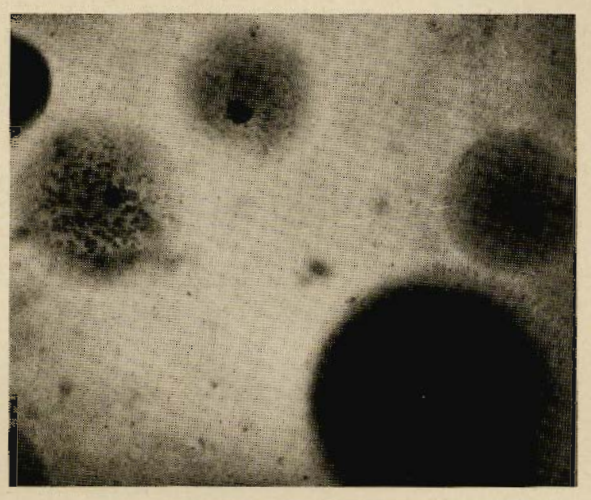
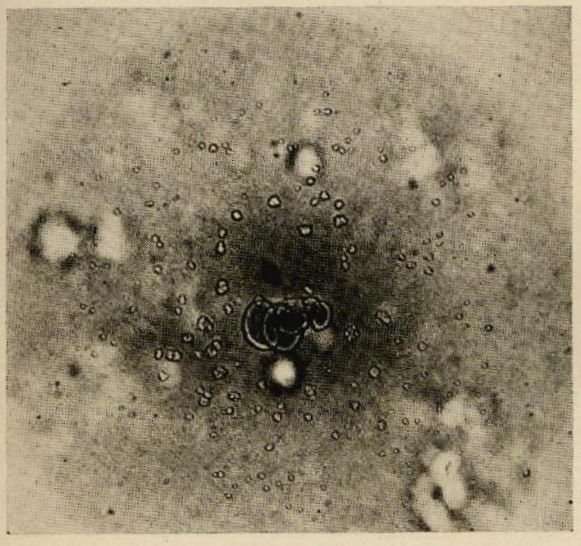
3. Horrocks (1905): Rep. Med. Fever Com. London.
4. Smith (1924): Jour. Exp. Med. **40**: 219.
5. Henry (1928): Proc. Soc. Exp. Biol. & Med. **24**: 100.
6. Plastridge and McAlpine (1930): Jour. Infect. Dis. **46**: 315.



Clear translucent colony growing in the deep layers of the agar pour plate.



Coarse granules appearing within the colony.



On aging small bodies are formed in the center of the colony.

Opaque colonies giving rise to clear type of cells.

Clear type of cells giving rise to opaque ones.