A "REVERSION" PHENOMENON IN BACTERIAL FERMENTATION.

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The publication of the following brief note is necessitated by the circumstance that, in a recent paper, Cunningham and King (1917)[1] make this remark in the course of a discussion of the question of acquisition of new fermentative properties by bacteria: "It would appear however that the characteristic once acquired is not absolutely permanent, for Ledingham has shown that the reverse process can take place, alkaline papillae appearing on colonies which have been thus trained." In a footnote the authors add, "We have been unable to find the reference to this piece of work. Col. Ledingham told one of us (J. C.) about it in the course of a conversation." This observation referred to has not hitherto been published as it emerged simply in the course of a larger investigation which was interrupted by the war. In their statement, Cunningham and King do not perhaps quite strictly express the nature of the reversion process as it was observed by me in a strain of *B. dysenteriae*, and consequently I give the relevant facts here while postponing their full discussion to a more convenient season.

SOURCE OF MATERIAL INVESTIGATED.

Two strains of *B. dysenteriae* (mannite-fermenting type) which had been isolated from cases of asylum dysentery by D. McKinley Reid (1913)[2] were given me for further study.

Both strains were agglutinated readily by stock Flexner-Y serum but a specific serum prepared by immunisation with one strain "A" had only feeble action on strain "B" and *vice versa*. The two strains differed also with respect to their fermentation reactions on certain carbohydrate media, the chief differences being in connexion with

[1] Dysentery in the jails of Eastern Bengal, *Indian Journ. Med. Res.* v. 103.
[2] On the Bacteriology of Asylum Dysentery in England, *Journ. of Ment. Sc.* lix. 621.

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raffinose, arabinose, and isodulcite. From extracts of my notes available here (Baghdad) I give the following particulars of these differences.

FERMENTATION PROPERTIES OF ORIGINAL STRAINS.

Raffinose:

Strain "*A*." Examination of 10 separate colonies inoculated simultaneously on tubes of raffinose peptone water. One gave full acidity in three days, one in four days, four in six days, one in seven days, one in eight days and two in twelve days. The limits of time, therefore, for development of full acidity, were three to twelve days.

Strain "*B*." Examination of 10 colonies as before. Seven gave full acid reaction in two days and the remaining three in four days.

Isodulcite:

Strain "*A*." Examination of 5 colonies. All gave full acidity in 24 to 48 hours.

Strain "*B*." Examination of 5 colonies. One showed full acidity on the fourteenth day while the remainder showed no change during a period of observation of 20 days. From the variant which produced a full acid reaction on the fourteenth day, was ultimately obtained one which gave full acidity on isodulcite in five days. When plated out on solid medium containing isodulcite, certain of the white colonies after prolonged incubation (nine days) developed red papillae—a phenomenon now well known and frequently studied. It will not be referred to further here.

Arabinose:

Strain "*A*." All colonies gave full acidity in 24 hours.

Strain "*B*." The ten colonies examined showed no change whatever at least in fourteen days but it should be added that an acid-forming variant was ultimately derived from this strain.

The differences noted above are grouped together for convenience in the table annexed.

	Raffinose	Isodulcite	Arabinose
"A"	Full acid in 3–12 days	Full acid in 24–48 hours	Full acid in 24 hours
"B"	Full acid in 2–4 days	Acid-forming variant selected by prolonged growth on medium (14 days). Development of red papillae on white colonies of ditto	No change in 14 days. Acid-forming variant selected by prolonged growth on medium

With these differences before us, the question arose whether variants could be obtained from the original strains "A" and "B," which would show a similar behaviour on the three substances employed and, if so, whether such approximation was accompanied by a serological *rapprochement* in cross-agglutination experiment. From a consideration of the original properties of the strains, it will be realised that the

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selection of a variant promptly fermenting raffinose, was readily evolved from "A" while from "B" it was at least possible to secure a variant rapidly attacking isodulcite and arabinose, by the simple expedient of working from acid papillae developing on white colonies after long or short periods of incubation. The method, however, was applied only in the case of "B" on arabinose, when a variant producing early acidity on this substance was ultimately obtained.

The peculiar behaviour of "A" on isodulcite plates afforded a much simpler method of securing identical variants so far as this medium was concerned. Strain "A" was inoculated on isodulcite peptone water, and at various periods of its growth the culture was pla'ed out on isodulcite agar containing bile salt.

RESULTS, WITH DESCRIPTION OF "REVERSION" PHENOMENON.

3 days' growth (full acid reaction). Plates yielded mainly white colonies on the first day of incubation but a few had an intense red colour. On the second day all the colonies were intense red. On the third day white papillae were appearing on the surface of certain of the red colonies. The papillae attained fairly large dimensions as incubation proceeded.

5 days' growth. Plates sown from the culture at this date, yielded mainly white colonies after 24 hours' incubation, with however a few intense red colonies. On the second day all the colonies had an intense red colour. On the third day white papillae appeared on certain of the red colonies.

7 days' growth. Plates at the end of 24 hours' incubation showed a mixture of red and white colonies. On the third day, all the colonies were red and papillae had appeared on some of these. It was observed that those colonies which developed an intense red colour in 24 hours, yielded no papillae.

10 days' growth. Plates yielded after 24 hours' incubation almost solely red colonies with only a few whites. After 48 hours, all the colonies were red. On the ninth day a minority of the red colonies had developed papillae, the proportion being 5 papillated to 30 non-papillated.

13 days' growth. Plating of one loop of the culture gave only two colonies after 24 hours, the one intense red and the other white. After 48 hours both were red. On the fourth day the colony which last became red was studded with white papillae while the original red colony showed none.

14 days' growth. Plating of three large loops of culture yielded on the first day reds and whites in the proportion of 13 to 54. On the third day all were red, but only those colonies which last became red had developed white papillae. The early intense reds showed none.

17 days' growth. Plating of 1 c.c. of the culture gave no apparent growth after 24 hours but on the second day many colonies of the less intense red type appeared. The majority of the colonies were, however, still white. On the fourth day all the colonies had developed a red colour and papillae had appeared on most of them.

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Plating of the isodulcite fluid culture after this date yielded no growth. The whole contents of the tube were inoculated into broth which remained sterile. The isodulcite fluid culture thus lived 17 days but not 20 days.

We thus see that strain "A," which, on analysis of several colonies had been shown to give a full acid reaction on fluid isodulcite medium in 24 to 48 hours, exhibited parallel variations on plates, some of the colonies showing an intense red colour in 24 hours while the others developed the red colour only at the end of 48 hours' incubation. Full acidity on the plates was rarely longer delayed. The strain would ordinarily be regarded as a fairly prompt fermenter of isodulcite. Plating however showed that the strain could be split up into two distinct elements, viz. (1) producing acid very rapidly from isodulcite and (2) producing somewhat less acid and only after 48 hours' incubation. The colonies of (2) also had the property of developing secondary white colonies on the surface, sometimes in enormous numbers, whereas the early acid-formers invariably produced none. On the tenth day of growth on fluid isodulcite, plating showed a marked preponderance of type (1) or the non-papillae-producing type, but after this date, plating of the isodulcite fluid showed a decline of the intense acid-formers and a relative preponderance of the papillae-producing types.

These were the last viable survivors on the seventeenth day.

Analysis of the two types.

The colonies taken for examination were those obtained by plating of the isodulcite fluid on its thirteenth day of growth (see above), viz. (1) intense red non-papillated colony, (2) red colony with white papillae.

Exp. 1. The red central portion of (1) was touched with a needle, inoculated on ordinary broth and finally plated on isodulcite bile salt agar. Result: On the first day all the colonies were intense red. On the second day the colonies were becoming alkaline. No further change occurred except that the colonies showed colour alterations pointing to alkaline reaction. All the colonies remained smooth.

Exp. 2. A portion of the white periphery of the same colony (1) was touched and plated. Colonies exactly similar to those of Exp. 1 resulted.

Exp. 3. A white papilla from (2) yielded white colonies solely, some of which on the sixth day showed extremely minute secondary white papillae on their surface.

Exp. 4. The red portion of (2) yielded on the first day, white colonies only. On the second day many had turned red and were showing white papillae. On the sixth day there still remained a number of pure white colonies in addition to the red types with white papillae.

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FURTHER TESTING OF COLONIES FROM EXP. 3.

Four white colonies from this experiment were inoculated on isodulcite peptone water. For 17 days no trace of acid reaction occurred in any. Between the seventeenth and thirty-first days two of the tubes began to show an acid reaction but, on the thirty-first day (the last day of observation) the medium in two of the tubes remained unaltered.

We have therefore demonstrated the presence of three different elements in strain "A" with respect to action on isodulcite, viz. (1) an element fermenting the substance rapidly (in 24 hours) with intense acid production and subsequently rendering the medium alkaline; (2) an element fermenting the substance less rapidly (48 hours) and throwing off subvariants (3) which have no action whatever on isodulcite and are detected by their occurrence as white papillae on the surface of their red ancestors.

The two elements (1) and (2) were obtained in a pure state and the non-acid-producing subvariants (3) remained stable for the period of 31 days during which the observations were continued.

I do not propose to discuss this interesting type of variation further at this stage as I have access here only to extracts from my original notes, but I may add that the two final variants selected from the original strains "A" and "B" and used for immunisation of rabbits gave the following reactions on the three substances raffinose, isodulcite and arabinose. Period of observation on fluid media, 21 days.

	Raffinose	Isodulcite	Arabinose
Variant from "A"	A (24 hours)	—	A (48 hours)
Variant from "B"	A (48 hours)	—	A (48 hours)

This was the closest approximation obtained when the experiments were interrupted. A more complete approximation was prevented by the development on the part of "A" of a tendency to throw off lactose variants and these lactose elements could not so far be eliminated from the final "A" variant used for immunisation.

Sera prepared from the final variants gave the following reactions in cross-agglutination experiment. The reactions with the sera prepared by immunisation with the original strains, are also adduced.

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End-point readings after 24 hours	
Serum from "A" (original) v. "A" (original)	1 in 6400
Serum from "B" (original) v. "B" (original)	1 in 6400
Serum from "A" (original) v. "B" (original)	1 in 200
Serum from "B" (original) v. "A" (original)	1 in 400
Serum from A′ (final variant) v. A′	1 in 12800
do. do. do. v. B′	1 in 400
Serum from B′ (final variant) v. B′	1 in 25600
do. do. do. v. A′	1 in 3200
Serum from "A" (original) v A′	1 in 3200
do. do. v. B′	1 in 100
Serum from "B" (original) v. B′	1 in 3200
do. do. v. A′	1 in 200

Thus, the sera prepared from the variants, though of higher titre than those prepared from the originals, behaved in a similar manner with respect to the variants, and absorption experiments not here detailed revealed little or no absorption of the specific agglutinins of the variant by digestion of its homologous serum with the heterologous final variant.

SUMMARY AND CONCLUSION.

Two strains of *B. dysenteriae* isolated from cases of asylum dysentery, were found to be readily agglutinable with stock Flexner-Y serum but serum prepared from the one had only a feeble action on the other. Absorption experiments revealed a like specificity. They differed in certain particulars in their action on certain carbohydrate media, and the attempt was made to obtain, by various procedures, variants from each strain giving fermentation reactions as alike as possible.

In the course of this work, an interesting and probably hitherto undescribed type of variation was met with, viz. the capacity exhibited by one strain which fairly promptly fermented the substance employed (isodulcite), of throwing off variants having no action on the substance. These variants appeared as white papillae on the surface of the original red ancestor and the description and analysis of this "reversion" process form the main interest of this paper. Though a fairly close approximation in fermentation characters was realised by selection of variants from each of the original strains, little or no approximation was apparent in respect of the serological affinities of these final variants.

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