A GAS-PRODUCING VARIETY OF *BACTERIUM ALKALESCENS* (ANDREWES)

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In the autumn of 1934 I received from Dr A. R. D. Adams, Superintendent of the Bacteriological Laboratory at Mauritius, 163 cultures of organisms. These had been isolated by him from the faeces of the inmates of the Beau Bassin Prison and Reformatory in a research on the intestinal flora and fauna of the general population in that island. In the course of the bacteriological examination the specimens of faeces had been cultured on plates of Endo's medium and incubated for 24 hours, at the end of which time non-lactose-fermenting organisms were picked off. A number of these strains were identified by Dr Adams as belonging to the typhoid, paratyphoid, or dysentery groups. One hundred and sixty-three strains which did not fall into these groups were sent to me for investigation.

It is well known that the classification of bacilli commonly called "non-lactose or slow lactose fermenters" is very unsatisfactory. Many have not been classified.

But in the course of this investigation twenty-one strains of an organism were found which by reason of its similarity in cultural and biochemical characteristics and of its close serological relationship to *B. alkalescens* (Andrewes) is of some theoretical interest and possibly of practical importance.

Morphology and cultural characteristics

Each of the twenty-one strains proved to be a Gram-negative bacillus morphologically similar to the majority of bacilli belonging to the colon, typhoid, and dysentery groups. It was found, however, that every one of the twenty-one strains was motile. The motility was well shown in young cultures in peptone water and, in addition, flagella were demonstrated in films stained by Kirkpatrick's method. On agar plates after 24 hours' incubation similar colonies were formed by all of the twenty-one strains. They were circular colonies with clear-cut edges and were like those of *B. alkalescens* though rather more glossy in appearance. On litmus lactose agar the colonies were blue and resembled those of *B. alkalescens*: with further growth the edges of the colonies remained clear-cut and the centres tended to become white.

Though minor differences, to which reference will be made, were observed, the biochemical reactions of this organism were very constant for all the twenty-

https://doi.org/10.1017/S0022172400043709 Published online by Cambridge University Press

one strains and are shown in Table I in which the reactions of *B. alkalescens* (Lister Institute strain) are also shown for comparison.

Table I. *Biochemical reactions of bacillus resembling* B. alkalescens. *Reactions of* B. alkalescens *shown for comparison*

	Lactose	Glucose	Mannite	Maltose	Saccharose	Dulcite	Milk	Indol	Haemolysis	Gelatin	Motility
Bacillus resembling *B. alkalescens* (Andrewes)	−a.g.	a.g.	a.g.	a.g.	alk.	$\frac{\text{a.g.}}{\text{alk.g.}}$	$\frac{\text{a.}}{\text{alk.}}$	+	+	−	+
B. alkalescens (Andrewes) (Lister Institute strain)	alk.	a.	a.	a.	alk.	a.	alk.	+	+	−	−

a.g. = formation of acid and gas.
−a.g. = late formation of acid and gas.
a. = formation of acid only.
alk. = formation of alkali.
$\frac{\text{a.}}{\text{alk.}}$ = acidity followed by alkalinity.
$\frac{\text{a.g.}}{\text{alk.g.}}$ = acidity and gas formation followed by alkalinity.

The reactions of this organism in the various media employed were observed for a period of 10 days. Certain of these reactions were constant. In every case acid and gas were formed in glucose, mannite, and maltose in 24 hours and this reaction remained unchanged. Saccharose was not fermented. Dulcite showed acid and gas formation in 24 hours but on the 5th or 6th day the acidity gave place to an alkalinity which increased. The reactions obtained in lactose showed considerable variation. In nineteen of the twenty-one strains there was late fermentation in lactose, acid and gas being produced on the 5th, 6th, 7th, or 8th days, which reaction remained unchanged. In the case of two strains, however, lactose was not fermented, and at the end of 10 days the reaction was unchanged.

A variable reaction was also obtained in milk. In the case of four strains after a preliminary acidity the milk became alkaline and remained so for 10 days, but in the remaining seventeen strains on the 7th, 8th, or 9th days the alkalinity gave place to acidity. All the twenty-one strains were haemolytic and all produced indol.

It will be seen that in certain respects these reactions strongly resemble those of *B. alkalescens*. Glucose, mannite and maltose are attacked by both though in the latter case no gas is formed. Both produce indol in peptone water and both are haemolytic. *B. alkalescens* differs in its non-effect upon lactose, in the characteristic strongly alkaline reaction produced in milk and in the fact that it is non-motile.

The production of haemolysis appears to be an important reaction. Using sheep's red cells Topley & Wilson found that *B. alkalescens* produced a slight degree of haemolysis. With human red cells all strains of *B. alkalescens* examined by me in 1934 produced marked haemolysis and the same result was obtained

with each of the twenty-one strains of this group. Indeed, others of the 163 strains showed similar biochemical reactions but they were non-haemolytic and failed to show any antigenic relationship.

AGGLUTINATION REACTIONS

An agglutinating serum was prepared against one of the twenty-one strains (B 4392) by the intravenous inoculation of a rabbit. An injection of 100 millions of a saline suspension of living organisms from a young agar culture was made with subsequent doses of 200 and 500 millions at intervals of 5 days. By this means an antiserum of sufficiently high titre was produced. It was found that this serum agglutinated a saline suspension from a live agar culture of the homologous organism in a dilution of 1 in 10,000. The serum agglutinated equally well all the remaining twenty strains. It was remarkable that all were agglutinated in similar suspensions to an almost uniform titre of 1 in 10,000. In view of the fact that all these organisms were motile alcoholized suspensions were made in order to eliminate flagellar agglutination. The growths on agar slopes were removed with absolute alcohol, kept as thick suspensions in 50 per cent. alcohol, and suitably diluted with saline when required. The serum agglutinated these suspensions to the same titre. In performing these agglutination tests incubation was done in a water bath at 52° C. for 24 hours (which period was recommended by Andrewes for *B. alkalescens*) for, otherwise, the reactions were not complete.

In order to demonstrate flagellar agglutination a series of seven or eight subcultures at frequent intervals was made in beef broth and the final culture was divided into equal parts. One part was heated at 75° C. for 20 min. A comparison of the agglutination reactions with antiserum B 4392 in various dilutions was made using both heated and unheated antigens. After 2 hours' incubation in the water bath flagellar agglutination was observed in the tubes containing the unheated antigen, and at the same time the fine granular agglutination of somatic type was just starting in the tubes containing the unheated antigens. The same procedure was carried out with five others of the twenty-one strains and in each case a similar result was obtained.

In addition these twenty-one organisms were also tested with various other agglutinating sera. Amongst those employed were sera prepared against the V, W, X, Y, Z, strains of *B. dysenteriae* Flexner, kindly supplied by Dr A. D. Gardner, serum *B. dysenteriae* Sonne, serum *B. alkalescens*, serum *B. dispar*, and a serum prepared against an organism B 5659. This organism B 5659 is a non-motile bacillus isolated by Dudgeon from a case of acute infection of the urinary tract and belongs to the slow-lactose-fermenting group (atypical *B. coli*). Agglutination was obtained only with serum *B. alkalescens* and to a small extent with serum *B. Flexner* X. All the other sera which were tried gave completely negative results. As all of the twenty-one strains gave almost precisely similar results the reactions of the strain B 4392 alone are shown

here. It was also found that antiserum B 4392 agglutinated suspensions of *B. alkalescens* in high titre. The reciprocal cross-agglutination between these two organisms is shown in Table II.

Table II. *Showing the cross-agglutination reactions between* B. alkalescens *and B* 4392

Dilution of serum ...	50	100	250	500	1000	2000	5000	10,000	Antigen
Antiserum *B. alkalescens*	I.C.	I.C.	I.C.	I.C.	I.C.	M.	—	—	*B. alkalescens*
	I.C.	I.C.	C.	C.	C.	C.	M.	Tr.	B 4392
	I.C.	I.C.	C.	C.	C.	C.	M.	Tr.	B 4392 (alcoholized)
Antiserum B 4392	C.	C.	C.	C.	C.	I.C.	I.C.	M.	*B. alkalescens*
	I.C.	I.C.	I.C.	I.C.	I.C.	M.	M.	—	B 4392
	C.	C.	C.	C.	C.	C.	C.	M.	B 4392 (alcoholized)

C. = complete sedimentation. M. = marked agglutination.
I.C. = incomplete sedimentation. Tr. = trace.

So striking were the results (shown in Table II) obtained by cross agglutination between *B. alkalescens* and B 4392 representing the twenty-one strains of an organism showing such differences in biochemical reactions that it was considered possible that B 4392 was a motile gas-producing variety of *B. alkalescens*. Absorption tests were therefore undertaken.

Antiserum *B. alkalescens* (homologous titre 1 in 4000) was diluted fifty times. A portion of this diluted serum was reserved for control purposes and the remainder absorbed with an equal quantity of a thick suspension of B 4392. The agglutination reactions after absorption as compared with those of the control serum are shown in Table III.

Table III. *Showing effects of absorbing antiserum* B. alkalescens *with B* 4392

Dilution of serum ...	100	250	500	1000	2000	4000	Antigens
Control antiserum *B. alkalescens*	C.	C.	C.	C.	C.	Tr.	*B. alkalescens*
	C.	C.	C.	C.	C.	M.	B 4392
Antiserum *B. alkalescens* absorbed with B 4392	M.	Tr.	—	—	—	—	*B. alkalescens*
	M.	Tr.	—	—	—	—	B 4392

Antiserum B 4392 (homologous titre 1 in 10,000) was also diluted fifty times. A portion of this diluted serum was reserved as control and the remainder absorbed with an equal quantity of a thick suspension of *B. alkalescens*. In testing the agglutination reactions in the case of B 4392 cultures in Dreyer's veal broth were employed as antigens, since it had been found that flagellar agglutination was better demonstrated in fluid media. Table IV shows the agglutination reactions after absorption as compared with those of the control serum.

From Table III it is evident that absorption of antiserum *B. alkalescens* with B 4392 removes practically completely the agglutinins both for the homologous organism and for B 4392. It is also shown in Table IV that by saturating the serum made from the motile organism B 4392 with a suspension

of *B. alkalescens* which is non-motile, the somatic agglutinins were removed but some agglutination, flagellar, remained. This is shown by the effect of heating the veal broth culture of B 4392 at 75° C. for 20 min. Absorption of antiserum B 4392 with *B. alkalescens* removes practically completely the somatic agglutinins for both organisms.

Table IV. *Showing the effect of absorbing antiserum B* 4392 *with* B. alkalescens

Dilution of serum ...	100	250	500	1000	2000	5000	10,000	Antigen
Control antiserum B 4392	C.	C.	C.	C.	C.	I.C.	M.	*B. alkalescens* (agar suspension)
	C.	C.	C.	C.	C.	C.	I.C.	B 4392 (Dreyer's veal broth)
	C.	C.	C.	C.	C.	C.	I.C.	B 4392 (Dreyer's veal broth heated 75° C. for 20 min.)
Antiserum B 4392 absorbed with *B. alkalescens*	Tr.	—	—	—	—	—	—	*B. alkalescens* (agar suspension)
	M.	M.	M.	M.	M.	Tr.	Tr.	B 4392 (Dreyer's veal broth)
	M.	Tr.	—	—	—	—	—	B 4392 (Dreyer's veal broth heated 75° C. for 20 min.)

It is concluded that these twenty-one strains are identical strains of one organism—a motile gas-forming variety of *Bacterium alkalescens* (Andrewes). They were isolated from the faeces of individuals who were not suffering from disease at the time. It is possible that this organism may be found in the excreta of persons living in this country, but up to the present time I have not encountered it.

ACKNOWLEDGEMENTS. I wish to thank Dr A. R. D. Adams for the cultures and Mr G. H. Trayford for assistance in this investigation.

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(*MS. received for publication* 6. v. 1936.—Ed.)