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ENVIRONMENTALLY INDUCED CHANGES IN *SALMONELLA* CULTURES

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INTRODUCTION

The recognition of *Salmonella cholerae suis* as a pathogen was followed by the isolation of related organisms to which confusing names were assigned. It was only after Andrews (1922, 1925) had elucidated the diphasic nature of flagellar antigens and White (1925, 1926, 1929) discovered S–R variation of somatic antigens, that a system of classification was adopted. The Kauffmann-White Diagnostic Scheme, introduced by White and modified by Kauffmann (1950, 1954) was accepted by the *Salmonella* Sub-committee of the International Congress of Microbiology (1934, 1940) thus recognising antigenically distinguishable serotypes. This scheme had to be supplemented from time to time (1957, 1958, 1959 a and b, 1960) due to the large number of *Salmonella* serotypes subsequently isolated. It forms the basis of modern typing in conjunction with the variation in biochemical characteristics.

No evidence of intertype mutations existed until the publication of the results of induction experiments by Stocker, Zinder & Lederberg (1953) and Baron's (1957) successful addition of somatic antigens to *Salmonella* by bacteriophage mediated transduction. Quadling & Stocker (1956, 1962) and Weiner & Swanson (1960) induced transition from flagellated to non-flagellated variants in *S. typhimurium* and *S. typhi*, respectively. William Smith (1955a, b, 1956 a, b), Gordon (1959) and Harbourne (1957) developed R-variants of *S. gallinarum* for immunogenic purposes.

Taylor, Lee, Edwards & Ramsay (1960) reported a third flagellar phase which was irreversibly changed to the diphasic state.

Similarity in behaviour of different *Salmonella* cultures with a complex antigenic structure was found by Douglas & Edwards (1962) to be due to a loss of a major antigenic compound rather than to a change from the third phase to the diphasic or monophasic forms.

In the light of the continued description of new serotypes the phenomenon of variation seems worthy of further investigation.

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Materials and Methods

Twenty-two strains of *Salmonella typhimurium* (6), *Salmonella gallinarum* (10), and *Salmonella dublin* (6) obtained from outbreaks of fowl typhoid and calf paratyphoid in various localities, were used in this investigation. The cultures were identified by serological and biochemical methods. Motility was determined microscopically (hanging drop) and by culturing in semi-solid agar. The presence of H-antigens was determined serologically.

Salmonella stock cultures obtained from Kauffmann, kept freeze-dried or in sterile wax-sealed agar tubes were used for preparation of diagnostic antiserum.

Media:—

(1) Serum agar.

(2) Nutrient agar.

(3) Broth (pH 5·5 and 7·5).

(4) In addition the following *non-metabolite media* were used:—

(*a*) A mixture of Bayol F and Arlacel A in the proportion of 9: 1 by volume.

(*b*) A liquid medium containing—

KH_2PO_4	1·0 g
$Na_2H.PO_4.12H_2O$	6·2 g
$Na_3C_6H_5O_7.2H_2O$	1·5 g
NH_4Cl	1·0 g
$MgSO_4.7H_2O$	0·01 g
$CaCl_2$	0·0004 g
$CuSO_4$	0·0001 g
$ZnSO_4$	0·0001 g
$FeC_6H_5O_7.3H_2O$	0·0006 g
Glucose	1·0 g
Water (dist.)	1 litre.

(*c*) A solid medium containing the same constituents as 4 (*b*) except that 2 per cent agar was added.

Cultivation.—Subcultures on nutrient agar, serum agar, solid non-metabolite (4*c*) and in broth (pH 5·5 and 7·5) and liquid non-metabolite 4 (*b*) were made at 8 and 24 hour intervals, respectively, and incubated at 44°C. Cultures of *S. gallinarum* in Bayol F and Arlacel A and non-metabolite media 4 (*b*) kept at room temperature were subsequently cultured on serum agar and in broth at weekly intervals. After 21 subcultures in broth at pH 5·5, strains of *Salmonella gallinarum* were transferred to non-metabolite 4 (*b*) and subcultured on nutrient agar, at weekly intervals. Biochemical and agglutination tests were carried out after every subculture.

Results

Large colonial variants were isolated from *S. dublin* and *S. gallinarum* strains, cultured at 8 and 24 hour intervals on nutrient agar incubated at 44°C (about one-third of which became mucoid). The majority of the remaining strains retained their antigenic and biochemical characteristics as well as their virulence even after 150 subcultures.

One of the *S. dublin* strains, showed a change in the somatic (O) as well as the flagellar (H) antigens with simultaneous fermentation of inositol (see Table 1).

Subcultures of *S. gallinarum* at 8 and 24 hour intervals resulted in no growth at all on the solid non-metabolite medium and a very poor growth in the liquid non-metabolite medium. When left in the latter medium for four to five days a progressive growth was obtained. Subcultures in the liquid medium at weekly intervals revealed the following changes in morphology, antigenicity and biochemical properties:—

(1) Two strains showed a change in morphology from a small, scanty to a large, sumptuous growth with acid as well as gas in glucose, dulcitol, mannitol, maltose, arabinose, rhamnose and trehalose. They were H_2S positive (T.S.I. agar), lactose and sucrose negative, retained their original O-antigens (1.9.12) but became actively motile:—

(*a*) One of the two with newly acquired motility did not agglutinate in any of the known H-antisera (1.9.12:—?). Only a small percentage of bacteria was actively motile while in the majority motility was limited to rotation movements (Table 2).

(*b*) The other showed the specific phase only (1.9.12: gq—). (Table 3).

(2) Three strains showed in addition to the already mentioned morphological and biochemical changes, an alteration of the O-antigens as well as an acquisition of H-antigens, viz.—

(*a*) (1.9.12:—) to (1, 4, 5, 12: i: 1, 2) (Table 4).

(*b*) (1.9.12:—) to (1, 4, 5, 12: gq, i: 1, 2) (Table 5).

(*c*) (1.9.12:—) to (1, 4, 5, 12: i:—)

In Arlacel A and Bayol F, strains of *S. gallinarum* plated on agar at weekly intervals, showed a change in colonial morphology (small to large) after the second week but they were antigenically and biochemically still specific. A very poor growth was obtained at the fifth week when acid was produced in glucose only and a weakened agglutination in O-antisera 9 and 12 was observed. At the sixth week a better growth was obtained with acid and gas production in glucose, dulcitol, mannitol, maltose, arabinose, rhamnose and sorbitol as well as in inositol, but not in trehalose. This culture was H_2S positive (T.S.I. agar), lactose and sucrose negative with newly acquired motility and its O-antigenics tructure as follows: (1, 4, 5, 9, 12:).

The seventh week's subculture revealed large and small colonies, serologically and biochemically identical, homogeneous very large rods with active hypermotility and pronounced 4 and negligible 9 O-antigens accompanied by i: 1, 2 H-antigens plus loss of virulence for fowls. After eight subcultures on nutrient agar at 37°C both large and small colonies retained their newly acquired characteristics, viz.:—

(1) Motility.

(2) Inositol+.

(3) Trehalose—.

(4) Antigenic formula: (1, 4, 5, 12: i: 1, 2).

(5) Loss of virulence (Table 6).

TABLE 1.—Salmonella dublin *strain* 2654, *daily subcultured at* 44°C

No Subcultures	Morphology	Antigens							Biochemical reactions												Motility	Changes
		O					H															
		1	4	5	9	12	1st Phase	2nd Phase	G	L	D	S	Mu	Ml	Ar	Rh	In	S	T	H_2S		
1	Smooth large	2+	–	–	4+	4+	gp	–	+	–	+	–	+	+	+	+	–	+	+	+	+	
72	Smooth large	1+	2+	2+	2+	4+			+	–	+	–	+	+	+	+	–	+	+	+	+	(1) 1.9.12: gp–to 1.4.5. 12: 1.5
80	Smooth large	2+	4+	2+	±	4+			+	–	+	–	+	+	+	+	+	+	+	+	+	(2) Inositol – to inositol +
90	Smooth large	2+	4+	2+	±	4+	–	1·5	+	–	+	–	+	+	+	+	+	+	+	+	+	
140		2+	4+	2+	–	4+	–	1·5	+	–	+	–	+	+	+	+	+	+	+	+	+	

TABLE 2.—Salmonella gallinarum *R2–1–21 subcultures in broth pH* 5·5 *then weekly subcultured in Inorganic Salt Medium*

No Subcultures	Morphology	Antigens							Biochemical reactions												Motility	Changes
		O					H															
		1	4	5	9	12	1st Phase	2nd Phase	G	L	D	S	Mu	Ml	Ar	Rh	In	S	T	H_2S		
1	S.S.S.....	2+	±	±	4+	4+	–	–	×	–	×	×	×	×	×	×	–	×	×	+		(1) O 1.9.12:—to O 1.9.12: motile (H?).
4	S.L.....	2+	–	–	4+	4+	–	–	+	–	+	–	+	+	+	+	–	+	+	+		(2) Acid only to acid and gas in glucose, dulcitol, mannitol, maltose, arabinose, rhamnose, sorbitol and trehalose.
8	S.L.....	1+	1+	1+	4+	4+	–	–	+	–	+	–	+	+	+	+	–	+	+	+	+	(3) 10th subculture—04, 5, 9 and 12 agglutination equally strong, without acid and gas in arabinose and inositol.
9	S.L.....	1+	–	–	4+	4+	–	–	+	–	+	–	+	+	+	+	–	+	+	+	+	(4) Smooth, small scanty to smooth and large colonies.
10	S.L.....	1+	2+	2+	2+	2+	not tested yet		+	–	+	–	+	+	–	+	–	+	+	+	RO++	

(1) Morphology:— S.S.S. = Smooth, small, scanty.
S.S. = Smooth, small.
S.L. = Smooth, Large.
L + S = Large + Small.
L = Large.
S to L.S. = Small to Large Smooth.
R.O. = Rotation movements.

(2) O-agglutination: 1 + = weak.
2 + = strong.
4 + = very strong and quick.

(3) Biochemical reactions:—
G = glucose
S = sucrose
Ar = arabinose
S = sorbitol
– = no fermentation
× = acid only
+ = acid and gas.

L = lactose
Ml = maltose
Rh = rhamnose
T = trehalose

D = dulcitol
Mu = mannitol
In = Inositol

TABLE 3.—Salmonella gallinarum 1 *R* (2)–21 *subcultures in broth pH* 5·5, *then weekly subcultured in Inorganic Salt Medium*

No Subcultures	Morphology	Antigens							Biochemical reactions												Motility	Changes
		O					H															
		1	4	5	9	12	1st Phase	2nd Phase	G	L	D	S	Mu	Ml	Ar	Rh	In	S	T	H_2S		
1	S.S.S.....	2+	±	±	4+	4+	–	–	×	–	×	–	×	×	×	×	–	×	×	+		(1) Smooth, small scanty to smooth large, to smooth, small to smooth large.
4	S.L.....	2+	–	–	4+	4+	–	–	+	–	+	–	+	+	+	+	–	+	+	+		(2) O 1.9.12:—to O 1.9.12: gq—(non-motile to motile).
8	S.L.....	1+	1+	1+	4+	4+			+	–	+	–	+	+	+	+	–	+	+	+	+	(3) Acid only to acid and gas in glucose, dulcitol, mannitol, maltose, arabinose, rhamnose, sorbitol and trehalose.
9	S.S.....	1+	–	–	4+	4+	gq	–	+	–	+	–	+	+	+	+	+	+	+	+	+	(4) 10th subculture shows a stronger agglutination in O4 antiserum (continued).
10	S.S. to S.L..	1+	2+	1+	4+	4+	not tes	ted yet	+	–	+	–	+	+	+	+	–	+	+	+	+	

(1) Morphology:—
S.S.S. = Smooth, small, scanty.
S.S. = Smooth, small.
S.L. = Smooth, Large.
L + S = Large + Small.
L = Large.
S to L.S. = Small to Large Smooth.
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1 + = weak.
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L = lactose
Ml = maltose
Rh = rhamnose
T = trehalose

D = dulcitol
Mu = mannitol
In = Inositol

TABLE 4.—Salmonella gallinarum *strain* 5503–21 *subcultures in broth pH* 5·5, *then weekly subcultured in Inorganic Salt medium*

No Subcultures	Morphology	Antigens							Biochemical reactions												Motility	Changes
		O					H															
		1	4	5	9	12	1st Phase	2nd Phase	G	L	D	S	Ml	Mu	Ar	Rh	In	S	T	H_2S		
1	S.S.S.....	3+	–	–	4+	4+	–	–	×	–	×	–	×	×	×	×	–	×	×	+	–	(1) Small, smooth, scanty to large luxurious growth.
2	L.....	1+	1+	1+	4+	4+	–	–	×	–	×	–	×	×	×	×	–	×	×	+	–	(2) O 1.9.12: to O 1.4.5. 12;
4	L.....	1+	4+	1+	1+	4+	–	–	+	–	+	–	+	+	+	+	+	+	+	+	+	(3) H—to i (gq): 1, 2.
7	L.....	1+	2+	2+	2+	3+			+	–	+	–	+	+	+	+	+	+	+	+	+	(4) Acid only to acid and gas in glucose, dulcitol, maltose, mannitol, arabinose, rhamnose, sorbitol and trehalose.
8	L + S.....	1+	4+	4+	1+	4+			+	–	+	–	+	+	+	+	+	+	+	+	+	(5) Acid and gas in inositol.
9	L.....	1+	4+	2+	±	4+	i (gq)	1, 2	+	–	+	–	+	+	+	+	+	+	+	+	+	(6) Virulent to non-virulent.

(1) Morphology:— S.S.S. = Smooth, small, scanty.
S.S. = Smooth, small.
S.L. = Smooth, Large.
L + S = Large + Small.
L = Large.
S to L.S. = Small to Large Smooth
R.O. = Rotation movements

(2) O-agglutination: 1 + = weak.
2 + = strong.
4 + = very strong and quick.

(3) Biochemical reactions:—
G = glucose
S = sucrose
Ar = arabinose
S = sorbitol
– = no fermentation
× = acid only
+ = acid and gas.
L = lactose
Ml = maltose
Rh = rhamnose
T = trehalose
D = dulcitol
Mu = mannitol
In = Inositol

TABLE 5.—Salmonella gallinarum *strain* 5677–21 *subcultures in broth pH* 5·5, *then weekly subcultured in Inorganic Salt Medium*

No Subcultures	Morphology	Antigens							Biochemical reactions												Motility	Changes
		O					H															
		1	4	5	9	12	1st Phase	2nd Phase	G	L	D	S	Ml	Mu	Ar	Rh	In	S	T	H_2S		
1	S.S.S.....	2+	–	–	4+	4+	–	–	×	–	×	–	×	×	×	×	–	–	×	+		(1) O 1.9.12—O 1.4.5 12: i: 1, 2.
2	L.....	1+	1+	1+	4+	4+	–	–	×	–	×	–	×	×	×	×	–	–	×	+		(2) Non-motile to motile (H—to i: 1, 2).
4	L.S.....	1+	4+	2+	1+	4+			+	–	+	–	+	+	+	+	+	+	+	+	+	(3) Acid only to acid and gas in glucose, dulcitol, mannitol, maltose, arabinose, rhamnose and trehalose.
7	L.S.....	1+	4+	4+	1+	4+			+	–	+	–	+	+	+	+	+	+	+	+	+	
8	S to L.S....	1+	4+	2+	1+	4+	i	1,2	+	–	+	–	+	+	+	+	+	+	+	+	+	(4) Acid and gas in inositol and sorbitol.

(1) Morphology:—
S.S.S. = Smooth, small, scanty.
S.S. = Smooth, small.
S.L. = Smooth, Large.
L + S = Large + Small.
L = Large.
S to L.S. = Small to Large Smooth.
R.O. = Rotation movements.

(2) O-agglutination:
1 + = weak.
2 + = strong.
4 + = very strong and quick.

(3) Biochemical reactions:—
G = glucose
S = sucrose
Ar = arabinose
S = sorbitol
– = no fermentation
× = acid only
+ = acid and gas.

L = lactose
Ml = maltose
Rh = rhamnose
T = trehalose

D = dulcitol
Mu = mannitol
In = Inositol

TABLE 6.—Salmonella gallinarum, *strain* 6128, *weekly subcultured in Arlacel and Bayol F*

No Subcultures	Morphology	Antigens							Biochemical reactions												Motility	Changes
		O					H															
		1	4	5	9	12	1st Phase	2nd Phase	G	L	D	S	Ml	Mu	Ar	Rh	In	S	T	H_2S		
1	S.S.S.....	4+	–	–	4+	4+	–	–	×	–	×	–	×	×	×	×	–	–	×	+	–	(1) Small, scanty, smooth large, rough to large, sm and small smooth.
2	R.L.....	2+	1+	1+	4+	4+	–	–	×	–	×	–	×	×	×	×	–	–	×	+		(2) O 1.9.12: to O 1.4.5.
5	R.L. (poor)..	1+	1+	1+	2+	2+	–	–	×	–	×	–	×	×	×	×	–	–	×	+	–	(3) Non-motile to motile. H H i: 1, 2.
6	S.L.....	1+	4+	1+	2+	4+			×	–	–	–	–	–	–	–	–	–	–	–		(4) Acid in glucose, dul mannitol, maltose, a nose rhamnose and treh to acid and gas in glu dulcitol, mannitol, mal arabinose, rhamnose, ino and sorbitol, but no trehalose.
7	S.L.....	1+	4+	1+	±	4+	i	1·2	+	–	+	–	+	+	+	+	+	+	–	+	++	
	S.S.....	1+	2+	1+	±	1+	i	1·2	+	–	+	–	+	+	+	+	+	+	–	+	++	
	1 × 8 S.L...	1+	4+	2+	–	4+	i	1·2	+	–	+	–	+	+	+	+	+	+	–	+	++	
	2 × 8 S.S....	1+	4+	2+	–	2+	i	1·2	+	–	+	–	+	+	+	+	+	+	–	+	+	(5) Virulent to non-viru (fowl).
																						(6) Pleomorph to homogen large rods to pleomorpl

(1 Morphology:— S.S.S. = Smooth, small, scanty.
S.S. = Smooth, small.
S.L. = Smooth, Large.
L + S = Large + Small.
L = Large.
S to L.S. = Small to Large Smooth.
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(2) O-agglutination: 1 + = weak.
2 + = strong.
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(3) Biochemical reactions:—
G = glucose
S = sucrose
Ar = arabinose
S = sorbitol
– = no fermentation
× = acid only
+ = acid and gas.

L = lactose
Ml = maltose
Rh = rhamnose
T = trehalose

D = dulcitol
Mu = mannitol
In = Inosito

Discussion

The morphological, biochemical and antigenic changes described above occurred only after long exposure of the *Salmonella* organisms to the conditions as obtained in the non-metabolite media. As an explanation for this phenomenon the assumption can be made that adaptation to the new environment took place after the available conventional nutrients had been exhausted.

Whether alterations in characteristics are due to adaptation or selection of mutants is still to be established. It would appear that acquisition of motility is followed by changes in somatic antigens, cultural and biochemical properties. Whatever the consecutive order, population changes due to a changing environment are brought about gradually and in stages due to the occurrence of transitional variants. To what extent transitional variants account for the occurrence of new *Salmonella* serotypes is not known.

If these changes can be brought about experimentally one would assume that they could also occur in nature. The possibility does exist that serotype specificity could develop under specific favourable environmental conditions. In an attempt to clarify this point further studies are now in progress.

Summary

Exposure to unfavourable conditions resulted in an alteration of characteristics in certain strains of *Salmonella:*—

(1) Different strains of *Salmonella gallinarum* yielded motile variants showing various H-antigens. In addition somatic antigenic components, not detectable in the parent strains, accompanied by altered morphological and biochemical characteristics as well as virulence were demonstrated.

(2) Strains of *Salmonella dublin* showed a change of O- and H-antigens and acid and gas production in inositol.

It has not yet been established whether the new serotypes observed represent transitional variants of adaptive or mutant nature.

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REFERENCES

ANDREWS, F. W., 1922. Studies in group agglutination. i. The *Salmonella* group and its antigenic structure. *J. Pathol. Bacteriol.* 25, 505.

ANDREWS, F. W., 1925. Studies in group agglutination. ii. The absorption of agglutinin diphasic *Salmonellas*. *J. Pathol. Bacteriol.* 28, 345.

BARON, L. S., 1957. *Salmonella* antigen addition by Bacteriophage. *Virology*, 3, 417.

DOUGLAS, G. W. & EDWARDS, P. R., 1962. Complex flagellar phases in *Salmonella*. *J. Gen. Microbiol.* 367–372.

EDWARDS, P. R. & EWING, W. H., 1955. Identification of Enterobacteriaceae. Minnesota: Burgess Publishing Co.

GORDON, R. F., 1959. The use of living attenuated vaccines in control of fowl typhoid. *Vet. Rec.* 71, 300.

HARBOURNE, 1957. The control of fowl typhoid in the field by use of live vaccines. *Vet. Rec.* 69, 1102.

KAUFFMANN, F., 1950. The Diagnosis of *Salmonella* types. Springfield, Ill.: C. C. Thomas.

KAUFFMANN, F., 1954. Enterobacteriaceae. Second ed. Copenhagen: Munsgardt.

KAUFFMANN, F., 1957. A revised simplified Kauffmann-White Scheme. *Acta Pathol. Microbiol. Scand.* 41, 242.

KAUFFMANN, F., 1958. Supplement to the Kauffmann-White Scheme (I). *Acta Pathol. Microbiol. Scand.* 43, 247.

KAUFFMANN, F., 1959a. Supplement to the Kauffmann-White Scheme. *Acta Pathol Microbiol. Scand.* 45, 406.

KAUFFMANN, F., 1959b. Supplement to the Kauffmann-White Scheme. *Acta Pathol. Microbiol. Scand.* 45, 411.

KAUFFMANN, F., 1960. Supplement to the Kauffmann-White Scheme (III). *Acta Pathol. Microbiol. Scand.* 49, 89.

QUADLING, C. & STOCKER, B. A., 1956. Occurrence of motile cells, which are not mutants, in some *Salmonella* O-strains. *J. Gen. Microbiol.* 14, 1956.

QUADLING, C. & STOCKER, B. A., 1962. An environmental-induced transition from flagellated to non-flagellated state in *S. typhimurium*. *J. Gen. Microbiol.* 28, 257–270.

SALMONELLA SUB-COMMITTEE, 1934. *J. Hyg.* 34, 333.

SALMONELLA SUB-COMMITTEE, 1940. *Proc. 3rd. International Congr. Microbiology*, New York. p. 832.

SALMONELLA SUB-COMMITTEE, 1949. *Proc. 4th. International Congr. Microbiology*, Copenhagen, p. 607.

SMITH, H. WILLIAM, 1955a. Observations on experimental fowl typhoid. *J. Comp. Pathol.* 65, 37.

SMITH, H. WILLIAM, 1955b. The chemotherapy of experimental fowl typhoid in fowls. *J. Comp. Pathol.* 65, 55.

SMITH, H. WILLIAM, 1956a. The use of live vaccines in experimental *Salmonella gallinarum* infection in chickens with observations on their interference effect. *J. Hyg.* 54, 419.

SMITH, H. WILLIAM, 1956b. The immunity of *Salmonella gallinarum* infection in chickens produced by live cultures of members of the *Salmonella* genus. *J. Hyg.* 54, 433.

STOCKER, B. A. D., ZINDER, N. D. & LEDERBERG, J., 1953. Transduction of flagellar characters in *Salmonella*. *J. Gen. Microbiol.* 9, 410.

TAYLOR, J., LEE, M. M., EDWARDS, P. R. & RAMSAY, C. H., 1960. A new type of flagellar variation associated with new antigens in the *Salmonella* group. *J. Gen. Microbiol.* 23, 583.

WEINER, M. LAWRENCE & SWANSON, R., 1960. Antigens of chloramphenicol-resistant strains of *Salmonella typhosa*. *J. Bacteriol.* 79, 863.

WHITE, P. B., 1925. Med. Res. Council, Gt. Brit. *Spec. Rep. Ser.* no. 91.

WHITE, P. B., 1926. Med. Res. Council, Gt. Brit. *Spec. Rep. Ser. no.* 103.

WHITE, P. B., 1929. Med. Res. Council, Gt. Brit. *System of Bact.* 4, 86.