

centrations recommended in plasma ascorbic acid analysis. These observations indicate that there is no reason for the addition of potassium cyanide in the determination of plasma ascorbic acid by the methods mentioned.

## 10264

**Immunological Differentiation of *Shigella Alkalescens* from  
*Shigella paradysenteriae* Flexner, V, W, X, Y, and Z.**

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When Andrewes<sup>1</sup> described for the first time a new member of the *Shigella* group that is referred to today as *Shigella alkalescens*, his main purpose was to differentiate this microorganism from true dysentery bacilli. The purpose of this differentiation still holds true, since *Shigella alkalescens* does not cause epidemic or endemic forms of bacillary dysentery in man as does the closely related *Shigella paradysenteriae* Flexner. Both microorganisms show similarities, not only in cultural characters but also in antigenic structure: *Shigella alkalescens* may be agglutinated by anti-Flexner sera and anti-*Shigella alkalescens* sera may agglutinate certain strains of *Shigella paradysenteriae* Flexner as demonstrated by Andrewes,<sup>1</sup> Smith and Fraser,<sup>2</sup> Popoff and Spanswick,<sup>3</sup> Welch and Mickle,<sup>4</sup> Gilbert and Coleman,<sup>5</sup> Neter and Rappole,<sup>6</sup> and Neter.<sup>7, 8</sup> Recently, it was found that type-specific anti-*Shigella paradysenteriae* Flexner sera differ markedly in their content of cross-agglutinins for *Shigella alkalescens*. While anti-*Shigella paradysenteriae* Y sera strongly agglutinated *Shigella alkalescens*, and anti-*Shigella paradysenteriae* V and X sera contained a moderate titer of agglutinins for this microorganism, anti-*Shigella paradysenteriae* W and Z sera may lack agglutinins for *Shigella alkalescens* in spite of a high titer of

<sup>1</sup> Andrewes, F. W., *Lancet*, 1918, **194**, 560.

<sup>2</sup> Smith, J., and Fraser, A. M., *J. Path. and Bact.*, 1928, **31**, 511.

<sup>3</sup> Popoff, N. W., and Spanswick, M. P., *J. Lab. and Clin. Med.*, 1931, **16**, 437.

<sup>4</sup> Welch, H., and Mickle, F. L., *Am. J. Publ. Health*, 1934, **24**, 219.

<sup>5</sup> Gilbert, R., and Coleman, M. D., *Am. J. Publ. Health*, 1934, **24**, 449.

<sup>6</sup> Neter, E., and Rappole, F., *Arch. Path.*, 1938, **25**, 298.

<sup>7</sup> Neter, E., *J. Bact.*, 1938, **35**, 202.

<sup>8</sup> Neter, E., *J. Immunology*, 1938, **35**, 339.

agglutinins for the respective, homologous types of *Shigella paradysenteriae* Flexner. It could be shown that certain strains of *Shigella paradysenteriae* Flexner absorbed their cross-agglutinins from anti-*Shigella alkalescens* sera, and that such absorbed sera still agglutinated *Shigella alkalescens*. It is well known that it is not possible to absorb completely anti-*Shigella paradysenteriae* Flexner sera with heterologous types of the Flexner group. The possibility has to be considered, that *Shigella alkalescens* may contain several antigenic components in common with certain types of the Flexner group and that the absorption of anti-*Shigella alkalescens* serum with only one type of the Flexner group may not remove all anti-Flexner agglutinins. It was decided, therefore, to absorb anti-*Shigella alkalescens* sera with all 5 Andrewes-Inman types, V, W, X, Y, and Z of the Flexner group in order to determine whether *Shigella alkalescens* contains an antigen characteristic of itself, which differentiates it immunologically from *Shigella paradysenteriae*, V, W, X, Y, and Z of the Flexner group.

The experiment was set up as follows: Anti-*Shigella alkalescens* serum, in a dilution of 1:25 was mixed with the sediment of heavy suspensions of (a) *Shigella paradysenteriae* V; (b) *Shigella paradysenteriae* W; (c) *Shigella paradysenteriae* X; (d) *Shigella paradysenteriae* Y; (e) *Shigella paradysenteriae* Z; (f) a mixture of *Shigella paradysenteriae* V, W, X, Y, and Z, and (g) *Shigella alkalescens*. The sediment was suspended in the serum and the mixtures were kept in the ice chest for 3 hours. Frequently the sediment was stirred up. Then the tubes were centrifuged at high speed for one hour, until the supernate was clear. The supernates, as well as the non-absorbed native serum dilution were mixed with the 5 types of *Shigella paradysenteriae* V, W, X, Y, and Z as well as with a suspension of *Shigella alkalescens*. The tubes were incubated in a water bath at 55°C for 24 hours. The resulting agglutination was read macroscopically and noted at various intervals. The result of this experiment is recorded in Table I.

It may be seen from Table I that the native anti-*Shigella alkalescens* serum agglutinated *Shigella alkalescens*; it also caused agglutination of *Shigella paradysenteriae* V, X, and Y. After absorption of the serum with *Shigella alkalescens*, no agglutination occurred with any of the strains tested. The table, furthermore, shows that after absorption with the suspension of one type of *Shigella paradysenteriae* Flexner, the agglutinins for this type were completely removed; such an absorbed serum, however, still agglutinated more or less other types of the *Shigella paradysenteriae* group. It is evident from Table I that after absorption of the anti-*Shigella alka-*

TABLE I.  
Agglutination of *Shigella paradysenteriae* V, W, X, Y, Z and *Shigella alkalescens* by Native and Absorbed Anti-*Shigella alkalescens* Serum.

Anti- <i>Shigella alkalescens</i> Serum.										
I Native					II Absorbed with <i>S. alkalescens</i>					
<i>S. paradysenteriae</i>					<i>S. alkalescens</i>					
V	W	X	Y	Z	V	W	X	Y	Z	
+	-	+++	+++	-	++++	-	-	-	-	-
III Absorbed with <i>S. paradys.</i> V					IV Absorbed with <i>S. paradys.</i> W					
<i>S. paradysenteriae</i>					<i>S. alkalescens</i>					
V	W	X	Y	Z	V	W	X	Y	Z	
-	-	-	++	-	++++	+	-	+	++	-
										++++
V Absorbed with <i>S. paradys.</i> X					VI Absorbed with <i>S. paradys.</i> Y					
<i>S. paradysenteriae</i>					<i>S. alkalescens</i>					
V	W	X	Y	Z	V	W	X	Y	Z	
+	-	-	++	-	++++	++	-	-	-	-
										++++
VII Absorbed with <i>S. paradys.</i> Z					VIII Absorbed with <i>S. paradys.</i> V, W, X, Y, Z					
<i>S. paradysenteriae</i>					<i>S. alkalescens</i>					
V	W	X	Y	Z	V	W	X	Y	Z	
+	-	+	++	-	++++	-	-	-	-	-
										++++

- = No agglutination.

+, ++, +++, +++++ = Increasing degrees of agglutination.

*lascens* serum with any of the 5 types, V, W, X, Y, and Z of *Shigella paradysenteriae* Flexner, the absorbed serum still agglutinated *Shigella alkalescens* and—as quantitative titrations revealed—to about the same titer as the native, non-absorbed serum. When the anti-*Shigella alkalescens* serum was absorbed with a mixture of the 5 types, V, W, X, Y, and Z of *Shigella paradysenteriae*, all the agglutinins for *Shigella paradysenteriae* V, W, X, Y, and Z were removed and yet the serum still agglutinated *Shigella alkalescens* without any demonstrable loss in its titer. Essentially the same results were obtained in several experiments, employing 3 different anti-*Shigella alkalescens* sera.

In view of the observations of Boyd<sup>9</sup> and others, that the Flexner

<sup>9</sup> Boyd, J. S. K., *J. Hyg.*, 1938, **38**, 477.

group comprises additional types, which are antigenically different from the Andrewes-Inman types, V, W, X, Y, and Z, further experiments are necessary in order to determine whether or not antigenic relations exist between *Shigella alkalescens* and the remaining types of the *Shigella paradysenteriae* Flexner group.

In conclusion, *Shigella alkalescens* contains in addition to antigenic components in common with *Shigella paradysenteriae* Flexner, an antigen that is not shared by the Andrewes-Inman types V, W, X, Y, and Z of *Shigella paradysenteriae* Flexner and that apparently is characteristic of itself.

### 10265 P

#### Functional Activity of Pancreatic Ampulla in Rabbit.

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In rabbit, the pancreatic ampulla is entirely separate from the biliary ampulla and its functional activity has not been described as far as we know; moreover both ampullae may be studied at the same time. This structure is located about 15 cm distal to the bile ampulla and varies between 5 x 3 and 7 x 3 mm. After exposure under sodium barbital narcosis little or no activity is generally seen. Intravenous injection of a crude "secretin" mixture, not assayed for histamin, in 0.5 cc per kg doses brings about a series of events in the pancreatic ampulla that are similar in general to observations made on the biliary ampulla by Westphal in guinea pig, rabbit and cat<sup>1</sup> and by Burget and Brocklehurst in guinea pig.<sup>2</sup> After a latent period of 15-30 seconds, a contraction less than 0.5 mm in width appears at the junction of the pancreatic ampulla and duct. This blanched area then relaxes, but the contractions reappear with increased frequency and increased strength, often involving more of the duct, until finally a peristaltic wave of contraction beginning at the duct sweeps over the entire ampulla changing it into a grayish-white, linear, puckered mass. Such a complete contraction may persist for 1-2 seconds and then relaxation sets in. The relaxation betrays itself as a pink flush that generally proceeds from the distal

<sup>1</sup> Westphal, K., *Z. f. Klin. Med.*, 1923, **96**, 52.

<sup>2</sup> Burget, G. E., and Brocklehurst, E. J., *Am. J. Physiol.*, 1927-28, **88**, 578.