lysozyme (Armour Company) was submitted to conditions under which blood hydrolysates exhibit their optimal antibacterial activities with the result that contrary to the pH range of 7.8 to 9.0 in which our products resist autoclaving, lysozyme activities were destroyed regardless of pH (5.4-9.0). Furthermore, the stability of lysozyme in acid solution and its inactivation in alkaline solutions is contrary to the characteristics of our product. Although the specific identity of our antibacterial substance or substances has not been determined as yet, it is plausible that the ultimate products obtained from hemoproteins by hydrolysis are peptides or amino acids. In its present crude form, the material is most probably a hydrolysate complex rather than an entity. As to the antibacterial substances of animal origin reported by others and previously mentioned by us, the bacteriostatic preparation from liver obtained by Konikova *et al.*(3) by acid precipitation is water-insoluble and apparently differs from our product inasmuch as they obtained no bacteriostatic fractions from processed blood. A sample of "erythrin" made available to us by Dr. Waksman was highly acid and insoluble in water.

It becomes apparent from the present study that the bacterial growth inhibition *in vitro* compares with the phenomenon induced by antibiotics. This similarity refers particularly to the selective antibacterial action of the substances released from red blood cells, affecting primarily the gram-positive organisms. Considering this selectivity and the biological source of origin of our products, their inclusion into a broader concept of antibiotics seems to be justified. This would be also in line with the recognition of antibiotic products from higher plants(7).

Summary. Red blood cells of various animals were submitted to enzymatic hydrolysis in alkaline medium, the resulting products exhibiting *in vitro* marked antibacterial activities. The latter covered a relatively wide spectrum of most gram-positive and a few gram-negative organisms. The highest activity was displayed by hydrolysates of human and bovine hemoproteins. Hydrolyzed bovine hemoglobin powder likewise exhibited antibacterial properties. These antibacterial products are heat-resistant and water-soluble. Their optimum activity lies in the pH range of 7.0 to 8.0. It is assumed that the active principle of the crude substance is a peptide-amino acid complex. This complex proved to be nontoxic to guinea pigs and albino mice. Its relation to other antibiotics is discussed.

7. Symposium on Antibiotics. *J. Clin. Invest.*, 1949, v28, 894.

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Antigenic Identity of *Shigella alkalescens* Type I and Kauffmann's Escherichia O Group 1. (17903)

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Antigenic relationships between the coliform bacteria and members of the genus Shigella have been studied by many workers (1-5). The sharing of minor antigenic components by members of the two groups has been noted frequently, but complete antigenic identity has been demonstrated in only a few instances. The closest relationships to the coliform group have been found in *Shig-*

1. Carpenter, P. L., and Stuart, C. A., *J. Immunol.*, 1950, v64, 237.

2. Felsenfeld, O., and Young, V. M., *Am. J. Dig. Dis.*, 1945, v12, 396.

3. Ferguson, W. W., and Wheeler, W. E., *J. Bact.*, 1946, v51, 107.

4. Kauffmann, F., *Acta Path. et Microbiol. Scand.*, 1944, v21, 72.

5. Wheeler, K. M., Stuart, C. A., and Ewing, W. H., *J. Bact.*, 1946, v51, 169.

ella alkalescens and *Sh. dispar,* both of which are generally considered nonpathogenic. Stuart and his co-workers(6) described a series of strains which included typical *Escherichia coli,* anaerogenic and slow lactose-fermenting paracolons, and biochemically typical *Sh. alkalescens,* all with identical O antigens. Ewing(7) has described a similar biochemically intergrading series between *E. coli* and *Sh. dispar,* with indistinguishable O antigens. The systematic analysis of antigens shared by *E. coli* and other bacteria has been handicapped by the serological complexity of Escherichia, and the lack of a formal classification of the antigens of this genus. Such a classification has recently been compiled by Kauffmann(8). Strains representing his O groups 1 through 110 were sent by Dr. Kauffmann to this laboratory, and form the basis of the present study.

Methods and results. Only the heat-stable O antigens were studied in the present experiment. All antigens were prepared from 18 hour extract broth or agar cultures, and heated in flowing steam for one hour. Agglutination tests were incubated at 50°C for 18 hours. Antigens of type strains of all the Kauffmann O groups 1 through 110 were tested for agglutination in a 1:100 dilution of *Sh. alkalescens* Type 1 antiserum with a titer of 1:1600. The only strains which gave more than partial agglutination were those of O groups 1 and 31. Further tests showed that *Sh. alkalescens,* O 1 and O 31 were agglutinated to the same titers in antiserums prepared from each of the 3 strains. Agglutinin absorption tests were performed, and resulted in complete reciprocal absorption between *Sh. alkalescens* and O 1, while absorption of the same serums with O 31 failed to lower the titer for either of the other organisms.

Fifteen locally isolated strains of *E. coli* previously identified as belonging to O group 1 on the basis of direct agglutination, and ability to agglutinate in O 1 antiserum absorbed with the O 31 type strain, and nine strains of *Sh. alkalescens* were included in the study. Among the local strains was a paracolon isolated from the feces of a normal person and biochemically closely related to *Sh. alkalescens.* The paracolon, like *Sh. alkalescens,* fermented glucose, maltose, mannitol, xylose, arabinose, glycerol, rhamnose, and sorbitol with acid but no gas, produced indol, failed to ferment sucrose and salicin, to liquefy gelatin, grow in Koser's citrate medium, or to produce H_2S or acetylmethylcarbinol. It differed from *Sh. alkalescens* in producing acid from lactose after 6 days' incubation, in giving delayed but permanent acidity in milk, failing to ferment dulcitol and being slightly motile. This organism, together with a biochemically typical *E. coli* belonging also to O group 1, was present in large numbers in the feces of this individual over a period of several months. All of the strains tested were agglutinated to approximately the same titer by 3 *E. coli* O group 1 antiserums and by 3 *Sh. alkalescens* antiserums. Each of the coli antiserums was absorbed with several different strains of *Sh. alkalescens* and the alkalescens antiserums with several strains of *E. coli.* In all cases complete absorption of agglutinins was obtained. A representative series of results is shown in Table I. Results with other strains were similar to the ones used as examples.

The Kauffmann type strains were also tested for agglutination in single dilutions of antiserums of *Sh. dysenteriae,* ambigua, sonnei, and all the Flexner and Boyd types of *Sh. paradysenteriae.* Though minor relationships were found several times, the Escherichia strains were rarely agglutinated to more than a fraction of the titer of the serums when titrations were done. In 2 cases, however, very close relationships were found. Preliminary experiments suggest that Kauffmann's O group 53 and *Sh. boydii* IV (P274), and O group 79 and *Sh. boydii* V (P143), may be antigenically identical, but the number of strains tested was too small to warrant definite conclusions.

Discussion. So far as can be determined, a correlation between the common antigens

6. Stuart, C. A., Rustigian, R., Zimmerman, A., and Corrigan, F. V., *J. Immunol.,* 1943, v47, 425.
7. Ewing, W. H., *J. Bact.,* 1949, v58, 497.
8. Kauffmann, F., *J. Immunol.,* 1947, v57, 71.

TABLE I.
Agglutination Titers of Representative Strains of *Shigella alkalescens* and *Escherichia coli* in Unabsorbed and Absorbed Serums of Both Species.

Antiserum	Absorbed by	O antigens: *Escherichia coli* K1	*E. coli* SS1	*E. coli* Paracolon	*Shigella alkalescens* Fong	*Sh. alkalescens* Wagner	*Sh. alkalescens* Gordon
		E. coli					
K1*	—	12,800	12,800	6400	6400	6400	6400
"	Fong	0†	0	0	0	0	0
"	Wagner	0	0	0	0	0	0
SS1	—	6400	6400	6400	3200	3200	3200
"	Fong	0	0	0	0	0	0
"	Wagner	0	0	0	0	0	0
Paracolon 1	—	3200	3200	3200	3200	3200	3200
"	Fong	0	0	0	0	0	0
"	Wagner	0	0	0	0	0	0
		Sh. alkalescens					
Fong	—	3200	3200	1600	1600	1600	1600
"	K1	0	0	0	0	0	0
"	SS1	0	0	0	0	0	0
"	Para. 1	0	0	0	0	0	0
Wagner	—	6400	6400	6400	3200	3200	3200
"	K1	0	0	0	0	0	0
"	SS1	0	0	0	0	0	0

* Kauffmann's strain U5/41.
† Negative at 1:40 dilution.

of *Sh. alkalescens* and certain coliform and paracolon strains has not previously been made in relation to Kauffmann's Escherichia O groups. The present experiments indicate the O antigens of Kauffmann's O group 1 and those of *Sh. alkalescens* are identical. Kauffmann(8) named group 1 among the 8 groups most frequently isolated by him from pathological materials. Strains belonging to this serologic group have been isolated many times in this laboratory, both from feces and from urine. Since they are apparently widely distributed, it is not surprising that they should occur fairly frequently together with *Sh. alkalescens*, though the marked biochemical differences between typical strains of *E. coli* and *Sh. alkalescens* make it unlikely that the two would be mistaken for each other. Knipschildt, as quoted by Kauffmann(8), has found that paracolon strains of varying biochemical reactions can be classified in the O groups as frequently as can true Escherichias, and the experience in this laboratory has been the same. Of particular interest is the occurrence of anaerogenic, slow lactose-fermenting members of the group, like the organism described here. The possibility of confusion between such strains and *Sh. alkalescens* is very great, since careful tests for motility and prolonged incubation of carbohydrate media may be necessary for differentiation. The motile, lactose-fermenting or gas-producing strains reported in the literature as *Sh. alkalescens* on the basis of their antigenic characters are presumably paracolons belonging to O group 1.

Summary. Agglutination and agglutinin absorption tests performed with fifteen *E. coli* strains of Kauffmann's O group 1 and nine strains of *Sh. alkalescens* demonstrated that their O antigens were indistinguishable.

Since this manuscript was submitted for publication an article by E. Frantzen has appeared in *Acta Path. et Microbiol. Scand.*, 1950, v27, 236. This article comes to the same conclusion as the present one concerning the antigenic identity of *Shigella alkalescens* Type I and Kauffmann's coli O group 1.

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