TABLE 1

Synergistic effects between fractions from Escherichia coli

	Total Quantity of Gas (µL) Evolved or Utilized								
Fraction	Formic hy- drogenlyase*		Formic dehy- drogenase†			Hydro- genase‡			
	5 min	10 min	15 min	5 min	10 min	15 min	4 min	8 min	13 min
Ps	0	0	0	10	24	41	7	19	36
Pm	0	0	0	6	15	30	13	51	110
SSN	11	30	59	10	18	29	3	9	16
Ps + SSN	74	181	279	40	80	120	36	79	128
Pm + SSN	59	150	240	40	81	121	115	206	—

All Warburg vessels contained 75 μ M sodiumpotassium phosphate buffer, pH 6.1, in final volume of 1.2 ml. Temperature, 30 C.

* CO_2 + H₂ production in the presence of 15 μ m sodium formate. Gas phase, helium. Where indicated, 1.86 mg (dry wt) *Ps*, 0.66 mg *Pm*, and 0.1 ml *SSN* were used.

† CO₂ production under the conditions noted for formic hydrogenlyase, but with $12 \,\mu M$ methylene blue added.

[‡] Utilization of gas under an atmosphere of H_2 in the presence of 12 μ M of methylene blue. Where indicated, 0.13 mg Ps, 0.66 mg Pm, and 0.1 ml of a 1:6 dilution of SSN were used.

Pm do not decay at the same rates during storage at 4 C. These observations suggest that a partial separation of insoluble cellular fractions with different metabolic activities was achieved.

Aerobic oxidation of citrate, α -ketoglutarate, and several other members of the tricarboxylic

acid cycle was also studied with similar preparations from cells grown as described by Swim and Krampitz (J. Bacteriol., 67, 419, 1954). The particulate fractions alone showed negligible oxidative activity with these substrates. Upon combining particulate and soluble components. disproportionate acceleration of the rates of O_2 utilization was observed. These stimulations are undoubtedly the result of coupling between soluble dehydrogenases and insoluble electron transport enzymes such as the cytochromes. The latter are invariably present in the insoluble fraction of E. coli (Gale, 1939; Gest, 1952). Moyed and O'Kane (Arch. Biochem. and Biophys., 39, 457. 1952) have described a very similar cooperative effect between soluble and insoluble components of Proteus vulgaris with regard to pyruvate oxidase activity. Considering the results noted in table 1, it seems likely that electron transport enzymes concerned with certain anaerobic processes are also present in the insoluble portion of the cell.

The particulate preparations described are much more stable than animal mitochondria with regard to changes in tonicity of the medium and show excellent retention of certain enzymatic activities upon storage at 4 C. It has been shown in one instance at least (Gest, 1952) that variation in enzymatic composition of intact cells with growth conditions is reflected by the particulate fractions. In view of these properties such preparations should be of considerable value for metabolic and related cytochemical studies.

A CULTURE OF ESCHERICHIA FREUNDII SEROLOGICALLY RELATED TO THE GENUS SALMONELLA

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Numerous relationships between the genera Escherichia and Salmonella involving O, K (sheath or envelop), and H antigens have been described. These relationships have been summarized by Kauffmann (Enterobacteriaceae, E. Munksgaard, Copenhagen, 1951). Perhaps the best known of these is the occurrence of the Viantigen of Salmonella typhi in cultures of the Ballerup type of Kauffmann and Møller (J. Hyg., 40, 246, 1940) and in other cultures of *Es*cherichia freundii (West and Edwards, Public Health Service Monograph, in press, 1954). The occurrence of Salmonella H antigens in *E. freundii* cultures first was noted by Gard (Z. Hyg. Infektionskrankh., 120, 59, 1937) and Gard and Eriksson (Z. Hyg. Infektionskrankh., 122, 54, 1939). Relationships between the O antigens of a variety of Escherichia cultures and those of Salmonella cultures have been reported by a number of workers. Most striking of these was the occurrence of the antigenic complex of Salmonella group B in coliform cultures observed first by Schütze (quoted by Kauffmann, 1951).

In spite of the numerous reports in the literature involving antigenic relationships between the Salmonella and Escherichia groups, no coliform culture yet has been reported which contained both O and H antigens in common with Salmonella strains. The present report deals with a culture (4598-53) which was a typical strain of E. *freundii* and which contained both O and H antigens found in Salmonella cultures. Culture 4598-53 was isolated from the stool of a "normal" adult in the course of a survey of food handlers.

Culture 4598-53 produced H₂S, acidified Jordan's tartrate agar, and grew in Simmons' citrate medium. It failed to produce indole, to hydrolyze urea, or to liquefy gelatin. The methyl red test was positive, and the Voges-Proskauer test was negative. In tests for growth in KCN medium and in tests for decarboxylase activity, the culture behaved as a typical strain of *E. freundüi.*¹ Acid and gas were produced promptly from glucose, arabinose, xylose, maltose, lactose, trehalose, rhamnose, mannitol, and sorbitol. Salicin was fermented after 14 days of incubation. Sucrose, raffinose, dulcitol, inositol, and adonitol were not attacked.

The organism was strongly agglutinated by O serum Salmonella group E_2 (3, 15) and to a lesser degree by serums for groups E_1 (3, 10) and E_3 (1, 3, 19). The relationship of the O antigen of 4598–53 to the O antigen of group E_2 is shown in table 1. Except for a very small fraction of antigen 3, which is common to groups E_1 and E_2 , 4598–53 possessed the whole antigenic complex of group E_2 . Further, as shown by absorption of its serum, 4598–53 possessed no O antigen other than that found in Salmonella group E_2 .

The H antigens 4598-53 were monophasic and were related to those of the Salmonella serotypes known as Weslaco and Macallen, both of which are represented by the symbol z_{36} . The relationships of these antigens are detailed in table 2. None of the three cultures possessed identical H antigens. The two Salmonella types shared an

¹ The writers are indebted to Dr. F. Kauffmann and Dr. Vagn Møller for the performance of the KCN and decarboxylase tests.

TABLE 1

O Antigen	s oj	Escherichia	jreunaii,	4098-03

	Antigens				
Serums	Salmo- nella E ₁ (3,10)	Salmo- nella E ₂ (3,15)	E. freundii 4598-53		
Salmonella E ₂ (3, 15) Unabsorbed Absorbed by	1,000	4,000	4,000		
4598-53	100	100	<20		
$\begin{array}{r} 4598-53 \ + \ Salmonella \\ E_1 \dots \dots \dots \end{array}$	<20	<20	<20		
E. freundii, 4598-53					
Unabsorbed	1,000	2,000	4,000		
Absorbed by Salmonella E ₂ (3, 15)	<20	<20	<20		

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H Antigens of Escherichia freundii, 4598-53

S an an	Antigens				
Serums	Weslaco (zm)	Macallen (zm)	4598-53		
Weslaco (z ₂₆)					
Unabsorbed	4,000	2,000	2,000		
Absorbed by		l í	,		
Macallen (z ₃₆)	1,000	<100	<100		
4598-53	2,000	1,000	<100		
Macallen (Z26)					
Unabsorbed	4,000	8,000	8,000		
Absorbed by			-,		
Weslaco (z ₃₆)	<100	4,000	500		
4598-53	2,000	4,000	<100		
4598-53					
Unabsorbed	1,000	4,000	16,000		
Absorbed by	,	,	,		
Weslaco (z ₃₆)	<100	500	8,000		
Macallen (Z26)	<100	<100	4,000		

antigen not found in 4598-53 and Macallen, and 4598-53 contained an antigen not shared by Weslaco. For practical purposes the antigenic formula of the *E. freundii* culture may be expressed by the Salmonella formula 3, 15: z_{36} .

SUMMARY

A typical culture of *Escherichia freundii* isolated from a normal food handler was found to have both O and H antigens in common with the Salmonella group. The antigens of the culture could be expressed by the Salmonella formula $3, 15: z_{36}$.