previously found in spontaneous phase variation. Addition of an anti-1,2,3 serum, however, brought out the hitherto missed i phase. Combined treatment with i and 1,2,3 antiserum immobilizes the culture without appearance of any other phase. On subculture the "induced" i phase reproduces 3 phases, the i, X, and 1,2,3, the latter 2 forming about 5% of the colonies. The action of 1,2,3 serum on the original 1,2,3 phase sets the X phase free, the i antiserum has no influence.

Altogether, 3 well defined phases were recovered by this method: X, i, and 1,2,3. Later in animal experiments a fourth variant was found: a well agglutinable i + 1,2,3 phase in single colonies, comparable to that found by Kauffmann in *S. paratyphi B* (b + 1.2).

Living cultures of the original strain and of the various phases were unusually virulent for animals. Of 5 rabbits, one guinea pig, and more than 100 mice which were fed or injected, only one mouse survived. Among the fatalities was one rabbit which had been immunized previously with a formolized culture. The minimal fatal doses used consisted of 10^{-8} dilutions of broth, representing about 50 organisms. Computed on the average time of survival, the X phase exerted the highest pathogenicity in mice, the 1,2,3 phase the lowest. The number of animals involved in the study of each phase was not large enough to allow statistical evaluation of results. When mice were fed only the X phase, both X and 1,2,3 colonies were recovered from blood and stool. When the 1,2,3 phase was fed, again both X and 1,2,3 colonies were isolated from blood and stool. Of the six mice fed the "induced" i phase, five died, one survived, and the cultures yielded i, 1,2,3, and X colonies from the blood and feces. From the one surviving mouse pellets of feces were taken after 19, 41, 70, and 103 days. The first specimen showed only i colonies, the specimen secured on the 41st day yielded i colonies and colonies which surprisingly turned out to be bi-phasic, i + 1,2,3. The specimen collected on the 70th day displayed i, 1,2,3, and i + 1,2,3 colonies, and the one collected on the 103d day yielded i and i + 1,2,3 colonies. In animals and on subculture the i + 1,2,3 phase yielded the i, 1,2,3, and i + 1,2,3 phases but never the X phase. This i + 1,2,3 phase seen in about 10% of the single colonies, might actually be identical with the X phase which now became agglutinable. A formolized 6-hour broth culture of this variant was used for rabbit immunization. The resulting antiserum was active against the O (IV.V), i and 1,2,3 antigens. The fact that after removal of the O-antibody by specific absorption the remaining H-antibodies did not agglutinate the X phase indicates there is no difference between this serum and that produced against the X phase—the X phase itself has retained its inagglutinability now for 11 months.

Summary and Conclusions. The natural appearance of an inagglutinable but antigen-producing "mixed" phase of a *S. typhi murium* is described. The phase i, the presence of which could not be elicited in the original cultures, was brought out by the use of Gard technic. An agglutinable "mixed" phase was produced *in vivo*. Data on the different phase variations of this Salmonella variant are given.

The described observations raise a number of theoretical questions unanswerable for the time being. This paper, therefore, limits itself to a statement of the facts.

14836

A New Salmonella Type: *S. Virginia.*

IVAN SAPHRA AND ERICH SELIGMANN. (Introduced by Ella H. Fishberg.)

From the Department of Bacteriology and Serology, Beth Israel Hospital, New York City.

Culture 2255 was received on October 10, 1944, from A. Corpening, Director of Laboratories, Department of Health, Richmond, Va., where it had been isolated by F. Spindle from

the stool of a 40-year-old woman. The patient had suffered from 4 episodes of nausea, diarrhea, and fever, each lasting 12-72 hours, in the period from June until October, 1944. Her blood serum, received through the courtesy of Dr. J. P. Lynch, St. Luke's Hospital, Richmond, Va., on October 23 and November 7, 1944, agglutinated the culture in a dilution 1:80.

The isolated organism is a gram-negative motile rod possessing the biochemical characteristics of the genus Salmonella. Dextrose, maltose, mannite are fermented with gas formation. Lactose, sucrose, salicine, are not attacked. Sorbitol, dulcitol, xylose, rhamnose, arabinose are promptly acidified. Inositol and *d*-tartrate show transitory acidification with subsequent reversion to alkalinity after 24 hours. H_2S is produced, no indol; gelatin is not liquefied.

On feeding, the culture is not pathogenic for mice, but it was still being excreted 35 days after feeding. Of 4 mice injected s.c. with 0.1 cc of a 20-hour broth culture, 2 died after one day, one after 3 days, and one survived. The organism was recovered from the heart blood of the dead animals.

The O-antigen. Boiled bacilli were agglutinated only by sera derived from *S. newport* (VI.VIII), *S. kentucky* (VIII.XX), and *S. amherstiana* (VIII); by the latter to its end titer. The presence of an VIII antigen is thus evidenced. This antigen in combination with the VI is found in the VI.VIII group (12 types are known by now) and associated with the XX antigen in *S. kentucky*. Only *S. amherstiana* (VIII;eh-1.7) recently described by Edwards and Bruner[1] displays the VIII antigen with no additional factors. Single factor antisera against VI or XX did not agglutinate the new strain, nor did sera containing VI antibodies (*SS thompson* (VI.VII), *carrau* (VI.XIV.XXIV), *onderstepoort* (I.VI.XIV. XXV)). In absorption tests the culture removed completely the VIII fraction from VI.VIII, VIII.XX and VIII sera leaving VI and XX antibodies untouched. This speaks for the presence of VIII either as the only antigenic factor (like in *S. amherstiana*)

[1] Edwards, P. R., and Bruner, D. W., *J. Immunol.*, 1942, **44**, 319.

TABLE I.

Antigen	Serum											
	S. newport		*S. kentucky*		*S. amherstiana*		*S. virginia*					
								Absorbed with				
	Unabsorbed	Abs. with *virginia*	Unabsorbed	Abs. with *virginia*	Unabsorbed	Abs. with *virginia*	Unabsorbed	*newport*	*kentucky*	*amherstiana*		
S. georgia	VI.VII	80	80	0		0		0				
S. newport	VI.VIII	**640**	80-160	160	0	800	0	2560	0	0	0	
S. kentucky	VIII.XX	160	0	**320**	160	800	0	2560	0	0	0	
S. amherstiana	VIII	80	0	160-320	0	**1600**	0	2560	0	0	0	
S. virginia		160	0	160	0	8-1600	0	**2560**	0	0	0	

Figures give highest agglutinating dilution.
0 = negative 1:40.

or in a new combination VIII . . .

An O-antiserum prepared with boiled culture material agglutinated the homologous strain and *SS. newport, kentucky, amherstiana* almost uniformly to its end titer, but did not attack types with the VI.VII, VI.XIV.XXIV, and I.VI.XIV.XXV antigens. VIII containing types (*SS. newport, kentucky, amherstiana*) removed all antibodies for themselves and for the new strain from the serum. Thus, the exclusive presence of the VIII antigen and its identity with that of the other types could be established. The newly found VIII seems even more complete than that of *S. amherstiana* which in Edwards and Bruner's experience did not fully cover the VIII of the *newport* type.

The H-antigen. The strain's flagellar antigen is a monophasic *d* as is present in *S. typhi* and *S. wichita*. Testing of numerous single colonies did not reveal another phase, nor did the culturing in semi-solid agar with addition of *d* antiserum (Gard's technic). Living and formolized antigens are readily agglutinated by sera prepared with types endowed with the *d* antigen (*SS. stanley, amersfoort, muenchen, typhi, shangani, niloese, wichita*). The sera agglutinate the strain 2255 to approximately 50% of their relative titers. On the other hand, this strain removes about 75% of their agglutinins by absorption. A serum prepared with formolized broth culture of the strain agglutinates all *d* containing types to high titers, some of them to the end titer. Slight differences observed are in agreement with previous statements by Kauffmann,[2] Hormaeche and Peluffo,[3] and Edwards and Bruner.[4] These authors found differences in the *d* of different types and emphasized the complexity of that flagellar antigen.

The strain 2255 represents a new pathogenic Salmonella type. It has been named *S. virginia* after its place of isolation.

Summary. *S. virginia,* a new Salmonella type, has been described. It was isolated from a case of human enteritis and has the formula VIII; d —.

[2] Kauffmann, F., *Z. Hyg.*, 1937, **120**, 177.

[3] Hormaeche, E., and Peluffo, C. A., *Arch. Urug. de Med. Cir y Esp.*, 1941, **14**, 217.

[4] Edwards, P. R., and Bruner, D. W., *Am. J. Hyg.*, 1942, **34**, 121.

14837

Cystine Inhibition of the Succinoxidase System. III. Effect of Dialysis.*

STANLEY R. AMES AND C. A. ELVEHJEM.

From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

During the course of a previous investigation on the inhibition of the succinoxidase system by cysteine and cystine, we showed that the concentration of four-carbon dicarboxylic (C-4) acids in the tissue homogenate was a determining factor.[1] Subsequently, we showed that with either cysteine or cystine added to the reaction mixture (cytochrome *c* present), the inhibition was entirely due to cystine.[2]

All inhibitors acting on the sulfhydryl group in succinic dehydrogenase will be affected by the presence of any C-4 acids or related substances. Since considerable importance has been attributed to the action of such inhibitors in determining whether or not enzymes function by means of sulfhydryl groups,[3,4] some means must be found to eliminate these interfering substances from the

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were aided by a grant from the Rockefeller Foundation.

[1] Ames, S. R., and Elvehjem, C. A., *Arch. Biochem.*, 1944, **5**, 191.

[2] Ames, S. R., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 109.

[3] Potter, V. R., and DuBois, K. P., *J. Gen. Physiol.*, 1943, **26**, 391.

[4] Barron, E. S. G., and Singer, T. P., *Science*, 1943, **97**, 356.