

Biochemical, Serologic, and Sensitivity Characteristics of Salmonellae After Long Survival in Soft Agar

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IN A PREVIOUS PAPER (1) we reported a 40-year survival of salmonellae in soft agar. No detailed examinations, however, had been performed on that sample. The present study was undertaken to learn whether any alterations in several characteristics of these micro-organisms had taken place during this period. Following are the results of various tests concerning the biochemical reactions, antigenic properties, and sensitivity to antibiotics of a new population of salmonellae after a 35- to 40-year survival in soft agar.

Micro-organisms. Sixty glass ampules from

the Bacterial Archives of the Anthropological Research Center of the Army of Greece were sealed by melting and were kept closed from 35 to 40 years. These ampules, containing pure cultures of salmonellae in soft agar, were examined for surviving bacteria in 1971. The ampules were kept throughout the years in well-closed boxes at room temperature and not opened until this investigation. The cultures for the detection of living salmonellae were made on common nutrient media. Of the 60 ampules examined, 46 were found to contain living salmonellae; specific tests were done on each of the 46 surviving salmonellae.

Biochemical reactions. The biochemical reactions examined (2) included motility, indole production, hydrogen sulfide, urease, beta galactosidase (ONPG), methyl red test, deamination of phenylalanine, and reduction of nitrates. The fermentation of lactose, glucose, mannitol, sucrose, and salicin was also tested.

Serologic tests. To determine the antigenic properties of the surviving salmonellae, we used Difco antisera. Polyvalent antisera were used for the determination of the somatic (O)

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antigens and univalent for the flagellar (H) antigens of both phases, as well as for the Vi antigen of *Salmonella* group D.

Sensitivity tests. Sensitivity tests were performed by using standardized disks on sensitivity test agar (Oxoid) (3). For this purpose we used a 4-hour culture of salmonellae in nutrient broth. The sensitivity to seven antibiotics was tested—streptomycin, kanamycin, chloramphenicol, colimycin, ampicillin, carbenicillin, and tetracycline. The results were compared with those obtained from testing a polysensitive control strain of *Escherichia coli* under identical conditions. The following criteria were used: sensitive (S)—diameter of inhibition equal to that of the control strain, or not smaller than 4 mm; resistant (R)—complete absence of inhibition; partially sensitive (PS)—intermediate inhibition. To compare the sensitivity of these strains to recently isolated strains from the Greek population, the sensitivity to the same antibiotics of 43 strains of salmonella isolated from 1970–72 infections in patients of the children's hospital "Aghia Sofia," Athens, was also tested. These strains were: 27 *Salmonella typhi* (D:Vi:d:-), 12 *Salmonella typhimurium* (B:i:-), and 4 *Salmonella paratyphi* B (B:b:-).

Results

Of the 60 ampules examined, 46 (77 percent) were found to contain living salmonellae. The analytical results obtained that concern the antigenic properties, biochemical reactions, and sensitivity of strains are shown in tables 1–3. The results of sensitivity testing of the recently isolated strains of *Salmonella* are cited in table 4.

Discussion

The percentage of surviving salmonellae found in the present study (77 percent) is close to that

Table 1. Antigenic properties of *Salmonella* in soft agar surviving in 46 glass ampules sealed by melting and kept closed from 35 to 40 years

Number of <i>Salmonella</i> strains	Group	H antigens		VI antigen
		Phase 1	Phase 2	
2.....	A	a	...	
2.....	B	b	1,2	
1.....	B	b	e, n, x	
1.....	B	i	(1, 6 weakly)	
18.....	D	d	...	Positive
16.....	D	d	...	Weakly positive
6.....	D	d	...	Negative

Table 2. Biochemical reactions of 46 strains of *Salmonella* surviving 35 to 40 years in soft agar

Test	Strains	
	Positive	Negative
Motility.....	46	0
Methyl red.....	46	0
Nitrate reduction.....	46	0
H ₂ S.....	16	30
Indol production.....	0	46
Phenylalanine deamination.....	0	46
Urease production.....	0	46
Beta galactosidase production.....	0	46
Fermentation		
Lactose.....	0	46
Glucose.....	46	0
Mannitol.....	46	0
Sucrose.....	0	46
Salicin.....	0	46

reported previously (1). The results of our investigation confirm that *Salmonella* organisms can remain viable for as long as four decades in a nonspecific medium such as soft agar.

The biochemical reactions were typical for salmonellae. This result indicates that no deterioration of the enzymatic mechanisms responsible for those reactions took place during the survival period. Unaltered biochemical reactions have also been reported for strains of *E. coli* (4) after 14-year storage at room temperature.

The reactivity to polyvalent and univalent antisera was generally good. Interestingly, of the 40 strains of *S. typhi*, 18 displayed a strong agglutination with the anti-Vi antiserum, 16 reacted weakly, and 6 were nonreactive, even after repeated subcultures on nutrient agar. This result may indicate a variable loss of Vi antigen in the latter two groups; in fact, it has been known since the early work of Kauffmann (5) that the Vi antigen content of any strain of *S. typhi* may vary without affecting its O or H antigens.

The results of the sensitivity testing, in a sense, were unexpected. No significant differences in the resistance to antibiotics were found between the old strains (table 3) and those recently isolated strains of *S. typhi* (table 4); that is, both populations were fairly sensitive to the antibiotics tested. This finding is at variance with some of our other results (6) concerning the sensitivity to antibiotics of old strains of *E. coli* (7) surviving for 40 years under conditions similar to those of the present study; among those strains 6 percent were resistant to antibiotics, as compared

Table 3. Sensitivity to 17 antibiotics of 46 strains of *Salmonella* surviving in soft agar for 35 to 40 years

Antibiotic	<i>Salmonella typhi</i>			<i>Salmonella</i> group A			<i>Salmonella</i> group B		
	S	PS	R	S	PS	R	S	PS	R
Streptomycin.....	39	1	0	2	0	0	4	0	0
Kanamycin.....	40	0	0	2	0	0	4	0	0
Chloramphenicol.....	37	2	1	1	1	0	4	0	0
Colimycin.....	40	0	0	2	0	0	4	0	0
Ampicillin.....	40	0	0	2	0	0	4	0	0
Carbenicillin.....	40	0	0	2	0	0	4	0	0
Tetracycline.....	39	1	0	2	0	0	4	0	0

S—sensitive, PS—partially sensitive, R—resistant.

Table 4. Sensitivity to 7 antibiotics of 43 strains of *Salmonella* isolated in Greece, 1970–72

Antibiotic	<i>Salmonella typhi</i>			<i>Salmonella</i> group B:b			<i>Salmonella</i> group b:i		
	S	PS	R	S	PS	R	S	PS	R
Streptomycin.....	24	3	0	1	1	2	6	4	2
Kanamycin.....	26	1	0	1	0	3	1	1	10
Chloramphenicol.....	21	6	0	1	0	3	1	2	9
Colimycin.....	26	1	0	4	0	0	12	0	0
Ampicillin.....	27	0	0	1	0	3	1	0	11
Carbenicillin.....	27	0	0	1	1	2	0	1	11
Tetracycline.....	24	3	0	1	2	1	9	2	1

S—sensitive, PS—partially sensitive, R—resistant.

with 30–40 percent resistance found among recently isolated *E. coli* strains from infections in the Greek population. It is of interest, however, that among group B salmonellae in this sample, a marked increase in resistance in recent strains can be noted, although the number (four) of the old group B strains is too small for definite statements to be made.

Summary

Of 60 strains of *Salmonella* kept in soft agar in sealed glass ampules for 35 to 40 years, 45 strains survived. The surviving strains were tested for biochemical reactions, antigenic properties, and sensitivity to seven antibiotics. Of these 46 strains, 40 were *S. typhi*, 2 were group A, and 4 were group B. No alterations in biochemical reactions and antigenic properties were found other than a complete loss of Vi antigen in six ampules and a variable loss in 16 of 40 ampules containing surviving *S. typhi*. The sensitivity testing revealed a uniform absence of resistance to the antibiotics tested; only one strain of *S. typhi* was resistant to chloramphenicol. Comparison with the sensitivity of *Salmonella* isolated from

infections in Greece 1970–72 revealed no difference in the sensitivity of *S. typhi*, but showed a marked increase in resistance of the group B strains.

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