OBSERVATIONS ON STRAINS OF A MONOPHASIC SALMONELLA VARIANT[1]

W. B. CHERRY,[2] L. A. BARNES,[3] AND P. R. EDWARDS

Enteric Pathogen Laboratory, Naval Medical School, Bethesda, Md., and Department of Animal Pathology, Kentucky Agricultural Experiment Station, Lexington, Ky.

Received for publication October 27, 1945

The first known isolation of the *Salmonella* type having the antigenic formula IV, V, XII: e,h- was made in 1941 by Dr. D. W. Bruner from the intestine of a hog affected with enteritis; in the list of Standard Strains of *Salmonella* (Edwards and Bruner, 1942) this monophasic e,h variant is assigned no. 150. On the basis of the slow fermentation of rhamnose and an unconfirmed report that cultures containing O-factor V could be recovered from the original *S. reading* strain (no. 19), the monophasic organism was considered to be a variant of *S. reading*. This designation may have been an error in nomenclature since subsequent developments suggest that the variant may have been derived from one of several, perhaps commoner, *Salmonella* types.

During 1942 and 1943 six additional representatives of this variant were isolated in California and submitted to the National Salmonella Center at Lexington for verification; more recently, three more strains were received from California. In April, 1944, the organism was recognized in a routine stool specimen taken at the Naval Medical School, and from January through May, 1945, twenty-nine cultures were isolated in various Naval establishments and subsequently submitted to the Enteric Pathogen Laboratory for identification; three of the latter were duplications so that only twenty-six individual strains are represented.

This recent accumulation of a rather remarkable number of members of the monophasic variant group led to a restudy of the epidemiologic, biochemic, and serologic characteristics of the cultures in the hope that some clue to the ancestry of the type might be found. For the purpose of completeness the available data on the 36 strains are listed below.

150 (Hog 24682)—isolated in 1941 by Bruner from intestine of hog affected with enteritis.

CDAI 157—isolated in May, 1942; CDAI 222 and CDAI 223—isolated in June, 1942; CDAI 363 and CDAI 368—isolated in May, 1943; from poults on a California turkey ranch.

[1] The opinions or assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

The investigation reported in this paper was conducted in part (by P. R. E.) in the laboratory of the Department of Animal Pathology, Kentucky Agricultural Experiment Station, and is published by permission of the director.

[2] Lieutenant (jg), H(S), USNR.

[3] Lieutenant Commander, H(S), USNR.

Cal. 605—Isolated in March, 1943, by Los Angeles County General Hospital Laboratory from the feces of a seven-month-old baby suffering with acute gastroenteritis.

CDAI 989—isolated in June, 1945, from a turkey in Lancaster, California.

CDAI 998 and CDAI 999—Isolated in June, 1945, from turkeys in Hemet, California.

100-73—Isolated in April, 1944, by NAMRU no. 2 from a stool specimen submitted to the Naval Medical School; no further information available.

106-10—Isolated in January, 1945, from the feces of a patient on the USS Orion at Pearl Harbor during an outbreak of gastroenteritis; turkey was incriminated as the vehicle of dissemination.

106-24—Isolated in March, 1945, from the skin of a frozen turkey in a lot that was transferred in November, 1944, from the supply ship USS Mizar to the USS Orion at Woendi.

106-44—Isolated in March, 1945, from a man on the USS Orion at Pearl Harbor during continued investigation of the January outbreak; clinical and epidemiological data not available.

106-70—Isolated in March, 1945, from a mild case of enteritis in man during the continued investigation of the January outbreak on the USS Orion.

106-126, 106-128, 106-129, 106-130, 106-131, 106-132, 106-133, 106-134, 106-135, 106-136, and 106-137—All isolated in April, 1945, from the feces of patients during an outbreak of gastroenteritis on the USS Courtland at Pearl Harbor; all had histories negative for previous diarrheal disease.

106-141 and 106-142—Isolated from the feces of two food handlers during the April outbreak on the USS Courtland; men were asymptomatic at the time but had typical symptoms in March, 1945, during a small epidemic aboard the ship.

106-145—Isolated from the feces of a food handler during the April, 1945, outbreak on the USS Courtland; was asymptomatic at the time and denied any history of previous diarrheal disease.

106-236—Isolated in March, 1945, from the feces of a food handler at Bachelor Officers' Quarters in Mokalapa, T. H.; no further information available.

10-273—Isolated in January, 1945, in the laboratory of the U. S. Naval Hospital at Pearl Harbor from the feces of a rheumatic fever patient who had fever and diarrhea.

10-274—Isolated in January, 1945, in the laboratory of the U. S. Naval Hospital at Pearl Harbor from the feces of a gastroenteritis patient stationed on the USS Baham; an outbreak of enteritis involving at least 21 men occurred on this ship in October, 1944.

10-275—Isolated in January, 1945, in the laboratory of the U. S. Naval Hospital at Pearl Harbor from the feces of a gastroenteritis patient; no further information available.

10-277—Isolated in January, 1945, in the laboratory of the U. S. Naval

Hospital at Pearl Harbor from the feces of a case diagnosed as duodenal ulcer, but with no symptoms of diarrheal disease.

82-111—Isolated in May, 1945, at a California Naval Station from the feces of a neuro-psychiatric patient who gave a history of mild diarrhea in August, 1941, while in Kamuela, T. H.

103-5—Isolated in March, 1945, from the feces of a Marine patient on Iwo Jima whose staging area was in Hawaii; case diagnosed as intestinal hemorrhage and had other symptoms of diarrheal disease; no information available on previous history.

It is unfortunate that scant information is available concerning the circumstances surrounding the original sources of several of these cultures. The first isolation (no. 150) was from a hog affected with enteritis, but it is not known whether other animals in the same herd were sick. Eight of the strains were isolated from poults and turkeys in California, but no data are available as to whether the birds were ill. Attempts have been made to determine whether the turkey from which culture 106-24 was isolated may have been procured in California, but so far the information has not been obtained.

In man, these e,h variants are capable of causing symptoms of gastroenteritis comparable with those incited by other *Salmonella* types. Some strains were from allegedly asymptomatic subjects with no history of diarrheal disease; others were found in mild cases; and still others were from individuals who exhibited moderately severe symptoms. At present, no known cases of enteric fever have occurred. In general, the symptoms described are fever of about 103 F, diarrhea with 10 stools per day, nausea, vomiting, and abdominal cramps; the duration of symptoms is about 2 days and the average stay on the sick list is approximately 5 days. A few cases may be ill enough to require sedation and intravenous fluids.

Although one culture (Cal. 605) was isolated from an apparent sporadic case and others may have been single cases, the available evidence also shows that under certain somewhat obscure conditions epidemics may be initiated. In one outbreak aboard a ship in the Pacific, 19 cultures were isolated from cases with acute symptoms; 4 cultures were recovered from individuals asymptomatic at the time. Three of these 4 individuals were food handlers, and 2 of them were ill with typical symptoms during an acute outbreak aboard the same ship a month previously. It seems possible that these two food handlers may have remained carriers following the first outbreak and could have been responsible for initiating the second epidemic. The organism was involved in an outbreak of gastroenteritis on another ship in the Pacific, but 3 other *Salmonella* strains, including a newly described type, *S. orion* (Barnes *et al.*, 1945), plus a concurrent shigellosis, complicated the picture.

The monophasic e,h strains appear to be endemic in California and the Pearl Harbor area of Hawaii, but further information is necessary before the geographic area from which the variants originated can be determined. At the time culture 106-24 was isolated, several other frozen turkeys from the Naval Supply Depot, Fremantel, West Australia, were cultured but no strains of *Salmonella* were found. The original source of the Fremantel turkeys is not available.

BIOCHEMICAL CHARACTERISTICS

All cultures studied exhibited the morphological and tinctorial attributes of the genus *Salmonella*. In view of the delayed fermentation of rhamnose observed in studying the original culture (no. 150) of these e,h variants (Edwards and Bruner, 1942), an extensive re-examination of the biochemical characteristics of the 36 strains was undertaken in the hope of detecting some significant disparity that might suggest possible ancestry. The results are summarized in table 1.

Remarkably consistent behavior is apparent from the biochemic study of these cultures; the only variations noted were in rhamnose, melibiose, and cellobiose. No culture was observed to ferment rhamnose in less than 2 days, but all cultures produced acid by the eighth day; 14 of the 36 strains produced a small volume of gas from rhamnose, but the remainder were grossly anaerogenic. Two cultures (CDAI 157 and 150) that initially required 6 and 7 days, respectively, to produce acid detectable in 1 per cent rhamnose broth containing Andrade's indicator were serially transferred in this medium, and following the first or second subculture both strains produced both acid and gas within 24 hours. This fact suggests that the delayed and incomplete fermentation of rhamnose first observed may be due to a predominance in the original culture of organisms possessing sluggish enzymes and that selection or adaptation of cells with more active rhamnose-splitting enzymes may result in more prompt and complete fermentation.

With the exception of 3 strains all cultures produced acid and gas in melibiose within 24 hours. Acid and gas production by CDAI 368 was observed first on the seventh day and by 10-273 and 106-24 on the third day. After 2 serial transfers in 1 per cent melibiose CDAI 368 produced acid and gas within 24 hours.

In cellobiose all cultures exhibited delayed activity; the time required for visible evidence of fermentation varied from 3 to 6 days. A single strain (106-10) remained anaerogenic during the period of observation, but in several instances gas production was not evident for 1 or 2 days after acidity was noticeable.

The increased rate of fermentation of a given carbohydrate subsequent to serial transfer of *Salmonella* strains in the medium is well known. Although no definite evidence is at hand of an alteration in antigenic composition correlated with a change in enzymatic activity of these organisms, the possibility of the two variations occurring coincidently should not be overlooked.

If it is assumed that the monophasic e,h strains under discussion were derived from some established *Salmonella* type as a result of a "loss variation" in antigenic composition, the most likely ancestors would appear to be in the group composed of *S. reading* (19), *S. chester* (17), *S. san-diego* (110, 111), *S. saint-paul* (108), *S. zagreb* (132), *S. kaapstad* (145), *S. kaposvar* (133), and *S. salinatis* (148). In order to test this hypothesis from the biochemic standpoint, 22 strains of *S. saint-paul* were inoculated into rhamnose broth. Ten of the cultures were isolated from 6 patients during an epidemic on board a ship in the Pacific, but the

TABLE 1

Biochemical characteristics of 36 strains of a monophasic Salmonella variant

	MANNITOL, GLUCOSE, XYLOSE, MALTOSE, DULCITOL, ARABINOSE, TREHALOSE, SORBITOL	RHAMNOSE	CELLOBIOSE	MELIBIOSE	LACTOSE, SUCROSE, INOSITOL SALICIN ADONITOL, MELEZITOSE	UREA, GELATIN, V. PROSKAUER, INDOLE, *i*-TARTRATE	STERNS, H_2S, MUCATE, CITRATE, *d*-TARTRATE, MOTILITY, METHYL RED	PURPLE MILK	TRIMETHYLAMINE-OXIDE	*l*-TARTRATE
10-273	AG_1	A_5	A_4G_6	AG_3	−	−	+	N	+	
10-274	AG_1	A_3	AG_4	AG_1	−	−	+			
10-275	AG_1	AG_5	AG_4	AG_1	−	−	+			
10-277	AG_1	AG_3	A_4G_6	AG_1	−	−	+			
82-111	AG_1	AG_5	A_4G_6	AG_1	−	−	+	N		
100-73	AG_1	A_2	AG_4	AG_1	−	−	+	N		+
103-5	AG_1	AG_5	A_4G_6	AG_1	−	−	+	N		
106-10	AG_1	A_3	A_3	AG_1	−	−	+	N	+	+
106-24	AG_1	A_4	AG_4	AG_3	−	−	+	N	+	+
106-44	AG_1	A_5	AG_5	AG_1	−	−	+	N	+	+
106-70	AG_1	A_4	AG_4	AG_1	−	−	+	N	+	+
106-126	AG_1	A_5	A_6G_7	AG_1	−	−	+			
106-128	AG_1	AG_6	AG_4	AG_1	−	−	+			
106-129	AG_1	A_6	A_6G_7	AG_1	−	−	+			
106-130	AG_1	A_3	A_4G_6	AG_1	−	−	+			
106-131	AG_1	A_6	AG_4	AG_1	−	−	+			
106-132	AG_1	AG_5	A_4G_6	AG_1	−	−	+			
106-133	AG_1	A_5	A_4G_6	AG_1	−	−	+			
106-134	AG_1	A_8	A_4G_6	AG_1	−	−	+			
106-135	AG_1	A_4	A_4G_6	AG_1	−	−	+			
106-136	AG_1	A_4	A_4G_6	AG_1	−	−	+			
106-137	AG_1	A_5	AG_4	AG_1	−	−	+			
106-141	AG_1	A_6	AG_4	AG_1	−	−	+			
106-142	AG_1	A_3	AG_4	AG_1	−	−	+			
106-145	AG_1	A_6	A_4G_6	AG_1	−	−	+			
106-236	AG_1	A_5	AG_4	AG_1	−	−	+			
24682	AG_1	A_7	AG_4	AG_1	−	−	+	N	+	+
CDAI 157	AG_1	A_6	AG_6	AG_1	−	−	+			+
CDAI 222	AG_1	A_4G_7	A_4G_6	AG_1	−	−	+	N		+
CDAI 223	AG_1	A_4G_7	A_4G_6	AG_1	−	−	+			+
CDAI 363	AG_1	AG_6	AG_4	AG_1	−	−	+			+
CDAI 368	AG_1	AG_4	A_4G_6	AG_7	−	−	+			+
CDAI 989	AG_1	AG_6	A_2G_4	AG_1	−	−	+			+
CDAI 998	AG_1	A_4G_6	AG_4	AG_1	−	−	+			+
CDAI 999	AG_1	A_4G_6	A_4G_6	AG_1	−	−	+			+
CAL. 605	AG_1	A_4G_6	A_4G_6	AG_1	−	−	+	N		−

Subscripts indicate number of days of incubation before reaction was apparent. Where no gas production is indicated, it was not apparent after 21–30 days' incubation.

Negative in carbohydrates = no acid or gas after 21–30 days at 37 C.

Urea negative = no ammonia production after 1–2 days at 37 C.

Gelatin negative = no liquefaction after 29–37 days at 37 C.

V-P negative = no acetylmethylcarbinol production after 24–28 hours at 37 C.

Indole negative = no production after 3 days at 37 C.

i-Tartrate negative = no utilization after 14 days at 37 C.

TABLE 1—*Continued*

Sterns positive = pale or deep violet during first 3 days of incubation.

H_2S positive = strong hydrogen sulfide production during first 24 hours at 37 C.

Mucate positive = Complete utilization during incubation at 37 C for 14 days.

Citrate positive = same as mucate.

d-Tartrate positive = same as mucate.

Motility positive = active motility.

Methyl red positive = pronounced acid reaction in presence of methyl red indicator following incubation at 37 C for 4 days.

N = characteristic *Salmonella* reaction of initial acidity followed by return to neutrality and subsequent increasing alkalinity. 11 cultures tested.

Trimethylamine oxide positive = marked production of trimethylamine within 24 hours. 6 cultures tested.

l-Tartrate + = complete utilization during incubation at 37 C for 14 days. − not utilized during incubation at 37 C for 14 days. 15 cultures tested.

balance were from widely separated geographic areas inside and outside the United States. In addition, 10 strains of *S. san-diego* from well-distributed sources were cultured in the same carbohydrate medium. All cultures fermented rhamnose overnight with the production of acid and gas.

Of interest are some observations concerning the fermentation of inositol. Nine strains of *S. saint-paul*, 2 strains of *S. chester*, and 1 of *S. kaapstad* all produced acid and gas promptly from this carbohydrate, but all 36 strains of the e,h variant, 5 strains of *S. san-diego*, and 1 strain each of *S. zagreb* and *S. kaposvar* failed to show any activity in the medium during the period of observation. It was noted, however, that, whereas the diphasic strain of *S. reading* (19) produced acid and gas in inositol, the monophasic e,h strain (150) produced no visible change. Such results appear to be consistent with a hypothesis that the ancestor of the e,h variants may be one of the 8 types mentioned. If this were the case, the focus of attention might be returned to *S. reading* on the basis of a correlated biochemic and serologic loss variation. This is not intended to infer, however, that *S. san-diego* or *S. saint-paul*, for example, should be excluded from consideration.

The utilization of certain organic acids was partially investigated. One strain of the e,h variant (Cal. 605) utilized *d*-tartrate, but not *l*-tartrate to a significant degree; this was an unexpected result, but observations with other cultures and the hypothetical precursors in *d*-, *l*-, and *i*-tartrate failed to reveal anything else noteworthy.

In general the biochemic studies failed to produce much encouragement in the search for an ancestor of the monophasic e,h variants; the observations do not, however, lead to an exclusion of any of the 8 diphasic types mentioned as possibilities.

SEROLOGICAL CHARACTERISTICS

The most direct method for designating the parent type of the e,h variants would be to isolate and identify a second flagellar phase from at least one of the monophasic strains. Another procedure entails the actual observation of the ancestor in the process of loss variation; this would obviously depend to a large extent upon chance.

A considerable amount of work has been done in both laboratories in attempts to demonstrate serologic relationship. All of the monophasic variants possess the somatic antigens IV, V, XII and the flagellar phase 1 complex e,h. Isolation of a second flagellar phase was attempted soon after receipt of the cultures. The method consisted in cultivating the organisms in semisolid medium (Edwards and Bruner, 1942) containing an amount of sterile, single-factor h serum adequate to suppress motility in phase 1 (e,h) but of insufficient concentration to immobilize a second phase if present (Gard, 1937). This procedure was later repeated with 29 of the 36 strains using larger inoculums; incubation at 37 C extended for 10 to 15 days. Under these conditions no evidence was obtained of the existence of a second phase. The same 29 cultures were cultivated in petri plates of the same medium containing an appropriate amount of h serum in order to determine if the increased surface would favor the separation of a second phase; these cultures also remained monophasic.

Seven of the cultures (150, CDAI 157, CDAI 222, CDAI 223, CDAI 363, CDAI 368, and Cal. 605) have been studied intensively in the Lexington laboratory as a part of a series of investigations on monophasic variants in the genus *Salmonella*. These strains have been cultivated in semisolid medium containing h antiserum for 1 year with biweekly transfers; also, all cultures were kept on agar slants for 1 year with monthly transfers. Cultures 150, CDAI 157, CDAI 222, and CDAI 223 were grown in broth containing 1:1,000 concentration of pure h serum and e,n. . . vaccine, and also in broth containing h serum and 1,2,3. . . vaccine. The vaccines were heavy suspensions from agar slants devitalized by heating at 58 C for 30 minutes; they flocculated well in homologous serum and were added in 0.1-ml amounts to tubes containing 2.0 ml of broth. The serum-vaccine-broth mixtures were inoculated with 1 loopful of a 1:100,000-dilution of a broth culture of the organism being tested; they were transferred at 5-day intervals and tested at each transfer for phase variation. Under none of these circumstances did a second phase appear.

Formalinized, motile broth cultures of all the monophasic variants flocculated well in a 1:1,000-dilution of e,h serum; when tested in suitable dilutions of single-factor serums a strong reaction occurred with h serum but not with n serum. Two of the strains from man (106-130 and Cal. 605) and one from turkey (CDAI 989) were used to test the extent of adsorption of agglutinins from the strong e,h serum prepared from the original monophasic hog culture 24682 (150). The results showed that all 3 cultures completely exhausted e,h agglutinins from the serum, thus indicating that the e,h phases of these 4 variants are very closely related if not identical. The same 3 cultures were employed in adsorbing the somatic antibodies IV, V, XII from *S. typhi-murium* (9) serum that initially agglutinated culture 106-130 to a dilution of 1:1,000. After adsorption by any one of the 3 strains (106-130, Cal. 605, CDAI 989) the *S. typhi-murium* serum failed to react in a 1:50-dilution with the usual alcohol-heat-treated antigens of any one of the 3 adsorbing strains or the homologous *S. typhi-murium*. The

O antigens of these monophasic strains are, therefore, very closely related to if not identical with those of *S. typhi-murium*.

The serologic studies here reported have, therefore, failed to shed any light on the derivation of the monophasic e,h variants.

DISCUSSION

The first known strain of the monophasic e,h *Salmonella* was isolated from a hog with enteritis in 1941. Subsequently the organism was recovered from turkeys on California ranches and on board a Naval ship in the vicinity of Pearl Harbor, T. H. It has also been isolated from the feces of an acute case of enteritis in man in California, and, more recently, a remarkably increased number of strains were isolated from Naval personnel in the Central Pacific. Some of the latter were asymptomatic at the time cultured (at least one of these denied any history of diarrheal disease); others were apparently sporadic cases of gastroenteritis; and still others were cases representing epidemics. Of further epidemiologic importance is the fact that food handlers were represented in the two categories, asymptomatic carriers and acute cases. Under these circumstances organisms of this type cease to be simple biologic curiosities and assume major public health significance as incitants of salmonellosis. The symptomatology of the infections is similar to the syndrome observed in other acute salmonelloses. Of the infections in man so far reported an appreciable number appear to have had their immediate origin in a lot of turkeys obtained from a Naval supply ship in the Pacific. At present, information as to the original source of these turkeys is not at hand. Evidence indicates, however, that this *Salmonella* type has been endemic on certain turkey ranches in California. Recently obtained data suggest also that the infection has been endemic in man in the Hawaiian area and at times becomes epidemic. An important epidemiologic principle will be demonstrated if subsequent information shows that the organisms were introduced into the Central Pacific by means of contaminated turkeys from California. Nothing is known regarding the source of the strain isolated at Bethesda, but it is entirely possible that the subject had duty either in California or Hawaii or both.

The abrupt increase in the number of typed strains of this organism and evidence of its widened dissemination revived interest in attempts to designate a diphasic ancestor of this monophasic variant. It is believed that the type developed as a result of "loss variation" in some other *Salmonella*, either known or as yet undescribed, and that the organism has become stabilized in monophasic form. Observations herein reported were concerned with the biochemic and serologic characteristics of 36 strains of the type. From the serologic standpoint, 8 established *Salmonella* types were considered as possible precursors of the variant because of their possession of the e,h complex in flagellar phase 1 and somatic antigens IV, XII or IV, V, XII. It is biologically possible, however, that the e,h factors were acquired by some other type through a process somewhat analogous to that described by Griffith (1928). This approach should be investigated further. The serologic observations described failed to shed any light on the question.

During a comprehensive restudy of the biochemic characteristics of the 36 e,h variants in comparison with those of some of the possible ancestors, particular attention was paid to the fermentation of rhamnose and inositol. The activity in rhamnose of the various organisms studied did not yield any new evidence, but an observation in inositol deserves comment. It was noted that in inositol the diphasic strain of *S. reading* (19) produced acid and gas, but the monophasic strain (150) was apparently inert. This finding is not inconsistent with a working hypothesis that the monophasic e,h type may have been derived from one of the diphasic organisms through a process of correlated variation (Topley and Wilson, 1938). The answer to this question will, however, have to await the discovery of more direct evidence, which may, perhaps, be obtained by chance observations.

SUMMARY

Attention is called to a recently observed increase in the prevalence and distribution of a monophasic *Salmonella* type with the antigenic formula IV, V, XII: e,h-. The organisms have been recovered from swine, fowls, and in man from asymptomatic carriers, sporadic cases, and epidemics of gastroenteritis. The strains appear to be endemic in California and Hawaii. It is suggested that the designation *S. reading* for this organism should be subjected to further scrutiny until more direct evidence of its probable diphasic ancestry is acquired. Certain of the clinical and epidemiological characteristics of infections due to this group of organisms were described. Extensive biochemic and serologic experiments cited were designed to detect, if possible, some clue as to the derivation of the monophasic variants. Serologic results were not fruitful, but the suggestion is made that the strains may have developed as a result of a biochemic variation correlated with an antigenic alteration. Some possible further lines of approach are mentioned.

REFERENCES

Barnes, L. A., Cherry, W. B., and Myers, W. A. 1945 A new *Salmonella* type isolated from man. J. Bact., **50**, 577–578.

Edwards, P. R., and Bruner, D. W. 1942 Serologic identification of *Salmonella* cultures. Univ. Ky. Agr. Expt. Sta., Circ. 54.

Gard, S. 1937 Ein Colistamm mit *Salmonella*-H-Antigen, zugleich ein Beitrag zur Frage der Definition der Salmonellagruppe. Z. Hyg. Infektionskrankh., **120**, 59–65.

Griffith, F. 1928 The significance of pneumococcal types. J. Hyg., **27**, 113–159.

Topley, W. W. C., and Wilson, G. S. 1938 The principles of bacteriology and immunity. p. 200. 2d ed. Wm. Wood and Co., Baltimore.