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Original Communications

STUDIES ON URINARY CARRIAGE OF ENTERIC GROUP ORGANISMS

VI.—ANTIGENIC VARIATION AND VIRULENCE OF ISOLATED STRAINS

BY

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THE fifth paper in this series (Archer, 1953) recorded the incidence of cultural and serological abnormalities among 58 strains of enteric group organisms isolated from the urine of carriers. The total was made up of 18 *Salm. typhi*, 30 *Salm. paratyphi A* and 10 *Salm. paratyphi C* strains. The opinion was also expressed that in many, but not all, cases, the deviation from the normal was transient and not due to dominant variation or persistent mutation. Cultural abnormalities in gas production and variants which grow as dwarf colonies on media lacking available sulphur were described in detail. Antigenic defects and the loss of virulence associated with roughness are described below. Strain numbers conform to strain numbers in the fifth, and excretor numbers in the second and fourth, papers in this series.

“H” → “O” VARIATION

It has long been known that *Salm. typhi* may have no “H” antigen on isolation. Bridges (1933) considered its absence fairly common, but very rarely prolonged *in vitro*. One of his strains remained aflagellate for about eight months. Colquhoun and Kirkpatrick (1932) described the use of soft agar to restore lost motility. More recently the lack of flagellar antigen in strains of *Salm. typhi* rich in Vi antigen and the effect of desoxycholate-citrate medium in suppressing flagellate growth have been observed. Hayes and Freeman (1945, 1946) note that

absence of motility and of flagellar antigens is often met in recently isolated salmonella strains, but that motility is usually rapidly restored by one or more sub-cultures in nutrient broth or by soft agar. (Such an "O" phase is probably a temporary adaptation to environment.)

Criteria of permanence of absence of flagella given by Hayes and Freeman are :

1. No microscopic evidence of motility after sub-culture in broth or soft agar.
2. Complete "H" inagglutinability.
3. Inability to absorb "H" antibody.
4. Inability to stimulate "H" antibody production.

A mutant should satisfy them all. The second and third are also met by variants. Variants will, however, eventually fail to meet the first ; for example, the 901-O variant of *Salm. typhi* becomes motile in soft agar with a varying degree of readiness which seems to depend upon the care which has been exercised on the maintenance of individual strains. Variants may also fail to satisfy the fourth of these criteria even while continuing to satisfy the second and third. Thus Bridges' most markedly "O" strain had possibly evoked an "H" titre of 1/150 and two rabbit sera contained "H" antibodies at a titre equal to 100 per cent. and 70 per cent. of the corresponding "O" titre after inoculation with a formolized suspension of a *Salm. typhi* 901-O strain, though this strain was still apparently adequate for the preparation of "O" suspensions. Even an alcoholized inoculum of such a strain was followed by the appearance of "H" antibody to 10 per cent. of the "O" titre, and similar results followed the use of formolized cholera "O" variants *in vivo*. On the other hand, an optimally maintained strain of *Salm. typhi* 901-O (kindly supplied by Dr. A. Felix, F.R.S.), though eventually acquiring motility as described above, evoked no trace of "H" antibody when directly sub-cultured to either broth or agar and used as an inoculum for rabbits, even after five doses of broth or of a saline wash from the agar.

In general, "O" variants seem to behave as though the "O" (non-flagellate) form is dominant, but a small minority of recessive "H" (flagellate) organisms normally continue to be produced. (A similar dominance of phase 2 has been postulated (Archer, 1938) in respect of salmonella phase 2 ("group") variants.) The excess of dominant "O" forms after growth under ordinary conditions will, on this hypothesis, always be such as to satisfy the second and third criteria quoted, but the recessive "H" forms which develop can spread in soft agar owing to their motility and hence be selectively isolated. If an "O" isolation is merely the result of an adaptation to environment, spread on soft agar should be rapid and uninterrupted, but "O" variation may be manifest by complete or local stoppage of spread and a clear-cut growth margin. The resumption of such arrested spread has been noted, with the production of a target-like growth effect on the plate. Enough recessive "H" forms may develop in simple cultures of an "O" variant to stimulate "H" antibody production *in vivo*.

Strains which fulfil the four requirements of Hayes and Freeman may be encountered. One is described in an early account of two carriers from our laboratory (Archer *et al.*, 1950), and Hayes and Freeman described two themselves (one *Salm. paratyphi A* and one *Salm. paratyphi C*). Our strain was, however, completely rough and degraded. Theirs would seem to be true mutants, but, unless actual development from a flagellate precursor has been studied, the alternative, that such an apparent mutant is a distinct aflagellate species, must be considered.

Isolations from Carriers

As might then be expected, strains lacking "H" antigens may be isolated from the urine of carriers. Nine of the eighteen *Salm. typhi* strains here considered showed this characteristic to some degree; of these, five also showed a tendency to roughness; six smooth strains of *Salm. paratyphi A* and four strains of *Salm. paratyphi C* (of which one tended to roughness) also showed this defect. (Certain rough *Salm. paratyphi A* strains may also have been primarily aflagellate, but this was not definitely shown. When dealing with such auto-agglutinable strains it was not easy to show an antigen lack without manipulations which of themselves might induce the development of flagella.) Some of such organisms readily become flagellate, while flagellate suspensions of others may be produced with more or less difficulty by the use of soft agar with subculture to similar media or to broth after spread, or by the direct preparation of suspensions by breaking up a soft agar spreading culture in saline and filtering through paper (Archer, 1941). It was thought that the latter method might be useful if reversion of sub-cultures to the "O" form were very rapid, or where spread had started and then stopped. It is often successful, but the necessity for it was not positively demonstrated during this work. Even *Salm. typhi* 901-O did not tend to revert to the "O" form once flagellate growth was induced. This was unexpected and is difficult to reconcile with a theory of "O" dominance in the strain.

Salm. typhi strains lacking "H" antigen

One smooth strain yielded "H"-agglutinable cultures from soft agar; there is no complete record of the other three and of one rough strain.

Another rough strain became "H"-agglutinable in soft agar. The remaining three rough strains at first failed to spread in it. Of these strain No. 20 twice failed to spread when first isolated; when tested from a later specimen it did spread; later still both the large and dwarf colony forms previously described failed to do so. Finally, seven of a number of cultures from carrier No. 20, made before starting hexamine treatment (Archer and Naylor, 1952), were inoculated into Craigie tubes. Flagellar antigen *d* could not be demonstrated from four of them after two, nine, ten and thirteen days respectively, but was found in the remaining three cultures after two, three and eight days respectively.

One strain (No. 10) which has not been included in this category (since on first isolation and on most other occasions the "H" antigen was present) was also re-isolated repeatedly before hexamine treatment of the patient. Cultures isolated from eight specimens were inoculated into Craigie tubes. Three of these showed no flagellar antigen after three, four and five days respectively.

Salm. paratyphi A strains lacking "H" antigen

Six smooth strains and one rough lacked "H" antigen on primary isolation. One of these smooth strains (No. 14) also showed anaerogenic tendencies. As with *Salm. typhi*, one strain of *Salm. paratyphi A* (No. 9), which is not included in the "H"-deficient category since it was flagellate on first isolation, was later frequently isolated as a prelude to treatment of the carrier. Two of eleven Craigie tube cultures took five and six days respectively before "H" antigen *a* was demonstrable. It is also of interest that, though this strain was anaerogenic on first isolation, these pre-treatment cultures produced gas regularly in MacConkey-mannite and in the appropriate peptone water "sugars."

Salm. paratyphi C strains lacking "H" antigen

Three smooth strains of *Salm. paratyphi C* were not "H"-agglutinable on isolation. Strain No. 6 (rough) would not spread in soft agar after first isolation. Cultures from two further urine specimens from this carrier were agglutinable by "H" serum. Two still later isolations each required passage of the supernatant of rough broth cultures through soft agar three times before antigen *c* could be demonstrated.

This series therefore shows, in general, little more than might be attributed to adaptation, though *Salm. typhi* strain No. 20 and *Salm. paratyphi C* strain No. 6 showed some evidence of a tendency to genuine "O" variation, as did, to a less degree, two other rough typhoid strains.

The influence of "H" antibodies in vivo

The effect of "H" antisera in inducing "O" growth *in vitro* is recognized and counteracted by soft agar in methods used to induce phasic variation (Archer, 1941).

Hayes and Freeman speculate on the possibility of an apparently unstable phasic variant of one of their strains of *Salm. paratyphi A* being due to "H" antibodies in the patient's blood. That this might be a determining factor for flagellar absence both *in vitro* and *in vivo* on isolation from inoculated persons had occurred to me with regard to "O" cultures isolated in 1933 from two cases of typhoid fever. Both these strains became "H"-agglutinable in soft agar, but both tended to revert to "O" later.

At that time tests were made on the effect of homologous "H" antibody on living flagellate *Salm. typhi* either growing in blood-bile and blood-taurocholate mixtures, or when injected intravenously into immunized rabbits. The blood mixtures were also titrated. Evidence on induced loss of "H" antigen was

inconclusive. Apparent damage to "H" antibody was noted in some, but not in all, preparations of both types of blood mixture. These experiments were, however, unsatisfactory in that both "H" and "O" antibodies were present in the immunized rabbits, a standard strain was not used as the living inoculum, and blood cultures from the rabbits were all in fluid medium.

Infection of immunized Rabbits and Mice

Further experiments using rabbits and mice immunized with *Salm. muenchen* to produce "H" but not "O" antibody for *Salm. typhi*, and a living inoculum consisting of a suspension of *Salm. typhi* 901-H (intravenously to rabbits and intraperitoneally to mice) were carried out. Rabbits were bled and mice killed at intervals. Cultures of rabbits' blood were made at approximately 1/10 dilution in 2 per cent. taurocholate solution, and in glucose broth, by adding 1.0 ml. blood to 9.0 ml. of each medium. Blood was also plated direct on agar and MacConkey. The blood titre of each immunized rabbit had been determined before the living dose was given and the titre of a number of rabbits' blood cultures was tested. Cultures of mouse heart-blood and peritoneal washings were made on agar and MacConkey. Blood was pooled from each immunized batch for estimation of its average titre.

Results: Rabbits.—1. Blood cultures from only one of four immunized rabbits were found to be positive. This animal had a titre of 1/2500 for flagellar antigen *d* and had received a dose of 1,000 million living *Salm. typhi*. The other animals had received 1, 10, and 100 million *Salm. typhi* respectively. The positive cultures were that in glucose broth made four hours, and those in both glucose and taurocholate made seven hours, after the living dose, sub-cultures from these gave a growth after one, three and five days' incubation respectively. All were "H"-agglutinable.

2. All other cultures (made four, seven, 24 and 48 hours after inoculation) were negative. Hence 2 ml. of blood contained no *Salm. typhi* four hours after doses of 1, 10 and 100 million. A dose of 1,000 million yielded no *Salm. typhi* from drops of blood on direct plates four hours after, and none from 2 ml. blood 24 hours after, it was injected.

3. Culture titres indicated little loss of "H" antibody in either medium after one to seven days in 12 of 14 tests. Of the remaining two, one showed early loss of more than two-thirds of the "H" antibody in sterile taurocholate, and the other a four-fifths fall after seven days in a positive glucose culture. "O" (*muenchen*) titres were <1/10 in taurocholate (indicating that less than one-fifth antibody remained), while all, two-fifths, and less than one-fifth of the added "O" antibody was present in three glucose broth cultures, after five to seven days.

Mice.—Batches of eight immunized mice received 5, 20 or 100 million living *Salm. typhi* 901-H and uninoculated control mice received 5 or 100 million, intraperitoneally, two immunized mice at each dose being killed after five, 24 or 48 hours. After five or 24 hours, two control mice were also killed.

After five hours: (i) Blood showed scanty to numerous organisms and the peritoneal washings a moderate to heavy growth. Fifteen heart-blood colonies from immunized animals and 15 from controls were inoculated to broth; all were "H"-agglutinable. Nine colonies from immunized animals were subcultured to agar; saline suspensions of all were "H." Growth on six original plates from immunized animals was washed off and the suspensions tested directly with "H" antiserum; all showed partial agglutination, though one only to a slight degree. The plates from the peritoneal washings were also washed off; the suspensions produced were all "H"-agglutinable. (ii) "H" titre of the blood-pool was 1/125.

After 24 hours: (i) Blood was almost cleared; only three colonies in all, from two of six immunized mice, were present on plates. All produced "H" suspensions after subculture to solid media and wash-off. Peritoneal growth was much reduced in these six cases. Broth and agar subcultures, and plate washings, were all "H." (ii) "H" blood-pool titres (two pools) were 1/1,000 and 1/500 tr.

After 48 hours: (i) Blood was negative in all six immunized mice; peritoneum was negative in three cases, the remaining three yielding a total of seven colonies. Five subcultured were "H." Two plate washes made after spreading from the colonies were only poorly agglutinated by "H" antiserum. (ii) "H" blood-pool titres (two pools) were 1/250 for each.

Clearance of Infection.—The blood of mice surviving intraperitoneal injection of 5-100 million *Salm. typhi* 901-H was generally cleared in 24 hours and invariably cleared in 48 hours. The peritoneum was almost totally cleared in 48 hours.

Comment.—No evidence of impressed "O" variation and little or none of "O" adaptation owing to "H" antibody in the host was obtained by experiments on rabbits and mice. In both groups of animals the blood was so rapidly cleared of living organisms as to offer no fair comparison with a human infection. "H" antibody does not appear to be damaged by sodium taurocholate in 2 per cent. solution. The possibility remains that in urinary carriers who are also excreting urinary antibodies these may play a part in producing an adaptative loss of "H" antibody or even an impressed variation. No support is afforded to this possibility, however, by *Salm. typhi* strain No. 17, which was "H"-agglutinable on two occasions when "H" antibody was present at titres of 1/10 tr. and 1/20 tr. in the urine from which isolation was made.

ROUGHNESS AND VIRULENCE

Roughness of strains of *Salm. typhi* passed by urinary carriers has been described by Dunbar (1948), and the roughness and low virulence of a presumed strain of *Salm. typhi* and the roughness of a *Salm. paratyphi C* strain by Archer *et al.* (1950) in previous reports from this laboratory. Indeed, it later came about that rough growth of an organism from urine would suggest to us that it was

probably a member of the enteric group. The number of strains showing some degree of a rough tendency among those here under consideration was 9 *Salm. typhi*, 9 *Salm. paratyphi A* and 2 *Salm. paratyphi C*.

For some time a discrepancy between native carrier incidence and military case incidence for different enteric species has been noticed. The first is illustrated by the respective numbers of the different species among the strains in this series and by the carriers found in 1949-1950, which were 31 T, 35 A, 1 B, and 25 C. Case incidence was 32 T, 15 A, 17 B, and 1 C in the latter year. The number of cases may well, of course, be a misleading index of the number of sources of infection as one such source may produce single or many cases, depending upon the circumstances involved (e.g., contamination of food consumed by one or many). Five of the paratyphoid A cases and 10 paratyphoid B cases among those quoted above appear, in fact, to have represented single incidents. Nevertheless the extreme rarity of para B carriers (while cases occur) and of para C cases (while carriers abound) needs explaining. Possible causes might include a low incidence of paratyphoid C fever because the carriers have been eliminated by discovery; by contrast, the very failure to discover excretors of *Salm. paratyphi B* could result in infections occurring due to their undetected presence. Such a difference in success in screening might in turn be due to general chronicity (hence easy detection) of *Salm. paratyphi C* excretors and a high proportion of transient or intermittent urinary carriers, or even a predominance of faecal carriage, of *Salm. paratyphi B*. In the latter connection the possible roll of *Salm. paratyphi B* as a cause of gastroenteritis rather than of enteric fever merits consideration since, when the latter has occurred, urinary excretion during convalescence apparently follows in 3.4 per cent. of patients—only slightly less often than it does in typhoid fever (Vogelsang and Boe, 1948). The paratyphoid B problem needs further investigation. With regard to *Salm. paratyphi C*, the follow-up tests recorded in the second paper of this series do not suggest a general chronicity among carriers of this species, and other possibilities are that low virulence of *Salm. paratyphi C* excreted in urine is the rule and that an excretor of an avirulent strain may present no risk as a source of infection. *Salm. typhi* also, however, often appeared degraded in carrier urine and certain strains have been found intractably rough.

It thus seems of some importance to determine the degree of degeneration and loss of virulence in carrier strains; the proportion of excretors of avirulent organisms among carriers; and the regularity with which the organisms passed by individuals among them are avirulent. Findings for this series of strains is given in Table I (the figures for rough cultures are minimal as the recording of rough growth may at times have been omitted); the numbers of organisms, and proportion of rough cultures, passed by different carriers are shown in Table II, and the virulence of certain strains in Table III.

In general, roughness was more often found in strains of *Salm. typhi* than in paratyphoid strains. Though marked roughness at all times of observed excretion was found in one typhoid and one paratyphoid A strain, lesser degrees of

roughness, and the isolation of the same strain from its carrier sometimes in a rough and sometimes in a relatively smooth condition, seem more common. Fully virulent strains of *Salm. typhi* and *Salm. paratyphi A* were met. *Salm. typhi* was more often found excreted in very large numbers than was *Salm. paratyphi A*. There seem to be no grounds for general disregard of excretors of rough organisms as harmless carriers; nor, though Findlay (1951) has shown agreement between mouse-virulence of strains and the severity of the disease caused by them in human outbreaks, can we be sure that strains of low mouse-virulence cannot, particularly in large doses, infect man and their virulence perhaps be enhanced in him. Indeed, analogous evidence in favour of the ability of apparently degraded enteric strains to produce true and severe infections is afforded by a recent test in which intraperitoneal doses of approximately 1,000 *Salm. paratyphi C* from an apparently very rough culture killed 11/24 mice in from eleven to fifteen days. Further examination of the *Salm. paratyphi C* strain (an old one) showed that roughness was by no means complete and the organism was recovered in a smooth state from the spleens of the five dead animals examined *post mortem*.

TABLE I.—INCIDENCE OF ROUGH STRAINS AND ROUGH CULTURES

Species	Proportion of Strains showing Rough Tendency		Frequency of incidence of Rough Cultures of Strains which were repeatedly isolated (R/total and r/total. R=very rough; r=slightly rough) [Also proportion of cultures of <i>Salm. typhi</i> tested for the presence of the Vi antigen which were found to contain it.]																																								
	All isolated Strains (R/total)	Strains which were repeatedly isolated (R/total)																																									
<i>Salm. typhi</i>	9/18 (1)	6/9 (1)	<table style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="5" style="text-align: center;">STRAIN</td> </tr> <tr> <td style="text-align: center;">No. 1</td> <td style="text-align: center;">No. 2</td> <td style="text-align: center;">No. 4</td> <td style="text-align: center;">No. 10</td> <td style="text-align: center;">No. 17</td> </tr> <tr> <td>R 10/19</td> <td>8/23</td> <td>8/19</td> <td>10/25</td> <td>5/5</td> </tr> <tr> <td>r 1/19</td> <td>2/23</td> <td>2/19</td> <td>3/25</td> <td></td> </tr> </table> <table style="width: 100%; border-collapse: collapse;"> <tr> <td colspan="5" style="text-align: center;">{ Vi agglutination +/No. of tests }</td> </tr> <tr> <td colspan="5" style="text-align: center;">STRAIN</td> </tr> <tr> <td style="text-align: center;">No. 1</td> <td style="text-align: center;">No. 2</td> <td style="text-align: center;">No. 4</td> <td style="text-align: center;">No. 10</td> <td></td> </tr> <tr> <td style="text-align: center;">2/5</td> <td style="text-align: center;">5/7</td> <td style="text-align: center;">6/11</td> <td style="text-align: center;">10/11</td> <td></td> </tr> </table>	STRAIN					No. 1	No. 2	No. 4	No. 10	No. 17	R 10/19	8/23	8/19	10/25	5/5	r 1/19	2/23	2/19	3/25		{ Vi agglutination +/No. of tests }					STRAIN					No. 1	No. 2	No. 4	No. 10		2/5	5/7	6/11	10/11	
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<i>Salm. paratyphi A</i>	9/29 (2)	2/10 (3)	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">R</td> <td style="text-align: center;">Strain No. 5 4/16</td> <td style="text-align: center;">Strain No. 51 8/8</td> </tr> </table>	R	Strain No. 5 4/16	Strain No. 51 8/8																																					
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<i>Salm. paratyphi C</i>	2/10	1/2	<table style="width: 100%; border-collapse: collapse;"> <tr> <td style="text-align: center;">R</td> <td style="text-align: center;">Strain No. 6 5/6</td> </tr> </table>	R	Strain No. 6 5/6																																						
R	Strain No. 6 5/6																																										

- (1) The totals of 18 and 9 contain Strain No. 13, *not* included in the number shown as having a rough tendency but which produced a rough culture once out of 11 tests (see also under "virulence" and "numbers excreted").
- (2) One strain omitted owing to insufficient record.
- (3) The total of 10 includes two strains which produced smooth growth on each of the only two and three occasions when cultures were positive.

TABLE II.—PROPORTION OF ROUGH ISOLATIONS FROM CASES AND NUMBERS OF ORGANISMS EXCRETED BY THEM

Species	Strain	Frequency of Rough Cultures (Sum of R and r in Table I)	Numbers Excreted	
			No. of Counts	Range
<i>Salm. typhi</i>	1	11/19	2	20,000—23,000
	2	10/23	3	1,500,000—20,000,000
	4	10/19	3	50,000—1,000,000
	10	13/25	2	5,000—160,000
	13	1/11	1	2,000,000
<i>Salm. paratyphi A</i>	5	4/16	1	68,000
	9	0/70	2	700—1,700,000
	12	0/54	1	500,000 (1)
	18	0/16	2	200,000—400,000
	19	0/43	5	1,000—50,000
	21	0/26	4	21,000—2,000,000

(1) An intermittent carrier showing evidence of very wide variation in numbers excreted.

TABLE III.—ROUGHNESS AND VIRULENCE

Species (normal A.L.D. (L.D.50) and M.L.D. (L.D.100) for mice)	Strain	Rough Tendency (R/total cultures)	Virulence for Mice				A.L.D. million
			Critical Doses				
			Lower		Higher		
			Dose*	Survivors, all Recipients	Dose*	Survivors, all Recipients	
<i>Salm. typhi</i> (L.D.100 : 75 mill., L.D.50 : 50 mill.)	13	1/11	million 100	5/10	million 200	0/10	100
	17	5/5	100	10/10	200	2/10	> 100 < 200
	20	(Frequently rough)			400	14/16 8 mice each, for "Large" and "Dwarf" types. 2 inoculated with "Large" died.	> 400
	31	Single isolation : Smooth	50	5/10	100	3/10	50
	32	Single isolation : Rough			200	8/10	> 200
<i>Salm. paratyphi A</i> (L.D.100 : 250 mill.)	9	0/70	150	4/10	300	2/10	150
	12	0/54	150	5/10	300	1/10	150
	21	0/26	300	1/6	600	0/6	< 300
	Controls	<i>Salm. typhi</i> Ty 2	50	3/6	100	0/6	50
		<i>Salm. paratyphi A</i>	150	4/6	300	1/6	< 300

* As determined by Brown's opacity tubes.

NOTES ON TABLE III

1. *Dosage*.—Virulence testing in mice was carried out by intraperitoneal injection of a suspension of growth on agar. The number of mice was too small for an accurate determination of the L.D.50, but results satisfactorily demonstrated either approximation to, or gross variation from, the normal. Dosage was determined by an opacity count using Brown's tubes. It is now commonly held that true counts are generally about twice those obtained by the use of Brown's tubes and tables. The doses here recorded, however, were checked by viable counts using drops of serial dilutions on plates.

Findings include the following :

<i>Salm. typhi</i>	Strain 13 } Strain 31 }	opacity and viable count agreed to within 5 per cent.
<i>Salm. paratyphi A</i>	Strain 20 (Large), opacity 133 per cent. of viable count. Strain 9, opacity 75 per cent. of viable count. Strain 12, opacity 66 per cent. of viable count.	

In counts on strains 17, 20 (Dwarf) and 32 this viable check failed. This was attributed to roughness, to the degree of which failure seemed to be proportional. Strain 32—the roughest—was examined microscopically. Long forms were common and the deposit in a broth culture remained clumped after mixing by drawing in and out of a pipette thirty times. To try to demonstrate the effect of clumping on colony counts, some were made on the rough strain 17 and the very rough strain 32 before and after shaking up the suspensions with beads in an attempt to disperse such aggregations. No significant difference was found in the counts on strain 17 : that on strain 32 was four times greater after shaking than before.

2. *Correlation between Roughness and Virulence*.—*Salm. typhi* (strains 13, 17, 31 and 32). As might be expected, this correlation was close. The table shows an ascending A.L.D. of these strains in the order 31, 13, 17 and 32 : strain 31 was the smoothest, growing with little deposit in broth ; strain 13 was also smooth but showed some deposit in broth ; strain 17 grew with slight turbidity in broth and took over one hour to start clearing (without coarse flocculation) after shaking ; strain 32 grew as a deposit, the supernatant remaining clear. When this culture was shaken up it began to settle again with coarse flocculation in a few minutes.

Salm. paratyphi A: Though 30 per cent. of the strains showed a tendency to roughness (and strains 51 and 55 were so rough as gravely to hinder identification), the three strains whose virulence was tested were unfortunately all smooth.

Salm. paratyphi C: No virulence tests were carried out on the strains of this species under study. An A.L.D. of a rough strain was, however, reported as of the order of 400 million in an earlier paper (Archer *et al.*, 1950) and this was considered as low for the species. The determination of mouse-virulence for *Salm. paratyphi C* is complicated, however, by the fact that, as Kauffmann (1936) has shown, this species is capable of setting up a true infection in mice so that quite small doses (1,000–10,000 organisms) may prove fatal in five to twelve days. He also demonstrated that with very high dosage (500 million organisms) rapid death is produced (as by the other enteric group species), but it is not possible from his figures to estimate an A.L.D. for this rapid (?toxic) killing effect. Quick death was all that was noted in respect of the rough strain referred to above. Recent observations suggest, however, that the rapid death (within 72 hours) mouse L.D.100 of *Salm. paratyphi C* is approximately 200 million organisms and the L.D.50 (A.L.D.) 100 million organisms, hence the earlier recorded A.L.D. of 400 million for the rough strain is probably correctly considered as indicating low toxicity, though no deduction can be drawn as to the invasiveness of that strain since observation was not sufficiently prolonged, and doses below 100 million were not used.

3. *Degrees of Roughness*.—The greater instability of *Salm. typhi* strain 32 than of strain 17 in broth suspensions and the marked roughness of *Salm. paratyphi A* strains 51 and 55 have already been mentioned. Other evidence of marked loss of the smooth somatic antigen is afforded by the record of sub-cultures necessary before suspensions became sufficiently stable for agglutination tests to be performed. Thus, using serial cultures in broth, plates and Craigie tubes, results with four strains were as follows :

<i>Salm. typhi</i>	After isolation of strain 54, five serial broth cultures were needed before one was stable enough to test.		
	After one of the isolations of strain 17	} 4 broths, 3 Craigie tubes and 2 plates	} were used before sufficient stability for an "H" antigen test, even in 0.45% NaCl, was reached.
<i>Salm. paratyphi A</i>	With a sub-culture after isolation of strain 34		
	After one isolation of strain 51	} 6 broths, 3 Craigie tubes and one plate	

SUMMARY AND CONCLUSIONS

1. Lack of flagellar antigen and rough change among 58 enteric group strains isolated from urinary carriers in Egypt, and the virulence for mice of eight of the strains (5 *Salm. typhi* and 3 *Salm. paratyphi A*), is recorded.

2. Nineteen of these strains were found on occasion without flagella. In two of them there was some evidence that the strain showed dominant variation to the aflagellate form. Tests on rabbits and mice gave no evidence that "O" adaptation or variation may be impressed by pre-formed antibody *in vivo*. Though this at first seems surprising, being against experience *in vitro*, absence of signs of multiplication with, on the contrary, rapid elimination of the injected organisms suggests that the tests were inadequate as an index of what might occur in human infections.

3. Twenty of the strains were isolated rough on one or more occasions. Both fully mouse-virulent and apparently completely degraded strains were met, but degradation, when present, was more commonly incomplete, and individuals produced cultures at different times that were unlike in the apparent extent to which the organisms were degraded. The numbers of times on which a strain was found rough or smooth when isolated from different specimens from an individual may have depended upon the state of all (or at least the great majority) of the organisms in each such specimen or, perhaps more probably, have been mainly the result of chance when picking colonies from plates. If chance were the chief cause of the differences noted, they will have arisen from the random picking of either rough or smooth colonies from plates on which both were always present in a similar ratio, the plates having been inoculated with urine specimens containing rough and smooth organisms in relatively constant, rather than widely differing, proportions.

In either case the ratio of rough to smooth cultures found should afford an indication of the over-all degree of strain degradation. The degree of its fluctuation from specimen to specimen, however, remains to be assessed. In the present state of our knowledge many tests would be needed to prove degradation complete and we cannot ignore, as harmless, the excretor whose organism is found to be rough on routine screening.

No adequate explanation for the low incidence of Service cases of paratyphoid C fever in the Canal Zone of Egypt, in spite of the presence of *Salm. paratyphi C* carriers, is put forward. It does not appear to be due to carrier strains of this species generally degraded.

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**NON-OPAQUE FLOATING GALL-STONES AND THEIR
RADIOLOGICAL DEMONSTRATION**

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INTRODUCTION

This article may well begin with a quotation from Kerley's (1950) chapter on the Biliary Tract in "British Authors": "There are an increasing number of reports of floating gall-stones, i.e., small stones floating on top of concentrated bile, and it seems likely that with routine hospital technique these stones are being overlooked and the gall-bladders passed as normal." The literature is very scant on the subject. Brailsford described the condition in an article in the *British Journal of Surgery* in 1938, when he observed: "In the routine cholecystographic examination it is advisable to take one or more of the serial radiographs with the patient in the *erect* position, for this will sometimes bring out features which may be unrevealed or unsuspected from the radiographs taken in the prone position." (The italics are mine.)

COMPOSITION OF GALL-STONES

Gall-stones usually consist of differing amounts of bile salts, cholesterol, and calcium salts. When there is bilirubin-calcium present, the stones will be opaque. In its absence, or, in other words, when they are composed wholly of cholesterol, the stones will be non-opaque. It is with these latter stones that we are concerned.

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Studies on Urinary Carriage of Enteric Group Organisms: VI. —Antigenic Variation and Virulence of Isolated Strains

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