

TWO URINARY CARRIERS OF ENTERIC GROUP ORGANISMS PRESENTING SOME INTERESTING FEATURES

BY

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WHILE in general it would appear that faecal carriers of enteric group organisms are a greater source of danger than urinary, both on account of their higher incidence, and greater liability to chronicity or permanence (Vogelsang and Bøe, 1948), it has been shown that in Egypt urinary carriage is much more frequently detected than faecal (Ashton, 1947; Walton, 1949). It is also likely that, other things being equal, a urinary carrier will be the more dangerous type as a foodhandler since unnoticed contamination of the hands is more probable.

It was therefore decided to investigate further a number of urinary carriers detected in Egypt with special reference to the following aspects of urinary carriage :

(1) The duration of passage of the organism concerned in the urine and the possibility that this may be related to pre-existing urinary disease, especially schistosomiasis. Certain workers have suggested a possible relationship between these conditions, and a comparable correlation between pathological conditions of the gall-bladder and prolonged faecal carriage has been demonstrated (Vogelsang and Bøe).

(2) The virulence of the organisms voided in the urine. One of us (Dunbar, 1948) has shown that the organisms found in such cases may be rough and phage-contaminated. The influence of phage in inducing bacterial variations is known to be considerable and such modified strains may well be attenuated in virulence.

(3) The number of organisms passed ; since this is likely to influence the degree of infectivity and may be of importance in determining whether or not a concentration technique such as filtration, with use of the filter pad as an inoculum (Ashton), precipitation (Rappaport and Rosenknopf, 1948), or the use of selective media is necessary in the detection of carriers. Since Vogelsang and Bøe base their figures on direct plating their findings might not be comparable with those of workers using a concentration technique if such should prove to be necessary.

(4) The value of serological methods such as the demonstration of the presence

of urinary antibodies, faecal antibodies (Burrows and Havens, 1948; Harrison and Banvard, 1947) or, in appropriate cases, Vi antibodies in the blood, in the detection of carriers, and the estimation of their probable chronicity.

Though this investigation is in its early stages two cases are recorded presenting the following interesting features: carriage so far for over nine months; the passage of great numbers of organisms of low virulence; the presence of urinary antibodies; concurrent urinary schistosomiasis.

TECHNIQUE

(1) *Culture*.—Urine was collected in sterile bottles on three successive days at fortnightly intervals from each individual and brought straight to the laboratory. The first two specimens were kept in the refrigerator until the third had been obtained, when a pooled specimen of 30 ml. was prepared from the three from each person, incubated with an equal volume of selenite medium and subcultured. In addition all specimens were examined by adding known volumes of fresh urine to melted nutrient agar at 55°C., mixing by inversion, pouring plates, and incubating.

Isolated organisms were inoculated into "sugar" media and when necessary an attempt was made to promote flagellar agglutinability by passage through soft agar in Craigie tubes or plates. Viable counts in urine were made by using serial dilutions by the method of Miles and Misra, or by the inoculation of molten agar which was then poured as plates.

(2) *Virulence*.—A suspension was prepared by washing off the growth from overnight agar slopes. The bacterial content was determined by the use of Brown's opacity tubes. The suspension was tested for purity by inoculation of sugars and plates and 16–20 mice were inoculated intraperitoneally in batches of 4–6 with various doses of organisms contained in 0.5 ml. suspension and the survival rate after three days determined. Although it was realized that these numbers were too small for an accurate determination of the A.L.D., no more were available, and it was thought that the test as performed would indicate any gross change in virulence.

(3) *Urinary Antibodies*.—These were investigated by testing the urine against standard agglutinable suspensions by Dreyer's method without preliminary dilution. Tests were also carried out on urinary globulin concentrates. The results were compared with serum titres, and the albumen/globulin ratio in serum and urine was determined.

(4) *Schistosomiasis*.—The centrifuged deposit of the urine was microscopically examined for the ova of *Schistosoma hæmatobium*.

RESULTS

Case 1.—History.—This man, an Egyptian aged 19 years, was examined as a possible carrier in August 1948, apparently in connexion with a small outbreak of Paratyphoid B fever. An organism was isolated from his urine, which was thought to be *Bacterium paratyphosum B*. This was sent to the Central Pathology Laboratory, M.E.L.F., for typing. Results obtained were inconclusive and the strain, with others of established identity, was sent to the Central Enteric Reference Laboratory, London. It was found

to be rough and was passed to the Salmonella Reference Laboratory. Here the evidence of the "C" somatic structure (antigens VI and VII) was suggestive but owing to persistent roughness even after mouse passage identity could not be confirmed.

Present Investigation.—Examination of the urine was resumed in April 1949. In plates containing 3.0 ml. and 0.5 ml. urine a very heavy growth occurred. This consisted of an apparently pure culture of an organism which fermented glucose, mannite and dulcitol—but not lactose or saccharose—with the production of acid and gas. Indol was not formed. The growth in broth consisted of a granular deposit, the supernatant medium being generally quite clear. Attempts to determine the antigenic structure of this organism showed at first an absence of flagellar antigens c, b, 1, 2 or 5. In tests for somatic antigens some suspensions seemed sensitive to C. "O" serum but a tendency to auto-agglutination of reacting suspensions made these results inconclusive. Use was therefore made of soft agar cultures, by which means the antigen c was readily demonstrated. Certain subcultures also grew in broth with a degree of general turbidity and reacted satisfactorily with Para. C. "O" serum. This organism was provisionally identified as *Bact. paratyphosum C*. Subcultures were submitted for confirmation of identity to the Army Vaccine Laboratory. Here the flagellar antigens c, 1, 5 were identified but repeated attempts to differentiate between the somatic structure of *Bact. paratyphosum C* and *Bact. cholerae suis* were unsuccessful. As regards biochemical activity the strain fermented arabinose but failed to ferment trehalose. This organism was invariably found in examinations carried out as described over a period of eight weeks.

Concurrent Disease.—All specimens of urine examined were turbid due to the presence of pus. Ova of *S. hæmatobium* were found on microscopic examination.

Virulence.—The average lethal dose (L.D. 50) for mice inoculated as described was found to be of the order of 400 million organisms. Recovery of organisms from the heart-blood of a mouse dying after inoculation with 1,000 million of the organism under investigation showed it to be still rough.

Weight of Infection.—A viable count carried out on one specimen of urine showed the presence of 40–50 million organisms per ml. urine. A blood culture was sterile.

Urinary Antibodies.—The urine passed on three occasions was tested for the presence of antibodies at titres of from 1/2.5 upwards. Serum antibodies were also investigated, at titres rising from 1/20. No antibodies were detected for the antigens b, VI or VII in either urine or serum but a standard Para. C. "H" specific suspension (antigen c) was agglutinated to 1/12.5 by each specimen of urine and to 1/320 by the serum which also agglutinated a VI typhoid suspension to the same titre. The last-mentioned reaction suggests that the isolated organism was in fact *S. paratyphosum "C"* while the absence of antibodies to b. adds support to the suggestion that this individual never was a carrier of *Bact. paratyphosum B*. Differential urinary and serum protein estimations showed an inverse A/G ratio in both; that for the serum being 0.65 : 1, and for urine 0.5 : 1.

Urinary percentages were approximately 1/10 of the serum figures. Titration of 10 times concentrated urinary globulins against antigens c and VI and VII gave positive results at 1/320 and 1/20 respectively.

Case II.—History.—There was some possibility of mistaken identity in this case as in June 1948 *Bact. typhosum* was isolated from a native foodhandler whose name was recorded as I. H. I.; on reporting this, however, it was discovered that no person of this name was known but that there was a cook I. M. I. Specimens from the latter were therefore examined with a negative result. Following the occurrence of cases of typhoid in June 1949, however, the urine of I. M. I. was again tested with the following result.

Present Investigation.—A very heavy growth of an organism fermenting glucose mannite and dulcitol—but not lactose or saccharose—with the production of acid only was obtained. Indol was not formed in peptone water. All attempts to demonstrate flagellar or somatic antigens of *Bact. typhosum* failed even following repeated cultures in soft agar, and colony selection. Two rabbits were therefore immunized with formalized cultures but subsequent

tests failed to demonstrate the production of antibodies to Typhoid "H" or "O" antigens, and the culture was insensitive to Vi phage. The biochemical reactions therefore remain the sole evidence of identity. The organism was found on each of the three series of examinations carried out.

Concurrent Disease.—Ova of *S. haematobium* were present in the urine.

Virulence.—All six mice survived a dose of 150 million organisms intraperitoneally for seventy-two hours, though the A.L.D. of a fully virulent strain of *Bact. typhosum* is of the order of 40–50 million organisms. Two mice were killed five days after inoculation and the heart blood cultured. Very scanty growth of rough organisms giving the biochemical reaction of the inoculated strain was obtained.

Weight of Infection.—A viable count carried out in the urine of this individual showed that the specimen contained approximately 60 million organisms per ml.

Urinary Antibodies.—The urinary protein was approximately one-eighth of the serum protein concentration. In this case the A/G ration was almost three times as high in the urine as in the serum. Serum antibodies showed a titre of 1/20 for T. "O" and 1/250 tr. for T. "H." Solutions of urinary protein of 10 times the concentration found in the urine gave titres of 1/20 for T. "O" and 1/40 for T. "H." The presence of these antibodies supports the identification of the isolated organism as *Bact. typhosum*.

Further observations on the weight of infection in urinary carriers.

Viable counts of organisms in the urine of 6 other carriers were made. In 3, ova of *Schistosoma haematobium* were also present. These included 1 carrier of *Bact. typhosum* whose urine contained 200 million organisms per ml., and 2 of *Bact. paratyphosum A.* with 7 million and 1 million organisms per ml. respectively. A fourth, in whose urine large quantities of blood and pus were present though ova were not seen, yielded 6 million *Bact. paratyphosum A.* per ml. The remaining two, both carriers of *Bact. typhosum* with no evidence of schistosomiasis, passed urine containing 9 million and 3 thousand organisms per ml. respectively.

SUMMARY AND CONCLUSIONS

(1) Two cases have been described in which there was prolonged urinary carriage by individuals with urinary schistosomiasis of enteric group organisms which were antigenically rough, of low virulence, and regularly present in enormous numbers.

(2) The influence of pre-existing disease on the persistence of the carrier state requires further investigation. Vogelsang and Bøe find that urinary excretion can in most cases be stopped by urinary disinfectants. Recent improved methods for the rapid and effective treatment of urinary schistosomiasis suggest the desirability of experimental treatment of all enteric urinary carriers liable to this complication with suitable urinary antiseptics to ascertain if, in those in whom it is actually present, cure of the schistosomiasis is an essential preliminary to an attempt to eliminate bacteria from the urine.

(3) The two organisms tested showed a low virulence for mice. This was associated with obvious antigenic roughness. It is perhaps not without significance in assessing the role of urinary carriers that whereas carriers of *Bact. paratyphosum C.* are relatively common as compared with carriers of the other enteric group organisms the incidence of paratyphoid fever due to this species is almost nil in the population at risk under observation.

(4) Urinary antibodies were demonstrated in low titre in the two cases

described. The ratio between urinary and serum antibody, however, would appear to suggest a leak of serum globulin into the urine from lesions of the urinary tract rather than the active antibody production there postulated by Burrows and Haven following findings in guinea-pigs.

Such a leak in the first case is further suggested by the identity of the A/G ratio in serum and urine.

(5) The presence of urinary antibodies may account for the antigenic deficiencies of the organisms passed by favouring the multiplication of a rough non-motile mutant, or such an effect may be produced by the influence of bacteriophage.

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