STUDIES ON URINARY CARRIAGE OF ENTERIC GROUP ORGANISMS

III.—URINARY AGGLUTININS IN UNINOCULATED CHRONIC URINARY CARRIERS

BY

Colonel G. T. L. ARCHER

AND

Surgeon Commander W. SLOAN MILLER, R.N.

(Of the Central Laboratory, Middle East Land Forces, and U.S. Naval Medical Research Unit No. 3, Cairo, respectively)

It has been shown in a previous paper (Archer et al, 1952) that homologous H-specific agglutinins are generally present in the urine of chronic urinary typhoid and paratyphoid carriers in a TAB vaccinated Egyptian population having a high incidence of schistosomiasis.

Heterologous urinary agglutinins were also sometimes found in such carriers, as were agglutinins in the urine of non-carriers. Homologous agglutinins were rare for Salm. paratyphi C in transient carriers of that species. Salm. paratyphi C is absent from the vaccine used to inoculate these people.

It was suggested that such homologous agglutinins either resulted from the plasma leak of schistosomal bladder lesions or were locally produced. Conclusive evidence in support of this latter hypothesis has since been obtained by Naylor and Caldwell (1952). It was further suggested that a primary stimulus, as found necessary for local antibody production by Oakley et al (1949, 1951), might be required. Previous inoculation with TAB vaccine could possibly stimulate the early appearance of urinary agglutinins in carriers, although the chronic carrier state might of itself provide effective stimulation.

It thus seemed desirable to examine urine specimens from chronic urinary carriers known to be free of artificial immunization by anti-typhoid inoculation. For this purpose samples of urine were collected from four chronic urinary typhoid carriers and from one chronic urinary Salm. dublin carrier, none of whom had had injections for typhoid prophylaxis. Bacteriological examination of urine and stool specimens from these cases had been made at approximately monthly intervals for the preceding two years, and serological examination of blood samples rather less regularly. At almost every examination the infecting organism was present in the urine, usually in large numbers; on no occasion was it recovered from the faces. In all five cases there was unequivocal evidence of urinary schistosomiasis. The total duration of the carrier state as estimated from their clinical histories varied between three and eight years.
The urine samples examined were mostly early morning specimens, but in some instances serial samples were collected later on the same day. They were obtained during the course of a ten-day period of chloramphenicol therapy or in the subsequent week, and all were bacteriologically sterile when fresh.

**METHODS**

The H-specific antigens used were as follows: *Salm. paratyphi A* (a), *Salm. paratyphi B* (b), *Salm. paratyphi C* (c), *Salm. typhi* (d); a formalinized broth suspension of the patient’s own organism was used for testing the serum of the *Salm. dublin* carrier (..g, p..) and a suspension of *Salm. enteriditis* (.. g, m..) for his urine.

In testing for O agglutinins a *Salm. typhi* O suspension (IX, XII..) was used, and Bhatnagar’s strain Vi I for Vi agglutination.

Tests for H agglutinins in urine were carried out as previously described, 1/2 being usually the lowest dilution used. These tests were read independently by two or three observers; the last generally using the criterion of a demonstrably floccular deposit overnight; this usually gave slightly lower readings than immediate observation on removal from the water bath. Finer floccules, though clearly visible under the latter conditions, apparently disintegrate on attempted resuspension.

Tests for O agglutinins in urine were made at 55° C. and 37° C., and for Vi agglutinins at the latter temperature. In each case the urine was first used undiluted, one drop of concentrated bacterial suspension being added.

The serological tests were made with standard reagents supplied through the courtesy of Lieut.-Colonel Bensted, Director of the Central Public Health Laboratory (see Bensted, 1951). Doubling saline dilutions were used from 1/5 to an end point. The H and O agglutination tests were finally read after twenty-four hours at 52° C., and the Vi after two hours at 37° C. and twenty-two hours at 4° C. The H agglutinin titres were read naked eye, and the O and Vi with the aid of a concave microscope mirror.

**RESULTS**

The results of the agglutination tests are summarized in the table. It will be noted that relatively high titre H-specific agglutinins are present in the sera of all five cases, homologous with the infecting organism. Heterologous H-specific agglutinin is present in the serum of only one case (Case 4, “a” agglutinin) in low titre.

Heterologous H-specific agglutinins were found in none of the 14 specimens of urine examined for them. Homologous H-specific agglutinins are present in 45 of the 50 urine specimens examined. In only a single case was their presence inconstant (Case 1), where half of the samples examined were negative by the technique employed. However, homologous H-specific agglutinin was demonstrated in one “negative” sample from this case, in a few experiments where drops of concentrated *Salm. typhi* H suspension were added to the undiluted urine.
**TABLE SHOWING THE SERUM AND URINE AGGLUTININ TITRES (EXPRESSED AS RECIPROCAL OF HIGHEST REACTING DILUTION) OF FOUR CHRONIC URINARY Salm. typhi CARRIERS (CASES 1–4) AND ONE CHRONIC URINARY Salm. dublin CARRIER (CASE 5)**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Urine numbers indicate individual samples tested</th>
<th>Agglutinin Titre</th>
<th>O</th>
<th>Vi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H—specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>a    b    c    d</td>
<td>g, m/p . . *</td>
<td>IX, XII .</td>
</tr>
<tr>
<td>1</td>
<td>SERUM</td>
<td>&lt;5    &lt;5    &lt;5   320</td>
<td></td>
<td>40 20</td>
</tr>
<tr>
<td></td>
<td>URINES</td>
<td>&lt;2    &lt;2    &lt;2   2</td>
<td></td>
<td>2   2</td>
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<td>&lt;2    &lt;2    &lt;2   2</td>
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<td>2   2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>(Geometric mean 2.0)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>SERUM</td>
<td>&lt;5    &lt;5    &lt;5   640</td>
<td></td>
<td>640 320</td>
</tr>
<tr>
<td></td>
<td>URINES</td>
<td>&lt;2    &lt;2    &lt;2   2</td>
<td></td>
<td>4   4</td>
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<td></td>
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<td>4   4</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(Geometric mean 5.5)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>SERUM</td>
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<td></td>
<td>80 5tr</td>
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<td>URINES</td>
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<td></td>
<td>4   4</td>
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<td>&lt;2    &lt;2    &lt;2   10tr</td>
<td></td>
<td>10 10tr</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>(Geometric mean 6.1)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>SERUM</td>
<td>10    &lt;5    &lt;5   640</td>
<td></td>
<td>320 160</td>
</tr>
<tr>
<td></td>
<td>URINES</td>
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<td></td>
<td>4   4</td>
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<tr>
<td></td>
<td></td>
<td>&lt;2    &lt;2    &lt;2   10tr</td>
<td></td>
<td>10 10tr</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>(Geometric mean 9.5)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>SERUM</td>
<td>&lt;5    &lt;5    &lt;5   &lt;5</td>
<td>2560</td>
<td>80 5</td>
</tr>
<tr>
<td></td>
<td>URINES</td>
<td>&lt;2    &lt;2    &lt;2   &lt;2</td>
<td></td>
<td>20 20</td>
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<td>&lt;2    &lt;2    &lt;2   &lt;2</td>
<td></td>
<td>20 20</td>
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<td>&lt;2    &lt;2    &lt;2   20tr</td>
<td></td>
<td>20 20</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td>(Geometric mean 18.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Formalinized broth suspensions of Salm. dublin (patient's own strain, . . g, p . . ) were used as antigen for the serum tests, and of Salm. enteriditis ( . . g, m . . ) for the urines.

In calculating the geometric mean no correction has been made for "trace" readings. If these are assessed at 70 per cent. standard the means for cases 3, 4 and 5 become 5.5, 8.8 and 16.5 respectively.
No urinary O agglutinin was detected in any of three specimens from each of Cases 2, 3 and 4, either diluted or undiluted, despite its presence in their sera. Nor was Vi agglutinin detected in two urine specimens from Case 2 and one from Case 4, notwithstanding the relatively high serological Vi titres in these two cases.

Of 11 instances where the urinary H agglutinin titre was compared between two samples from the same case collected two to three hours apart, between 0730 and 1200 hrs., the later specimen showed a slightly higher titre on six occasions, but was never lower.

Two specimens of urine from Case 3 showed non-specific flocculation or turbidity and are not included in the results.

**DISCUSSION**

The source of the urinary antibody demonstrated in these cases is a matter of some interest. In a larger group of uninoculated chronic urinary carriers which have been followed up for almost three years (W. S. M.), homologous H-specific agglutinins have been constantly present in their blood. Although wide variations occur in agglutinin level between one case and another, the serological titre generally remains at a fairly constant level in any given case over periods of many weeks. Blood is frequently found in the urine in these cases, most of whom suffer from urinary schistosomiasis, and it might therefore be supposed that the urinary agglutinin results from simple mechanical leakage of blood.

This is not the case, however, because microscopical examination of the urinary deposits from the specimens here considered showed all but two of them to be free of red cells, and it is known that this test will reveal small traces of blood. Both haematuria specimens were from Case 5, and in each there was a heavy deposit of red cells clearly visible naked eye. The H titres for these two samples were 1/20 and 1/20 trace respectively, no higher than that found in seven other urine samples from the same patient which were entirely free of macroscopic and microscopic evidence of blood. The highest ratio of urinary to serological antibody level, as measured by H-specific agglutinin titre, occurs in Case 3. Urinary agglutinins were demonstrated twice in this case in specimens free of blood at a dilution of 1/10, but the corresponding serological titre has never exceeded 1/160. If this is a valid measure of antibody level, and on the hypothesis that the urinary antibody derives entirely from vesical bleeding, it would mean the addition of approximately 1 part of blood to 16 of urine, which is absurd.

In any given case there is a general consistency in urinary agglutinin titre between samples collected on different days over a ten-day period, although considerable variations occur between one case and another. For example, in Case 1 the urinary titre never exceeded 1/2, whereas in Case 5 it was consistently 1/10 or higher. Although this latter patient had the highest serological titre and consistently gave the highest urinary titre, it should be noted that the antigen used here differs from that in the other cases. This apparent correlation might
therefore be due merely to the better sensitivity of the bacterial suspension used, as compared with that used in the other cases.

There is no other correlation between urine and serum antibody level as measured by agglutinin titre. The following list (titre expressed as reciprocal) shows this:

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Geometric Mean Urine H-antibody Titre</th>
<th>Homologous Serum H-antibody Titre</th>
<th>Ratio Urine/Serum Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0 (5 samples)</td>
<td>320</td>
<td>1/160</td>
</tr>
<tr>
<td>2</td>
<td>5.5 (12 samples)</td>
<td>640</td>
<td>1/116</td>
</tr>
<tr>
<td>3</td>
<td>6.1 (8 samples)</td>
<td>160</td>
<td>1/26</td>
</tr>
<tr>
<td>4</td>
<td>9.5 (8 samples)</td>
<td>640</td>
<td>1/67</td>
</tr>
<tr>
<td>5</td>
<td>18.0 (12 samples)</td>
<td>2560</td>
<td>1/142</td>
</tr>
</tbody>
</table>

It will be noted that the five urine samples from Case 1 giving no reaction at 1/2 have been omitted and in this case the real mean titre is obviously less than 1/2. Although the cases are listed in order of increasing urine titre there is clearly no such corresponding order in serum titre. However, there is less variation in the urine/serum titre ratio of the different cases, which is about six-fold, than there is between the serum titre of the different cases which is sixteen-fold.

The tendency towards slightly higher urinary agglutinin titres in samples collected nearer noon may be due to increased urinary concentration as the day gets hotter or to the effect of work or exercise in causing more leakage of antibody from the bladder wall.

It has previously been reported (Archer et al 1952) that H agglutinin apparently deteriorates in certain specimens of urine from carriers, and that acidity is not the cause of its disappearance. In the present series of tests such deterioration was not observed. On the contrary 15 specimens were re-tested seven to eight weeks after being passed and four to five weeks after the first test. In 10 the titre had not fallen. In the remainder it was only reduced by about 50 per cent. This difference in behaviour may be due to the fact that many of the specimens in the previous investigation contained the homologous infecting organism in large numbers, whereas those here considered, owing to chloramphenicol therapy, were mostly sterile and none contained the infecting organism. Specific bacterial absorption of agglutinin or antibody protein degradation by bacterial action could account for its loss in the former studies, and could not be factors in those here described.

Failure to demonstrate O and Vi antibody in urine containing H antibody, despite the presence of all three antibodies in the patient's serum in relatively high titre, requires some explanation. Firstly, it is known that O antibody, as measured by agglutination, is more labile than H antibody. The shortest time between collection of urine and O agglutination tests was fifteen days and it is possible that any O agglutinin present was destroyed during this period. On the other hand, it might be considered another argument against the hypothesis that the source of urinary H agglutinin is blood or whole plasma. It has been shown that typhoid H and O agglutinins are contained in different plasma fractions which can be separated by physico-chemical means (Oncley et al, 1949). These are both part of the gamma-globulin fraction, however, and it seems
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unlikely that the part containing O agglutinin should be selectively withheld in any plasma leak into the urine.

Owing to long-standing schistosomal inflammation, and possibly other lesions caused by the superadded pathogenic bacterial infection, the urine in these cases is in contact with cellular elements normally shielded by an intact mucosa. It is conceivable that local antibody production occurs under such conditions.

The diagnostic value of urinary antibody tests in an inoculated community has been discussed in the previously quoted paper. Their practical use for detecting carriers in an uninoculated population cannot be assessed without information on the prevalence of urinary agglutinins in the non-carriers of this population. The results here described, however, are sufficiently promising to warrant further investigation.

SUMMARY AND CONCLUSIONS

Blood and urine samples from four chronic urinary carriers of Salm. typhi and from one of Salm. dublin have been examined for the presence of a, b, c, d, and g H-specific, and for typhoid O and Vi agglutinins. These patients had never received anti-typhoid inoculations and all had urinary schistosomiasis.

Relatively high titre H-specific agglutinins, homologous with the infecting organism, were constantly present in the blood in each case, and some had fairly high O and Vi titres. In the blood of only one patient heterologous H-specific antibody was present in low titre and to a single antigen only.

Urinary homologous H-specific agglutinins were found at low titre (1/2) in five out of ten specimens from one patient. But such agglutinins were constantly present in all of 8—12 urine samples from each of the other four cases. The titres ranged from 1/4 to 1/20, but were remarkably constant for any particular case. Higher titres were obtained with more samples collected nearer noon than earlier in the day. Heterologous H-specific and O and Vi agglutinins were not found in any of the urine specimens examined for them.

No significant correlation was found between urinary and blood H-specific antibody level, as measured by agglutinin titre. In at least some instances the urine/blood antibody ratio was sufficiently low to preclude simple mechanical leakage of whole blood as the sole source of the urinary antibody. Failure to demonstrate O and Vi antibody in urine containing H antibody, despite the presence of all three antibodies in the corresponding serum, might negate the hypothesis that the urinary antibody derives from plasma. But the apparent absence of urinary O and Vi agglutinins could also be explained by their lability to storage.

The practical use of urinary antibody tests in the detection of urinary typhoid and paratyphoid carriers in an unvaccinated population requires further investigation.

ACKNOWLEDGMENTS

We thank S./Sergt. A. Ritchie, R.A.M.C., and HM1 J. R. Baranski, U.S.N., for technical aid; Lieut.-Colonel H. J. Bensted for the supply of standard reagents; Lieut.-Colonel M. H. P. Sayers for preparing and sending us a special
concentrated *Salm. typhi* H suspension for use in urine; and Major-General A. J. Beveridge, O.B.E., M.C., Q.H.P., D.M.S. M.E.L.F., and the Medical Director-General of the Navy for permission to publish this work.

**REFERENCES**


**Obituary Note**

It was with profound regret that we heard of the tragic death of Surgeon Commander W. Sloan Miller very shortly after the completion of this paper, at the hands of the Cairo mob.

Sloan Miller was a laboratory worker of high achievement and even greater promise, who had already most worthily upheld the reputation of his Service in international circles. He had a boldly original and keenly analytical mind, and his help and advice were ever available to colleagues, not only of his own Service, but also of the other Medical Services of the Crown, to whom he was in addition a kind and generous host when they visited Cairo.

His passing is a sad loss not only to his friends and to the Medical Service of the Royal Navy, but to the Armed Forces and to the Medical profession.—Ed.

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**EXPLOSIVE OUTBREAK OF \( \beta \)-HæMOLYTIC STREPTOCOCCAL SORE THROAT**

**BY**

Captain F. J. C. ROE, B.M., B.Ch.

*Royal Army Medical Corps*

This is an account of an outbreak of \( \beta \)-Hæmolytic streptococcal sore throat, occurring in a group of approximately 130 men, acting as an independent unit in the field. Altogether 61 men were affected and admitted to hospital, that is to say almost half the strength of a battery. It was considered worth while describing the outbreak at some length both because of its unusual mode of onset and because several points arise which serve to emphasize the ever-constant need of vigilance in the matter of hygiene during exercises in the field.

**Mode of Onset of the Outbreak**

The whole of a Field Regiment went on manœuvres for a period of seven days ending at 1500 hrs., 20/7/51. Throughout this period the individual batteries acted as entirely separate units. The only battery affected by the out-
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