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REFERENCES


INFECTIVITY OF EGYPTIAN URINARY ENTERIC CARRIERS

BY

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The importance of the chronic carrier of enteric organisms in initiating epidemics and outbreaks of typhoid and paratyphoid fever is well recognized. Less information is available concerning the exact mode of spread of the organisms from carrier to victim. The infectivity of the hands of carriers is a problem well worth investigating, and advantage has been taken of the existence of several urinary enteric carriers amongst Egyptians in order to experiment along these lines.

There is evidence that normal skin has disinfectant properties. Arnold and Gustafson (1930) and Karns and Arnold (1931) report that Salmonella typhi could not be recovered from the palmar surface of the clean hand ten minutes after immersion in a culture, although under the nails and along their lateral margins the bacteria persisted longer.

Squire, Cruikshank and Topley (1950) and Ricketts, Squire and Topley (1951) found that factors prejudicing the survival of organisms on the skin were: (1) drying; (2) the presence of unsaturated fatty acids (notably oleic acid); the influence of the former being predominant against Gram-negative organisms such as Bact. coli and Ps. pyocyanea, the latter against Strep. pyogenes, both factors contributing to the elimination of Staph. aureus.
Payne (1949) found that the death rate of Bact. coli on the skin depended on the rate of drying, being maximal at humidities between 40 and 50 per cent. Survival at lower humidities was slightly longer and at higher humidities markedly prolonged.

Goffe (1950) found Salmonella paratyphi A on the hands of one of three urinary carriers five minutes after micturition, but no organisms could be recovered after twenty minutes. Of four carriers of Salmonella typhi, none showed organisms on their hands five minutes after micturition.

**Preliminary Investigation of the Infectivity of a Single Carrier of Salmonella paratyphi A**

*Salmonella paratyphi A* having been isolated from the urine of a native foodhandler subjected to routine bacteriological examination, the following investigation was performed on the carrier, on each of two successive days.

A specimen of urine was obtained, and cultured as a check on the passage of organisms on the day of the experiment. Immediately after micturition, moistened swabs were rubbed over the palmar surfaces of the carrier’s hands. The swabs were numbered 1–12 and taken from various sites on the hands as depicted in the diagram. The swabs were then cultured, and a number of significant isolations made, *S. paratyphi A* being obtained from—

(a) Swab No. 1 on one day.
(b) Swab No. 2 on one day.
(c) Swab No. 3 on both days.
(d) Swab No. 11 on both days.

Thus, six of twenty-four swabs taken immediately after micturition, from the hands which had not been subjected to previous washing, yielded *S. paratyphi A*. The urine samples collected on the days of the experiment gave fairly heavy growths of *S. paratyphi A*.

Indications were therefore evident that carriers might be infective by virtue of transmission of organisms on the fingers, and the following is an account of a series of experiments designed to enlarge upon the original observations.

**Investigations on the Infectivity of a Number of Carriers**

(A) Principle and Outline of Method

1. Control swabs were taken from the right and left hands of carriers immediately prior to micturition, to exclude the presence on the hands of extraneous
Salmonellae. In some cases (see below) scrapings were taken from the under-surface and lateral margins of the fingernails and the material obtained was cultured.

2. Specimens of urine were collected from the carriers and cultured as a check on the passage of organisms on the day of the experiment.

3. Various manœuvres were performed to determine the infectivity of these carriers.

   (i) (a) Swabs were taken from the hands.
   (b) Nail scrapings were taken from those carriers who had been subjected to corresponding control tests.
   (ii) Carriers were made to rinse their hands in previously sterilized samples of milk, which had been poured immediately prior to handling into a sterile petri dish, the milk being taken as an example of foodstuff frequently handled by kitchen workers, and important as a vehicle in the spread of enteric infections.
   (iii) Carriers rubbed their hands on sterile pieces of gauze cloth.

4. All the materials used for these tests were then cultured. It was considered that recovery of organisms from the hands, or transmission to the milk or cloths, would have the same significance in the assessment of carrier infectivity. Only one of these three manipulations was performed on any one carrier on a given day, the carriers being changed round so that each one would have his infectivity assessed by each of the three different methods. Certain comparative studies were introduced into the experiment.

   (i) Time Factor. — The infectivity tests were in the first instance performed within two minutes of the carriers passing urine. Those carriers showing positive results under these conditions were subjected to similar tests delayed until twenty minutes after micturition.
   (ii) State of the Hands. — Experiments in which infectivity was tested for by hand swabbing were performed both on “clean” and dirty hands, on separate days. For the “clean hand” experiments, hands were washed immediately prior to taking of the control swabs.
   (iii) Atmospheric Conditions. — Figures were obtained for the shade temperature and relative humidity prevailing during some of the experiments.

Quantitative Investigations

A small amount of quantitative work was done during the experiment in an attempt to relate the infectivity of the carriers to the number of organisms passed in the urine. Unfortunately, on the days on which viable counts were done on the urine specimens no pathogen was isolated from the hand swabs or milk samples. The record of results (see below) does, however, contain some quantitative data concerning cloth handling.

(B) Bacteriological Technique

1. Culture. — The principles of culture of all the specimens (urines, swabs, nail-picks, milk, cloths) involved —
Infectivity of Egyptian Urinary Enteric Carriers

(i) Direct plating, immediately after collection or handling, on MacConkey agar.

(ii) Incubation in selenite broth, it being found that when the carriers' hands were not previously washed, an inhibitory medium was necessary to prevent an undesirable amount of colonies of Coliforms, Sarcinae and other contaminants appearing on the plates.

(iii) Plating of selenite broth cultures, on MacConkey agar, after incubation six, twenty-four and forty-eight hours. Modifications adapted to the nature of some of the materials and specimens were as follows:

(a) Urines.—The whole of a specimen was passed into a sterile blood transfusion bottle. Two samples were immediately taken into sterile universal containers, one for a viable count and the other for culture. As all the urines yielded significant growths after six hours' culture in selenite broth if not on direct plating, twenty-four hours' platings were unnecessary.

(b) Swabs.—These were stored in sterile tubes, to which, a few minutes before the use of swabs, sufficient broth (MacConkey-mannite broth—Archer & Ritchie, 1950—in all experiments except the preliminary one, where papain broth was used) was added thoroughly to moisten the surface of the swab. After rubbing over the hands the swabs were replaced in these sterile tubes and selenite broth culture carried out in situ.

(c) Nail-picks.—These consisted of sharpened orange-sticks moistened and stored in the same way as the hand swabs. It was found that they could be rubbed over the surface of the plates without creating too much disturbance in the surface of the medium.

(d) Milk samples.—After being handled by the carriers, these were placed in sterile universal containers, diluted approximately one in four with sterile isotonic saline to avoid the material to be subsequently spread on agar being too dense, plated directly, and then incubated in a roughly equal volume of selenite broth. Further platings on MacConkey agar were made at intervals six, twenty-four and forty-eight hours after addition of selenite broth.

(e) Cloths.—These consisted of pieces of gauze about four inches square, sterilized and stored in universal containers. Immediately before use the cloths were moistened with selenite broth. The carriers removed the cloths and rubbed their hands on them, care being taken to see that the interdigital spaces were included in the handling. The cloths were replaced in the containers by the natives; 5 ml. of selenite broth were now added to the container and after vigorous shaking plated directly on MacConkey agar. Further plating was carried out at the same time intervals as used for the milk samples.
2. Controls.—It was considered that swabbing of the hands was sufficient to control subsequent milk and broth experiments. Handling of a control sample of milk would necessitate subsequent washing and drying of the hands, which would involve more risk of altering their state before the infectivity tests were carried out.

3. Reading of Plates.—This was carried out at intervals of twenty-four and forty-eight hours after inoculation. Suspicious colonies were subjected to slide agglutination, at once if the growth was sufficiently profuse, or after subculture if only one or two colonies were present. The identity of positive reactors was confirmed by their biochemical behaviour and agglutination to titre.

4. Viable Counts.—These were performed by a modification of the Miles and Misra (1938) surface count method, using 40 dropper pipettes, dilutions of urine ranging from $10^{-1}$ to $10^{-6}$, and MacConkey agar in six-inch diameter Petri dishes. Plates were read after twenty-four hours, no advantage being found in incubating for forty-eight hours.

Results

No organisms of the Salmonella group were isolated from any of the control swabs taken prior to micturition.

Of the 12 carriers taking part in the experiment, 7 were shown to be infective, following micturition, by virtue of carrying the organism excreted in their urine on their hands (4 cases) or by transmitting it to milk (1 case) or cloth (2 cases).

Analysis of results along several different lines brought out a number of interesting facts.

(a) Species

(i) Of 5 carriers of S. typhi, 3 were shown to be infective, 1 by transmission to milk and 2 by contamination of cloths.

(ii) Of 7 carriers of S. paratyphi A, infectivity was demonstrated in 4, in all cases by the recovery of the organisms directly off the hands.

(b) Time Factor

The 7 “positive” carriers were all infective when tested two minutes after micturition, whereas only 1 of 6 positive carriers tested twenty minutes afterwards carried organisms (Salmonella paratyphi A) on his fingers, those carriers who had transmitted bacteria to milk and cloths two minutes after micturition not repeating the performance when tested at twenty minutes.

(c) State of the Hands

Three of the infective carriers had “clean” hands at the time of testing, these all carrying S. paratyphi A on the hands. The remaining four, one with S. paratyphi A on the hands, one transmitting S. typhi to milk and two transmitting the same organism to cloths, had dirty hands. No tests were done with the milk and cloths when the hands were clean.
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(d) Atmospheric Conditions

Table I shows the proportion of positive isolations from a number of tests performed within two minutes of micturition, at various temperature ranges.

<table>
<thead>
<tr>
<th>Temp. Range °C</th>
<th>No. of tests</th>
<th>No. of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.5–18.5</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>21.0–24.5</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>25.5–32.0</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

Table II shows a similar analysis for two different humidity ranges.

<table>
<thead>
<tr>
<th>Humidity range (percentage)</th>
<th>No. of tests</th>
<th>No. of positives</th>
</tr>
</thead>
<tbody>
<tr>
<td>19–29</td>
<td>16</td>
<td>5</td>
</tr>
<tr>
<td>38–56</td>
<td>24</td>
<td>1</td>
</tr>
</tbody>
</table>

(e) Frequency of Positives

Although 7 of 12 carriers proved infective, the 8 significant isolations (1 carrier positive on two occasions) were only made from a total of 62 infectivity tests, distributed as follows:

- Swab tests: 27, 5 positives. All *S. paratyphi* A
- Nail tests: 13, No positives.
- Milk tests: 11, 1 positive. *S. typhi*
- Cloth tests: 11, 2 positives. Both *S. typhi*

The total number of infectivity tests performed on the positive carriers was 42.

Quantitative Results

The two carriers of *S. typhi* who transmitted organisms to pieces of cloth were excreting, on the day of the test, 50,000 and 470,000 organisms per ml. respectively. On the same day, three *S. paratyphi* A carriers, tested in the same way but all with negative results, were passing respectively 8,000, 4,000 and 8,000 organisms per ml., but on another day two of these three *S. paratyphi* A carriers when passing 300,000 and 45,000 organisms per ml. did not transmit organisms to cloth when tested, as above, within two minutes of micturition. The previous state of the hands (unwashed) did not vary noticeably from one day to the other.

Regarding the extension of the quantitative data to the transmission of organisms, it was noted that the number of organisms obtained off the hands was usually small, the one exception to this being in the case of the preliminary investigation, when on one occasion a profuse growth of *S. paratyphi* A was obtained from the palm of the right hand (Swab No. 11). By contrast, the positive milk and cloth cultures yielded numerous colonies on plating at corresponding time intervals. This suggests that these methods of assessing infectivity might lend themselves to quantitative analysis, especially in the case of the milk, where dilutions could be easily made and viable counts performed at different times after handling.
Discussion

It will be seen from the results that eight significant isolations were made from forty-two tests on the carriers whose infectivity was proved. This indicates that one of these carriers would, on the average, be capable of contaminating some suitable article of food once in every five occasions on which he returned to foodhandling duties immediately after micturition. Assuming, conservatively, the latter event to occur once during a day's working hours in the cookhouse or canteen, each of these carriers might, over a period of five days, introduce pathogenic organisms into the food and so initiate a separate epidemic. The fact that epidemics do not occur with this frequency may well be due to chance events such as delays in returning to duty after visiting the latrine, cookhouse procedures, such as heating of infected foodstuffs and precautionary measures such as washing the hands or immersing them in disinfectant.

Factors determining the infectivity of a given carrier at any one time might include—

(i) species of organisms carried;
(ii) number of organisms passed;
(iii) state of cleanliness of the hands;
(iv) the use of disinfectants;
(v) season and climate.

Taking the first three of these factors together, the results show that although there was no marked difference between the proportion of _S._ _typhi_ and _S._ _paratyphi_ _A_ carriers proved infective, the distribution of positive results among the various tests of infectivity was interesting, in that all the _S._ _typhi_ isolations were made from cloth or milk, whereas the paratyphoid organisms were obtained solely off the hands by direct swabbing. Again, while "clean" and dirty hands showed no appreciable difference of infectivity, it is noteworthy that all the typhoid isolations were made from carriers who had dirty hands at the time of testing, while the _S._ _paratyphi_ _A_ organisms were isolated from "clean" hands in three of the four cases.

The recovery of organisms from the cloths handled by two _S._ _typhi_ carriers excreting 50,000 and 470,000 organisms per ml. set against the apparent non-infectivity of three _S._ _paratyphi_ _A_ carriers passing much smaller numbers of organisms (4,000–8,000 per ml.) suggests the influence of numbers in determining infectivity. But on the other hand, the fact that two of these _S._ _paratyphi_ _A_ carriers were also non-infective when excreting organisms in numbers comparable to those passed by infective typhoid carriers (i.e., 45,000 and 300,000 per ml.) might lead one to believe that species is important in determining infectivity, _S._ _typhi_ being more readily transmitted than _S._ _paratyphi_ _A_.

Varying behaviour according to species might account for the observed discrepancy, in Egypt, between the number of carriers of _S._ _paratyphi_ _C_ (frequent) and the incidence of cases of disease caused by this organism (rare). Unfortunately, the only _S._ _paratyphi_ _C_ carrier available at the time of these
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experiments was passing organisms intermittently, and therefore could not be relied upon for infectivity tests.

The number of observations made, however, is small, and the figures are not suitable for statistical analysis. More quantitative observations are necessary. It would be interesting to observe the influence of disinfectants, especially those commonly used in cookhouses, in reducing the infectivity of these carriers. To determine a significant difference, however, a large number of tests would have to be done, bearing in mind the low frequency of positives (1 in 5) with undisinfected hands.

In view of previous experiments, by Payne (1949) on the influence of humidity and by Ricketts, Squire and Topley (1951) on the effect of drying, on the survival time of organisms on the skin, it might be thought that the carriers would be more infective at high temperatures, when their hands would be more moist from sweat, and also that they might be least dangerous at a humidity of 46 per cent. or thereabouts, their infectivity increasing slightly at lower humidities and markedly at higher ones. Table II presents an interesting comparative study on the influence of the lower humidities. It must be borne in mind, of course, that Payne's experiments are concerned with Bact. coli in broth culture diluted with Ringer's solution, whereas the present series involves enteric pathogens in urine.

Summary and Conclusions

1. Of 12 Egyptian urinary enteric carriers, 7 were proved infective after micturition by virtue of the carriage of organisms on their hands. The carriers were found capable of infecting milk and pieces of cloth, from either of which articles infection might be further transmitted so as to initiate epidemics.

2. On the average, infectivity of these carriers was established once in every five tests.

3. The danger from the carriers within two minutes of micturition is very evident, but there is a sharp fall in infectivity during the succeeding fifteen to twenty minutes.

4. Both S. typhi and S. paratyphi A carriers were proved to be infective, there being slight and possibly unimportant differences in the behaviour of the two species.

5. The possible influence of a number of non-specific factors on infectivity is discussed.

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LACK OF VITAMINS IN THE WAR-TIME ARMY DIET

By

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[The following paper was accepted for publication in the JOURNAL OF THE ROYAL ARMY MEDICAL CORPS in June, 1946. Subsequently it was lost owing to circumstances beyond the Editor's or the author's control. A copy of the manuscript has now been retrieved and it is published in its original form, since revision in the light of the recent literature would not alter the conclusions made.]

The vitamin content of soldiers' food has received careful consideration during the last war, but vitamin deficiencies were still seen. This paper was written with the purpose of drawing attention to this fact, and it is hoped that comprehensive investigations will be stimulated. The observations were based on over four years' experience with the Army abroad, in India, Iraq, Persia, Palestine, Syria, Egypt, Sicily, Italy, Belgium, Germany, and on board H.M. Troopships. Nearly all this time was spent with one Infantry Division, mostly as Regimental Medical Officer. Gross deficiency diseases were not a problem in these areas, but mild varieties, often overlooked and sometimes suggesting neuroses or malingering, were prevalent. These were mostly seen in Regimental Aid Posts and Field Ambulances. Those better qualified for such studies did not have opportunities of observing them, and this is perhaps a sufficient excuse for writing this paper. It is a somewhat incomplete account, and inaccuracies may have crept in, for few publications were available when the observations were

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