THE STERILITY OF NORMAL URINE IN MAN.

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It appears to be widely believed that normal human urine as voided is sterile, and that the fact of sterility is capable of ready demonstration.

The main grounds for this belief are the well-known experiments on the subject by Lord Lister, and the innumerable negative cultural results that have since been obtained after inoculation of laboratory media with unincubated urine or its centrifugal deposits.

When, however, the experiments of Lord Lister are carefully studied it is at once apparent that they do not prove the sterility of normal urine, as he would have been to-day the first to acknowledge. For example many of the specimens of urine that he examined for evidence of sterility were allowed to stand at room temperature for considerable periods, and if no organisms were then found, or if the urine remained clear, it was assumed that none were present.

Evidence of this nature, however, is on theoretical grounds hardly admissible because incubation of the urine at body temperature might conceivably be necessary to allow of multiplication of infecting organisms to take place. This possible explanation of Lord Lister's deductions from his experiments on the sterility of normal urine was therefore put to the following simple test.

Experiment 1.

An ordinary specimen of male urine from a healthy subject was received into two sterile flasks, which were at once closed with sterile rubber corks. Flask 1 was incubated at body temperature for 48 hours, and its contents were then found to be turbid from the presence of large Gram positive dividing cocci. Flask 2 was allowed to stand at room temperature for 10 days, and at the end of this time the urine was found to be perfectly clear. This flask was then placed in the incubator for 48 hours, and the contained urine was at the end of this time found to be turbid from the presence of large Gram positive dividing cocci. Two peptone agar tubes, which had each been inoculated with the invisible centrifuged deposit of 5·0 c.c. of the clear

https://doi.org/10.1017/S0022172400006021 Published online by Cambridge University Press

urine immediately after its collection, were free from colonies at the end of a period of 14 days, during which incubation at 37·0° C. had been allowed to proceed without interruption.

From this experiment it is clear that the absence of turbidity in unincubated urine after a lapse of several days does not necessarily indicate its sterility, and evidence of this nature therefore cannot be held to be satisfactory.

There remains for consideration the evidence afforded by the negative cultural results that often follow the inoculation of laboratory media with unincubated urine or its centrifuged deposits.

On theoretical grounds it would appear unsafe to assume the sterility of specimens of normal urine merely from failure to infect the ordinary laboratory media. For example, a given specimen might contain organisms which were unable to adapt themselves to a new environment, or could only do so if firmly established on urine first. And this would particularly apply to attempts to inoculate solid media. Moreover, the possibility of an infection of the total volume of urine discharged, for example, in 24 hours could hardly be excluded by failure to cultivate from the small fractional samples usually placed into tubes containing nutrient agar, or capable of reception by the ordinary centrifuge. And finally the degree of infection of normal urine might be so slight as to escape detection in the case of centrifuged deposits from clear urine unless these were transferred without loss to the medium employed as indicator. And this is a difficult feat to achieve.

Notwithstanding these theoretical objections to the use of laboratory media as indicators of the absence of infection of normal urine, the frequent negative results obtained are widely quoted as evidence of its absolute sterility. The value of laboratory media as indicators of sterility was therefore submitted to the following tests.

Experiment 2.

Clear urine from a healthy male was discharged into two sterile tubes, each receiving about 15·0 c.c. Tube 1 plugged with sterile wool was incubated at body temperature for 48 hours, and was then found to contain urine turbid from the presence of dividing cocci in groups. Tube 2 immediately after filling was centrifuged for 15 minutes in a high speed machine, and the supernatant urine carefully pipetted off. As much as possible of the slight deposit of mucus was then transferred in a platinum loop to two agar tubes, which were incubated for 14 days, and examined daily. One tube remained sterile throughout, the second showing one colony of cocci at the end of 76 hours, no further colonies appearing within 14 days.

From this experiment, confirming a similar observation in Experiment 1, it is clear that the inoculation of a solid medium such as nutrient agar with the centrifuged deposit from normal urine cannot be relied on to demonstrate its sterility.

Experiment 3.

The same conditions were observed as in Experiment 2 with the exception that the centrifuged deposit was transferred to two tubes of broth. The control tube of uncentrifuged urine and one of the tubes of broth were found to be turbid with growth after incubation for 48 hours, whilst the second tube of broth was still clear at the end of 21 days.

This experiment suggests that it is not safe to assume the sterility of a sample of normal urine from the absence of growth in broth inoculated with the centrifuged deposit of clear urine.

It may, however, be reasonably objected that the simplest method of determining if a given specimen of urine is sterile or not is to inoculate broth or agar, or other suitable medium, with fractional volumes of uncentrifuged urine. The validity of this objection was therefore tested.

Experiment 4.

Clear urine was discharged into a sterile flask, and 1·0 c.c. was at once pipetted over the surface of the medium in each of three agar tubes, the flask and the three tubes being subsequently incubated at body temperature for 76 hours, care being taken to prevent movement of the urine in the agar tubes during incubation. At the end of 76 hours the surfaces of agar were free from colonies, whilst the urine in the flask and in the three tubes was turbid with dividing cocci.

This experiment shows that if an inoculum of urine in an agar tube be undisturbed during incubation a fallacious result may be obtained if the laboratory medium alone be used as the indicator of sterility of the inoculum. And it also suggests that the urine is a more sensitive indicator of its own infection, as might be expected from its liquid nature, than is nutrient agar.

Experiment 5.

Clear urine from a healthy male was next discharged into a sterile test tube, and two tubes of agar were each inoculated with 1·0 c.c. of the urine obtained, a different pipette being used for each inoculum. During incubation the urine was repeatedly flushed over the surface of the agar, and in 48 hours each surface was covered with countless colonies of large dividing cocci. The control tube containing incubated urine alone also contained at the end of 48 hours large dividing cocci.

This experiment suggests that once the organisms found had firmly established themselves on urine they had no difficulty in adapting themselves to the solid medium if opportunity were allowed for re-inoculation.

Experiment 6.

Clear urine was then discharged direct into tubes of broth, and in 48 hours the incubated mixture was in the three tubes inoculated turbid with large dividing cocci.

Taken as a whole these six experiments suggest that the evidence hitherto relied on in favour of the sterility of normal urine should be abandoned, and that the fact of sterility of normal urine, if it be a fact, still awaits demonstration. And they also suggest that urine when incubated may be a better and more convenient indicator of its infection in the fresh state than the ordinary laboratory media whether liquid or solid.

If, however, incubated urine be used as the indicator of its absolute sterility, complete exclusion of extraneous organisms during the process of collection is essential. Unfortunately this is not an easy matter owing to our ignorance of the commonest source of extraneous infection, the difficulty of collecting human urine in such a way as to exclude it, and the time required in any given case to determine whether organisms found in, or cultivated from, the urine are extraneous or not. By extraneous infection is here meant infection which occurs after the escape of urine from the bladder. The possible sources of infection of male urine (with which alone this paper is concerned) occurring during and after discharge appear to be the urethra including the vestibule, the surface of the glans and of the lips of the meatus, the air through which the urine is collected, and the receiving flask. In all the experiments here cited, unless otherwise stated, infection from the receiving flask was excluded by exposing it to dry heat at 180·0° C., each flask being plugged with wool before being heated. Infection from the urethra and vestibule and from the lips of the meatus was excluded as far as possible by adoption of the following technique. The surfaces of the glans and of the lips of the meatus were washed with sterile wool soaked in a 1 in 1000 solution of perchloride of mercury in water. The glans was then immersed in this solution for one minute, a few ounces of urine being finally discharged under the surface of the solution in order to flush the urethra and vestibule. Assuming these precautions to be efficient, the only two sources of extraneous infection which remain are the urethra, including the vestibule in so far as it cannot be

cleansed by the flushing referred to of organisms that are supposed normally to inhabit it, and the air through which discharge takes place.

In spite of these precautions however, as already shown, it was not found possible to obtain a sterile specimen of urine. The two methods of exclusion of extraneous infection usually relied on by different observers are the serial method of collection and the catheter method. Both these methods were, therefore, carefully tested in order to determine if by the adoption of either of them sterile specimens of normal urine could be obtained, using as the indicator of sterility the incubated urine itself.

By the serial method is here meant collection into a numbered series of flasks, or other receptacle, only the last members of the series being reserved for study.

Experiment 7.

After careful washing of the genital surfaces and after preliminary flushing of the urethra under perchloride of mercury, urine was discharged through air by a healthy male into a series of narrow mouthed flasks numbered 1 to 4. After incubation for 48 hours each flask was found to be turbid with organisms. Each flask contained about 100·0 c.c. of urine.

This experiment was repeated by the same individual five times in one laboratory, once in a second laboratory and twice in a private house. Of the 24 flasks incubated not one remained sterile at the end of three days. All were turbid, the organisms found in each series being constant to the site of collection of each series, but differing in each site. One series, for example, in one laboratory gave small cocci only in groups, a second in a different laboratory gave a short bacillus, cocci in groups and chains, a streptothrix and yeast-like cells, and a third series gave large cocci alone.

Experiment 8.

In this experiment urine was discharged by a healthy male into a series of four numbered filter flasks, the side piece being plugged with sterile wool. In each case the glans was placed within the mouth of the flask to exclude air as far as possible, and each flask was finally closed with a sterile rubber cork. At the end of a period of incubation lasting ten days each flask, containing about 60·0 c.c. of urine, was found to be heavily infected. This experiment was repeated 15 times by ten healthy subjects with varying volumes of urine, extreme care being taken with the preliminary toilet of the genital surfaces and vestibule. Of a total of 64 flasks incubated for 14 days not one contained sterile urine at the end of that time. Great irregularity was noted as to the time of appearance of turbidity, which was often independent of the position of a flask in the series, even when the volumes were constant.

Experiment 9.

Into three series of flasks, each series containing 12 members, were collected, in the same way as in Experiment 8, volumes of urine varying from 10 to 40 c.c. Of the 36 specimens of urine incubated not one was found to be sterile at the end of 14 days.

Thus out of a total of 128 flasks of normal urine in these experiments a sterile specimen was not once obtained.

There are three alternative interpretations of these results:

1. Normal urine, contrary to general belief, is not sterile when it leaves the bladder, that is, before it enters the urethra.

2. The serial method of collection cannot be trusted to exclude urethral organisms from urine during its passage along the urethra, assuming such organisms to exist in the healthy subject.

3. A common source of extraneous infection of urine is the air through which it passes after it has left the urethra.

The experiments, in other words, throw no certain light on the source or time of infection of the various samples of urine examined, though from Experiment 7 the possibility of air infection is certainly suggested. They do, however, clearly show that the serial method of collection through unsterilized air is of no value in attempting to obtain sterile specimens of normal urine.

The catheter method of collection was next examined.

Experiment 10.

Specimens of normal urine from ten healthy males were collected by catheterization, the urine being discharged through air in the ordinary way, the free end of the catheter being finally placed inside sterile flasks. The catheters were sterilized before use in liquid paraffin in the autoclave at a temperature of 125·0° C. in order to avoid the necessity for lubrication after sterilization[1]. In each case the first few ounces of urine discharged were rejected before the flasks were filled. After incubation of ten specimens obtained in this way turbidity due to the presence of organisms was present in all at the end of 14 days.

This experiment shows that the catheter method of collection as generally employed is no more reliable than is the serial method in securing sterile specimens of normal urine.

An attempt was therefore made to obtain a sterile catheter specimen by only allowing the urine to pass through sterilized air as it leaves the catheter.

Experiment 11.

A catheter specimen of urine was collected from a healthy male, the free end of the catheter, fitted with a clamp, being attached to the side piece of a filter flask before sterilization, the mouth being closed with a sterile wool plug. As soon as the

[1] Absolute sterilization being impossible to ensure by this method in the case of the presence of spore-bearing organisms, these were carefully searched for in the catheter specimens of urine obtained. Only non spore-bearing organisms were however cultivated.

specimen was obtained, but before withdrawal of the catheter from the bladder, the clamp was closed and the catheter cut off. After incubation for 21 days the urine was found to be clear, and appeared to be sterile so far as could be judged from film preparations of the centrifuged deposit and from inoculation of broth.

Immediately after separation of the catheter from the flask a few ounces of urine were drawn through unsterilized air into a second flask, and in 48 hours the urine was found to be turbid with growth of large dividing cocci.

This experiment, taken alone, is of little value as evidence of the sterility of normal urine in general, but it suggests that a normal specimen can be obtained in an apparently sterile condition if passed through sterile air.

That unsterilized air may be a factor in extraneous infection of urine during its collection is also suggested by an experiment of a different nature.

Experiment 12.

A specimen of urine passed in the ordinary way without any preliminary toilet was collected in a sterile flask. Twenty c.c. of the urine were then transferred in a sterile pipette into a test tube. This was at once boiled, and when cool ten c.c. were poured through air into a second tube. The flask and the two tubes were then placed in the incubator for 48 hours. The flask and the tube containing the poured urine were both infected with large Gram positive dividing cocci, whilst the second tube containing the boiled urine which had not been poured remained clear and sterile at the end of a month. This experiment was repeated three times with the same results.

CONCLUSIONS.

1. As an index of the sterility of normal urine absence of growth on the ordinary laboratory media inoculated with fresh urine or its centrifuged deposit is of little or no value, except in the case of broth inoculated with uncentrifuged urine.

2. The use of incubated urine as the medium of growth shows it to be a highly sensitive and reliable indicator of infection with organisms that appear to be extraneous in the sense defined.

3. It is difficult, if not impossible, to obtain sterile specimens of normal urine by the serial method or the catheter method of collection as ordinarily carried out.

4. Unsterilized air through which urine passes during the process of collection appears to be a frequent source of the extraneous infection of urine, whether the catheter or serial methods be employed or not.

5. The sterility of normal urine in man still awaits demonstration.

SUPPLEMENTARY NOTE BY EDWARD C. HORT AND W. W. INGRAM.

Working in conjunction we have repeated on a large scale involving several hundred observations the experiments above described, and have without exception obtained the same essential results.

It appears that the most convenient method of determining whether normal human urine is or is not sterile before it leaves the bladder would be to obtain a sample by suprapubic puncture during life. This procedure has obvious disadvantages in man. We have therefore applied a modification of the method to animals by puncturing the bladder immediately after death with the following results:

Owing to the kindness of the authorities of the Metropolitan Cattle Market we were able to examine the bladder urine of fourteen animals. Immediately after the death of the animals the bladder was exposed, its surface being then seared with a hot iron. A sharp sterile glass pipette was then pushed through the wall of the bladder and a few cubic centimetres of urine drawn up. The pipette was in each case at once sealed in the flame and within an hour placed in the incubator and kept there for two and a half months at a temperature of 37·0° C. Companion pipettes were withdrawn from the incubator at intervals and examined. The contents of these pipettes and of those kept in the incubator for two and a half months were perfectly clear and in no case could organisms be discovered in the centrifuged deposits, and in no case did inoculation of laboratory media with volumes of the incubated urine reveal infection.

We then repeated these observations with the urine of twelve apparently healthy guinea pigs, using the same technique. In each case the specimens examined remained sterile as far as could be judged by examination of the centrifuged deposit and by attempts at cultivation on the ordinary laboratory media inoculated with the incubated urine.

We conclude from these experiments that the normal urine of cattle, sheep and guinea pigs, provided that they are healthy, is probably sterile, and that normal human urine is also probably sterile. We have, however, not yet been able to prove the sterility of normal human urine.