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Mercury resistance of *Staphylococcus aureus*

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SUMMARY

Reasons for the accumulation of mercury-resistant strains of *Staphylococcus aureus* in hospital have been studied. A collection of paired strains, that is staphylococci similar in every respect except sensitivity to mercury salts, was made. Tests were made in an attempt to demonstrate a link between mercury resistance and some other factor which might aid survival, viz. resistance to drying and heat, production of bound coagulase, growth in the presence of sublethal amounts of tetracycline, survival in human blood at 37° C. and uptake by polymorphs at 30° C. and 37° C., development of resistance to antibiotics and competition in mixed cultures. It was not possible to demonstrate any consistent link between mercury resistance and any of these properties. Paper strips impregnated with the mercurial diuretic, Mersalyl, were shown to differentiate between mercury-resistant and -sensitive strains *in vitro*. Furthermore, development of resistance to mercury by passage in mercuric chloride-broth was demonstrated.

It is proposed that mercury resistance has developed as a result of exposure to the mercury ion. Mercurial diuretics have been frequently used in medical and geriatric patients and it is among these that the higher carrier rates of mercury-resistant strains are found even when the local endemic strain is disregarded. In obstetric patients, where mercurials are seldom used, mercury-resistant strains are rare.

Nasal carriage of factory workers exposed to mercury products showed that this group is likely to carry resistant or partially resistant strains.

INTRODUCTION

The origins of mercury resistance and the reasons for the accumulation of resistant strains of *Staphylococcus aureus* in the hospital environment might be due to one of the following causes:

(i) Mercury resistance may be a marker of epidemic propensity and may be linked genetically with multiple-antibiotic resistance and therefore automatically selected in hospital.

(ii) Resistant strains may be selected by some other factor, such as penicillinase,

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or some biological property which enables them to survive in the hospital environment.

(iii) Resistant strains may have developed as a result of exposure to mercury-containing products.

This paper sets out to assess these hypotheses.

The first suggested cause is partly answered by reason of the fact that epidemics of antibiotic-resistant mercury-sensitive strains have occurred in maternity hospitals (Moore, 1960). Therefore sensitive strains cannot be regarded as harmless from an epidemiological point of view. Furthermore although mercury-resistant strains are commonly also resistant to penicillin, tetracycline and often to other antibiotics, they are not invariably antibiotic-resistant, neither are they invariably of phage-types associated with epidemics (Moore, 1960; Akinlade, 1962; Turner & Willis, 1962; Jessen *et al.* 1963; Meyer, 1966). With reference to the second proposition, they are commonly high penicillinase producers but there is no constant link so that mercury resistance cannot be satisfactorily explained in these terms. Theoretically there is no reason why antibiotic-resistant high penicillinase producing mercury-sensitive strains should not predominate in hospitals, since such strains exist. If mercury resistance *per se* is no advantage in hospitals it is difficult to comprehend why these strains have not proliferated since they appear to possess all the other required properties.

A variety of tests was made in an attempt to demonstrate a link between mercury resistance and some other biological property which might aid survival and thus account for the predominance of mercury-resistant staphylococci in the hospital environment.

MATERIALS AND METHODS

Staphylococcal strains. A collection of 'paired strains' was made from clinical material and nose swabs. Each member of a pair was of the same phage-type, had the same antibiotic resistance pattern, but one was sensitive to mercury salts, the other resistant. They were tested in parallel.

Mercury impregnated paper strips. The method of preparation is described elsewhere (Hall, 1970).

Resistance to physical agents

Drying. Staphylococcal suspensions were filtered through membrane filters so that the cocci were retained on the membranes and these were cultured at intervals (days) to obtain a quantitative survival figure.

Exposure to heat (56° *C.*). Broth cultures were incubated at 56° C. and explanted to blood agar at 15 min. intervals to assess possible differences in survival.

Effect of incubation temperature on growth rate

Growth rate curves of broth cultures were plotted and compared during incubation at 30 and 37° C.

Capacity to develop resistance to inhibitory compounds

Antibiotic-sensitive strains were passaged on solid and in liquid media and the number of passages required to develop tolerance to tetracycline and erythromycin compared. Likewise mercury-sensitive strains were passaged in mercuric chloride-broth and tested for the development of resistance.

Growth of staphylococci

The effect of tetracycline on growth rate. Growth was compared in broth and tetracycline-broth to see if a sub-inhibitory dose of tetracycline might selectively stimulate the mercury-resistant strains to account for their predominance among those that are tetracycline-resistant.

Competition in mixed cultures. This was investigated to determine whether mercury-resistant strains might outgrow mercury-sensitive strains in the presence of small quantities of antibiotics. Small counted numbers of a mercury-resistant and mercury-sensitive strain (both resistant to tetracycline) were inoculated into broth and broth containing tetracycline and viable counts were made after 24 and 48 hr. incubation. As a refinement serum-broth was used in later experiments to resemble more closely conditions in the patient.

Coagulase

Bound coagulase was estimated by a direct assay method using human plasma. Washed suspensions of staphylococci were added to serial dilutions of plasma and shaken and the highest dilution of plasma in which visible clumping occurred was recorded as the titre. It was also estimated using the antibody inhibition test (Duthie, 1955).

Fate of staphylococci in freshly drawn human blood

Phagocytosis. Counted numbers of washed staphylococci were added to a leucocyte-rich preparation, prepared by a modification of the method described by Li, Mudd & Kapral (1963), in siliconed tubes. These tubes were placed on a roller drum and rotated at 30 and 37° C. After 10 and 30 min. intervals smears were made and the number of cocci in each cell of a counted number of cells per slide was recorded.

Survival in defibrinated blood. Counted numbers of staphylococci were added to freshly drawn defibrinated blood and explants were made to blood agar after hourly intervals of incubation at 37° C.

In vitro *and* in vivo *action of Mersalyl* (*an organic mercurial diuretic*)

Blotting paper strips were dipped in dilutions of Mersalyl ranging from undiluted (40 mg./ml) to 1/1000 (4×10^{-2} mg./ml). An agar-diffusion method was used to test the action of this compound against mercury-resistant and -sensitive strains.

The serum of patients receiving Mersalyl 30 min. after intra-muscular injection was tested in a similar way with blotting paper disks.

Investigation of factory workers handling mercury compounds

Since mercury resistance and antibiotic resistance are commonly found together and both are used in treatment, both are likely to act as selective agents but it is difficult to distinguish between these two factors in a hospital community. It is already well established that penicillin-resistant strains are prevalent in nasal carriers engaged in preparing penicillin (Gould, 1958). If mercury salts can also act as selectors in the same way it should be possible to demonstrate an increased prevalence of mercury-resistant strains in nasal carriers exposed to mercury compounds.

One large factory handling mercury compounds for battery manufacture provided 101 nose swabs. This factory was divided into four sections and the concentrations of mercury were as follows: amalgam room and cell assembly room 70 μg./m.^{3} air; depolarizing room 40–60 μg./m.^{3} air and packaging room 20 μg./m.^{3} air. Another factory in the same district was used as a control and yielded 108 nose swabs.

A chemical factory manufacturing mercury salts provided 11 swabs from workers handling these substances. In this factory, mercury concentrations were tested every 2 weeks and were 45 μg./m.^{3} air on average but had been as high as 100 μg./m.^{3} air on occasions. Full protective clothing including face masks was employed and showers were taken after leaving the exposed area. In addition urine levels were tested every 3 months. Other sections of this factory provided 37 controls.

A factory which handled mercury for instrument manufacture including thermometers and barometers provided 45 nose swabs. Here the concentration was said to be 'within safe limits' but no figure was given. Other departments of this factory provided 32 controls. A fifth factory manufacturing soap and glue provided 40 controls.

Although the nature of the investigation was explained to the employees, not all would volunteer to be swabbed. Very great difficulty was encountered in obtaining permission to take nasal swabs from factory workers. This factor and the limited time available made it impossible to expand the numbers. At the outset of the experiment the two factories which provided the bulk of the swabs were matched for size and district but this was not possible for the remainder. Many more men than women were included in the survey.

RESULTS

It was not possible to demonstrate any consistent link between mercury resistance and any of the biological properties listed in the foregoing section up to and including 'survival in defibrinated blood'. These results are described in detail elsewhere (Hall, 1966).

Mersalyl at a dilution of 1/200 (0·2 mg./ml.) differentiated between the mercury-sensitive and -resistant control strains. It gave a zone of the usual size, 1·5 mm. for the sensitive, and no zone for the resistant. Dilutions below 1/200 gave zones for

both strains and 1/300 and above gave no zone. This behaviour was similar to that of strips impregnated with inorganic salts such as mercuric chloride, 1/500 (w/v).

No demonstrable inhibition of growth was found when serum from patients receiving Mersalyl was tested, but this was not surprising since a dilution of 1/200 Mersalyl is 100 times more concentrated than the level that could be expected in serum.

Eight mercury-sensitive strains were passaged daily in mercuric chloride-broth and tested with mercuric chloride strips and an agar-diffusion method. They developed resistance slowly and tolerance was acquired after 23–26 passages. Plate 1 shows both a naturally occurring sensitive and resistant strain and a strain which acquired tolerance before and after passage.

Nasal carriage of factory workers handling mercury compounds

Of the 157 people swabbed in the exposed population, 61 (39 %) yielded *Staph. aureus* and of the 217 controls, 60 (28 %) yielded *Staph. aureus*. These carrier rates are within normal limits. None of the positive carriers had been in hospital during the previous two years.

Table 1. *Distribution of mercury-resistant* Staphylococcus aureus *among exposed and control populations*

	Mercury-resistant	Partially mercury-resistant	Mercury-sensitive
Exposed	4	21	36
Controls	0	1	59

The *Staph. aureus* fell into three groups; those fully resistant, those fully sensitive and a partially resistant group whose zone was smaller than that of the Oxford Staphylococcus. When titrated in mercuric-chloride broth, 17 of these strains had a minimum inhibitory concentration of 1/160,000 and five strains had a minimum inhibitory concentration of 1/320,000 by comparison with the Oxford Staphylococcus of 1/640,000. These strains were bracketed together and classed as the partially resistant group.

Table 1 shows the distribution of the strains in the three categories. The χ^2 value for this table is 27·74, which is very highly significant since it exceeds the 0·1 % point of the χ^2 distribution on 2 degrees of freedom, namely 13·81. Although the number of individuals carrying fully resistant strains is small, the error so introduced in using the χ^2 will be small, since the calculated value of χ^2 is so large. Tetracycline resistance was not allied with mercury resistance; in the exposed group, one of the partially resistant strains and five of the fully sensitive strains were tetracycline-resistant. In the control group only one strain in the fully sensitive category was tetracycline-resistant.

DISCUSSION

It has been shown that the frequency with which hospital staphylococci are isolated from nasal carriers admitted to different departments varies (Hall, 1970). The prevalence of these cocci in these departments is related to the previous hospital history of the patients admitted and their average duration of stay (Stokes, Hall, Richards & Riley, 1965). In geriatric wards where almost all have had previous hospital history and a history of antibiotic therapy, and in which patients stay for a long time, the number of hospital staphylococci is high. In obstetric wards where patients seldom have been in hospital previously and seldom have received antibiotics and in which stay is short, few carriers are admitted. When the non-endemic strains from nasal carriers were studied it was found that geriatric patients carried the highest proportion of mercury-resistant strains.

The results from the clinical work indicate that medical and geriatric wards may often be the source of antibiotic-resistant and mercury-resistant staphylococci. It seems likely that a medical or geriatric patient will acquire a tetracycline-resistant strain in the nose, especially when on antibiotic treatment, and retain this for a certain period so that when readmitted he may still be carrying a resistant strain. On the basis of these observations the hypothesis may be put forward that medical wards act as the principal reservoirs for strains that are not only antibiotic-resistant but also mercury-resistant.

Originally the contention that contact with mercury salts might lead to the development of mercury resistance was considered but discarded (B. Moore, personal communication). Mercury is only rarely used in treatment in surgical wards where most studies on cross infection have been concentrated. Small quantities are employed as preservatives in some preparations for injection (British Pharmacopoeia). However, on closer examination it was found that medical and geriatric wards still use mercurial diuretics which contain a comparatively high concentration of mercury and moreover some patients receive them frequently and over long periods. Mersalyl ($C_{13}H_{16}HgNNaO_6$) was a commonly used diuretic in the past and a dose of one ampoule contains 40 mg. mercury; the usual dose is two ampoules given by injection. Other mercury-containing diuretics are Meralluride, Chlormerodrin, Mercuramide and Mercaptomerin.

Mersalyl is rapidly excreted by the kidney (85% appearing in the urine within 24 hr.) and is not easily broken down to inorganic compounds. When it is administered to elderly patients with some degree of renal failure the effective level of mercury present is prolonged.

It is proposed that mercury resistance indicates a strain which, having been exposed to the mercury ion, has developed resistance to it. This correlates with the known facts. Mersalyl is used in medical and geriatric wards and most patients receiving it are elderly and repeatedly admitted to hospital. Geriatric patients have the highest proportion of mercury-resistant strains and even when the local endemic strain is disregarded there is still a preponderance of mercury-resistant strains. In obstetric and ear, nose and throat patients, where mercurials are seldom used, mercury-resistant strains are less common (Hall, 1970). In the animal world,

mercury resistance is almost unknown among strains of bovine and canine origin (Meyer, 1966).

It has been shown in this paper that Mersalyl was inhibitory to mercury-sensitive strains *in vitro*. Paper strips impregnated with this compound behaved in the same manner as strips impregnated with inorganic salts when tested against sensitive and resistant controls. Furthermore, tolerance to mercury was acquired after many passages in medium containing mercuric chloride. In addition, data from factory workers suffering chance industrial exposure to mercury compounds indicate that these people are more likely to harbour mercury-resistant strains than is the general population. Exposure to mercury compounds may thus induce the emergence of mercury-resistant strains just as occurs with antibiotics, but it is unlikely that cocci come into contact with doses of mercury salts comparable with levels achieved in antibiotic therapy. With mercury compounds the concentration of available free mercury to act on staphylococci is unknown since the degree of ionization may vary, and furthermore the presence of protein in solution decreases the bacteriostatic action due to uptake of free mercuric ion by protein. It is known that large doses of mercury or its compounds cause profuse salivation and that mercury is concentrated in saliva and there may well be some accumulation in nasal secretions in addition. There may be a preferential excretion onto the skin although information on this point was difficult to ascertain.

It would be interesting to know the mercury reaction of strains isolated before the antibiotic era, since Mersalyl had been in use well before this time. It was introduced in the 1920's and indeed mercurials have been used in medicine for centuries. Unfortunately there is no large collection of cultures from the pre-antibiotic days in this country.

Moore claimed that mercury-resistant strains were more versatile and this would account for their presence in hospitals. He suggested that proneness to antibiotic resistance could be regarded as a reflexion of this versatility. However in the experiments described here they were not shown to spread or to survive drying better than mercury-sensitive strains. Moreover if his claim was correct mercury-resistant strains should be biologically dominant in the general population owing to their greater versatility.

Jessen *et al.* (1963) are of the opinion that, despite the lack of correlation between mercury resistance and virulence in the sense of causing a high mortality rate, production of α-lysin, hyaluronidase and egg-yolk reaction, the mercury reaction may still reflect an important metabolic or structural property of the strain. However, none of the properties investigated here were found to correlate with the mercury reaction.

Although it is claimed that there is a high correlation between penicillinase production and mercury resistance they are not always linked so that mercury resistance cannot be satisfactorily explained in these terms. It is recognized that multiple-antibiotic resistance is correlated with high penicillinase production and mercury resistance (Richmond, Parker, Jevons & John, 1964), while strains resistant to penicillin alone, which are now common in civilised populations, both in and out of hospitals, produce less penicillinase. Nevertheless, in strains tested

during the course of this investigation it was not uncommon to find mercury-resistant strains producing small amounts of penicillinase and mercury-sensitive strains producing large amounts. Richmond & John (1964) carry their contention a step further to say that mercury resistance is a marker of a high penicillinase producer. They were able to demonstrate co-transduction of these two properties, suggesting that the genes controlling penicillin synthesis and mercury resistance are often on the same plasmid. They do not claim an obligate connexion between the penicillinase genes and those responsible for mercury resistance; this would be the case if the mercury genes were actually involved in the synthesis or excretion of the penicillinase molecule. Paired strains were not tested for penicillinase production in this investigation because at the time co-transduction of penicillinase and mercury resistance had not been established. The alternative argument favoured here is that strains that have been circulating in hospitals for a time will be high penicillinase producers since these are the ones that will have been further selected by the environment. These are the strains, therefore, that are associated with hospitals, and mercury resistance may only be incidental.

Mercury resistance of staphylococci was discovered as an accident of investigation but it may not be a new phenomenon. Mercury resistance due to the use of mercury-containing compounds has probably ante-dated the phenomenon of antibiotic resistance although this cannot be stated categorically. If this be true, then in future years when mercurial diuretics are finally superseded, and a situation analogous to the withdrawal of antibiotics follows, mercury-sensitive strains should come to the fore and in time the association between antibiotic resistance, high penicillinase production and mercury resistance will no longer exist.

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REFERENCES

AKINLADE, N. D. (1962). A trial of mercuric chloride for the rapid identification of epidemic strains of *Staphylococcus pyogenes*. *West African Medical Journal* **11**, 35.

DUTHIE, E. S. (1955). The action of fibrinogen on certain pathogenic cocci. *Journal of General Microbiology* **13**, 383.

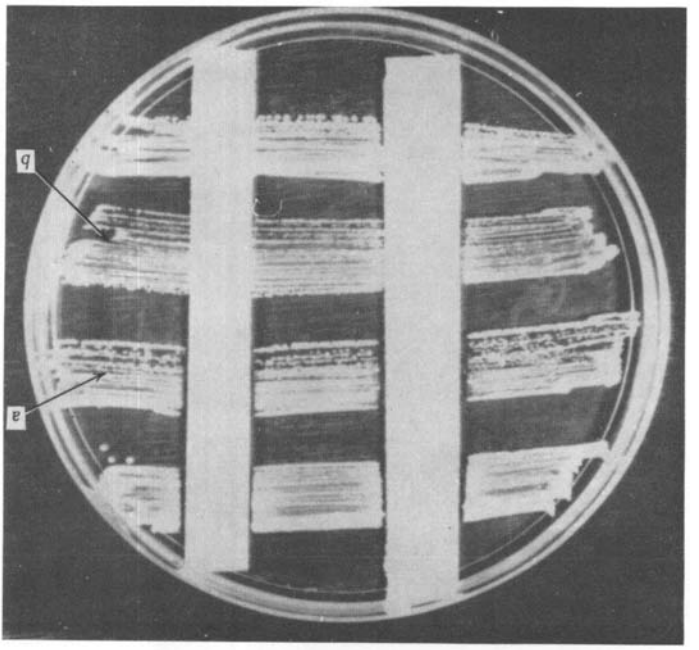
GOULD, J. C. (1958). Environmental penicillin and penicillin-resistant *Staphylococcus aureus*. *Lancet* i, 489.

HALL, B. M. (1966). A study of antibiotic-resistant *Staphylococcus pyogenes* in a hospital community with particular reference to Moore's mercury sensitivity test as a guide to strains of epidemic importance. Ph.D. Thesis, University of London.

HALL, B. M. (1970). Distribution of mercury resistance among *Staphylococcus aureus* isolated from a hospital community. *Journal of Hygiene* **68**, 111.

JESSEN, O., ROSENDAL, K., FABER, V., HOVE, K. & ERIKSON, K. R. (1963). Some properties of Staphylococcus aureus possibly related to pathogenicity. *Acta Pathologica et Microbiologica Scandinavica* **58**, 85.

LI, I. W., MUDD, S. & KAPRAL, F. A. (1963). Dissociation of phagocytosis and intracellular killing of *Staphylococcus aureus* by human blood leukocytes. *Journal of Immunology* **90**, 804.



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MEYER, W. (1966). Ergebnisse der Sublimatresistenzbestimmung bei Staphylococcus-aureus-Stämmen humaner und animaler Herkunft. *Zeitschrift für die gesamte Hygiene und ihre Grenzgebiete* **12**, 974.

MOORE, B. (1960). A new screen test and selective medium for the rapid detection of epidemic strains of *Staph. aureus*. *Lancet* ii, 453.

RICHMOND, M. H. & JOHN, M. (1964). Co-transduction by a staphylococcal phage of the genes responsible for penicillinase synthesis and resistance to mercury salts. *Nature* **202**, 1360.

RICHMOND, M. H., PARKER, M. T., JEVONS, M. P. & JOHN, M. (1964). High penicillinase production correlated with multiple antibiotic resistance in *Staphylococcus aureus*. *Lancet* i, 293.

STOKES, E. J., HALL, B. M., RICHARDS, J. D. M. & RILEY, D. J. (1965). Control of hospital staphylococci. *Lancet* ii, 197.

TURNER, G. C. & WILLIS, A. T. (1962). Staphylococcal invasion of a new surgical ward. *Journal of Pathology and Bacteriology* **84**, 349.

EXPLANATION OF PLATE

A nutrient agar culture plate showing a mercuric chloride strip (left) and a Mersalyl strip (right). A naturally occurring mercury-sensitive strain (top) and mercury-resistant strain (bottom) are included and a strain is shown before (*a*) and after (*b*) it has developed tolerance to mercuric chloride *in vitro*.

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