11952

## Recognition of Sulfapyridine-Fast Pneumococci.

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(Introduced by R. S. Muckenfuss.)

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The regularity with which pneumococci become resistant to the action of sulfapyridine under experimental conditions *in vitro* and *in vivo*[1-4] and the occurrence of such strains in infections in man, have led to the development of a method for their recognition through the use of blood agar plates, which is described below. The results of its application to stock laboratory cultures, to specimens obtained from patients who responded poorly to treatment with sulfapyridine, and to specimens from pneumonia patients submitted for routine bacteriological examination indicate that this test is suitable for use in connection with the control of chemotherapy.

*Method.* Specimens from pneumonia patients, or subcultures from such specimens, are inoculated on 3 blood agar plates (citrated horse blood, 5% in nutrient agar) to 2 of which sulfapyridine is added sufficient to make concentrations of 5 mg % and 10 mg %. The third blood agar plate, without sulfapyridine, serves as a control. Equal sized loopfuls of material are used in order to make the inocula as nearly as possible the same on all 3 plates. The growth on the plates is compared after 24 hours in the incubator, and pneumococcus colonies on the control plate without sulfapyridine are identified as to type by capsule swelling reaction and as to bile solubility by spraying dried bile over the colony.[5] The susceptible pneumococcus on the plates containing sulfapyridine produces some partial (alpha type) hemolysis where the inoculum is heaviest, but separate colonies are rarely visible and of minute size. Stained preparations from hemolyzed areas show aberrant bacterial forms, and subcultures sometimes show viable pneumococci. Pneumococcus growth on the plates containing sulfapyridine is seldom obscured by overgrowth of other organisms as these are also markedly inhibited. The presence of a resistant pneumococcus growing freely on the sulfapyridine plates is readily recognized.

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1 Ross, R. W., *Lancet*, 1939, **236**, 1207.

2 MacLeod, C. M., and Daddi, J., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 69.

3 Long, P. H., and Bliss, E. A., *Ann. Int. Med.*, 1939, **13**, 232.

4 Mulder, J., van der Berg, R., and Eimers, G., *Ned. Tijd. v. Geneeskunde*, 1940, **84**, 923.

5 Greey, P. H., *J. Inf. Dis.*, 1939, **64**, 206.

TABLE I.
Growth of Stock Pneumococcus Cultures on Blood Agar Plates with and without Sulfapyridine.

| Pneumococcus Type | Concentration of Sulfapyridine | | | |
|---|---|---|---|---|
| | None | 1mg% | 5mg% | 10mg% |
| 1 | Good | Good | Poor | Poor |
| 1 (Same as above but grown in broth containing increasing amounts of sulfapyridine) | " | " | Good | Good |
| 4 | " | Poor | Poor | Poor |

*Stock Laboratory Cultures.* The production of a sulfapyridine-resistant pneumococcus strain was undertaken in order to observe its growth on the differential media. A Type 1 pneumococcus culture, highly virulent for rabbits and mice and susceptible to the prophylactic action of sulfapyridine in both these animals, was grown in serum broth containing concentrations of the drug that were increased gradually from 2 mg % to 16 mg %. The resulting culture grew freely on blood agar plates containing as much as 16 mg % of sulfapyridine, whereas the growth of the parent strain was partially inhibited by a concentration of as little as 4 mg % of sulfapyridine. Table 1 shows the relative growth of the resistant strain and the parent susceptible strain, and for comparison the growth of a third strain, representing the greatest susceptibility found by these methods on blood agar plates containing various concentrations of sulfapyridine. The resistant culture was not changed perceptibly with regard to its type specificity or mouse virulence, nor in its susceptibility to the protective action of anti-pneumococcic serum.

Tests for growth on blood agar plates containing 1, 5 and 10 mg % of sulfapyridine have been carried out with 105 different strains, which include the 55 pneumococcal types described by Walter, *et al.*,[6] and 11 other types not yet completely studied. Some inhibition of growth of all strains occurred in the presence of 1 mg % sulfapyridine, although with 26 strains this was shown only by the smaller size of the colonies. Growth of all strains was inhibited to a marked degree by 5 and 10 mg % sulfapyridine.

*Cultures from pneumococcus infections unimproved by sulfapyridine therapy.* Brief summaries of diagnosis, outcome, therapy and bacteriological observations of 5 patients in whom the lack of therapeutic effect of sulfonamide drugs was particularly striking

[6] Walter, A. W., Guevin, V. H., Beattie, M. W., Cotler, H. Y., and Bucca, H. B., *J. Immunol.*, in press.

are given in Table II. The first 3 patients yielded cultures of sulfapyridine-resistant pneumococci late in the disease, after the patient had received comparatively large amounts of the drugs. Two of the pneumococcus strains, the Type 4 (case G.H.) and the Type 1 (case C.M.) grew freely on blood agar plates containing 10 mg % of sulfapyridine, while the Type 25 strain (case A.T.) grew freely in the presence of a concentration of 5 mg % of sulfapyridine, but not in a concentration of 10 mg %. The sulfapyridine-resistance shown by these 3 cultures, and that of the stock strains, made resistant *in vitro* are stable characteristics of the microörganisms and have persisted in culture media without sulfapyridine for over 3 months. In case C.M. a susceptible microörganism was recovered from a spinal fluid obtained the day before death and a resistant strain the day of death. Since 3 days intervened between the time of the spinal tap and the isolation of the pneumococcus from the fluid obtained on the day of death, the possibility that the resistance of this culture had developed in the specimen tube rather than the patient, is to be considered as a source of possible error.

*Application of culture methods to specimens submitted for bac-*

TABLE II.
**Sulfapyridine Resistance of Pneumococci from Infections Unimproved by Sulfonamide Therapy.**

| | | | | Sulfapyridine therapy | | | | Culture | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Before culture | | Total | | | | |
| Case | Diagnosis | Outcome Recovered or died | Days of disease | G | No. of days | G | No. of days | Days of disease | Pneumo. type | Reaction to sulfapyridine in blood agar plates |
| G.H. | Broncho pneumonia | Recov'd | 20 | 64 | 16 | 82 | 21 | 26 | 4 | Resistant |
| A.T.* | Meningitis Endocarditis | Died | 22 | 36 | 4 | 40 | 9 | 17 | 25 | " |
| C.M.† | Meningitis Bilateral Otitis Media (onset unknown) | " | 3 | 15 | 2 | 17 | 3 | 2<br>3 | 1<br>1 | Susceptible<br>Resistant |
| F.D. | Lobar pneumonia | Recov'd | 14 | 43 | 9 | 43 | 9 | 26 | 18 | Susceptible |
| G.A. | Lobar pneumonia Endocarditis | Died | 21 | 35 | 7 | 96 | 12 | 16 | 25 | " |

*Sulfanilamide 18 g in 2 days before sulfapyridine.
† " 8 g " 14 " " "

*teriological diagnosis of pneumonia.* A consecutive series of 105 specimens received for examination in the Health Department, Pneumonia Control Division Laboratories, were cultured, using one plate containing sulfapyridine 10 mg % and one control without sulfapyridine, to determine whether sulfapyridine-resistance occurs regularly and frequently in such specimens. Eighty-three of the 105 specimens contained pneumococci. The susceptibility of 35 of these pneumococcus strains was determined within 24 hours by direct inoculation of the specimens on blood agar plates, and of 29 by the use of subcultures which required 48 hours or longer. Subcultures were not obtained from 19 specimens. The direct inoculation of specimens on blood agar plates was successful most regularly (24 of 37 specimens) when the specimen showed a type-specific pneumococcus by Neufeld examination.

All of the microörganisms obtained from the 64 fully tested specimens (56 patients) were susceptible to the bacteriostatic action of blood agar plates containing 10 mg % of sulfapyridine.

The property of resisting the bacteriostatic action of sulfapyridine comparable in degree to that produced experimentally may appear as a characteristic of pneumococci in human pneumococcus infections where therapeutic response does not occur. Such resistance may be recognized by the observation of the growth on blood agar plates containing sulfapyridine. The diagnostic procedure outlined is recommended for use in pneumococcus infections that do not respond satisfactorily to chemotherapy with sulfapyridine.

## 11953 P

## Changes of Arterial Blood Pressure and Renal Hemodynamics by Injection of Angiotonin in Human Beings.

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Renin, the so-called "renal pressor substance" is vaso-inactive unless permitted to interact with a pseudoglobulin present in blood plasma, which has tentatively been denominated "renin-activator."[1] The interaction of renin and renin-activator yields a crystallizable,

[1] Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.