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THE SENSITIVITY OF ACTINOMYCES ISRAELI TO ANTIBIOTICS

BY

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Although sulphonamides have some effect in actinomycosis, penicillin has much more, and is now the standard treatment for this disease. There are nevertheless three reasons for inquiring into the possible usefulness of later antibiotics: they may conceivably be even more effective, a given patient's strain of *Actinomyces israeli* may be or become abnormally resistant to penicillin, or the patient may be intolerant of this drug.

There are records of the treatment of small numbers of cases of actinomycosis with antibiotics other than penicillin. Streptomycin was used successfully by Costigan (1947) in one atypical and not fully confirmed case, by Pemberton and Hunter (1949), who gave the drug by the mouth to a patient with a lesion in the rectum, by Torrens and Wood (1949), who treated three authenticated cases by a prolonged course of injections, and by Lambert (1951). Some of these patients had previously been given penicillin with no or only temporary benefit. Pulaski and Seeley (1948) mention a case in which the organism became resistant to the drug and the disease progressed: that a high degree of such resistance can develop *in vitro* has been shown by Boand and Novak (1949). All clinical reports on the response of the disease to aureomycin are enthusiastic: Wright and Lowen (1950) cured one case, McVay, Guthrie, and Sprunt (1951) three, Grant (1951) one, and Kelly (1951) one, which had previously not responded at all to streptomycin or penicillin, and only temporarily to chloramphenicol. Chloramphenicol was employed successfully together with penicillin and sulphadiazine in one patient by Littman, Phillips, and Fusillo (1950). There are also single case reports of the successful treatment of *Nocardia asteroides* infection with streptomycin, penicillin, and sulphadiazine (Jacobson and Cloward, 1948) and of *Nocardia madurae* infection with aureomycin and "diasone" (Thompson and Wax, 1950).

Only two of these papers mention *in vitro* tests of sensitivity to the drug. Littman *et al.* tested their own strain and five obtained from Holm against chloramphenicol, and found them to be inhibited by 0.5–3 μg./ml. McVay *et al.* say that tests showed their strains to be sensitive to aureomycin, but the only figure given is failure of inhibition by 50 μg./ml. Three strains of "*Actinomyces bovis*" (two human and one bovine) were tested by Strauss, Kligman, and Pillsbury (1951) against five antibiotics and six other drugs: concentrations as high as 200 μg./ml. of streptomycin, 100 μg./ml. of aureomycin, and 50 μg./ml. of penicillin were required to inhibit growth completely. Such results, if accepted at their face value, would preclude any hope of useful therapeutic effect, and they may be attributable to an inappropriate choice of method. Incubation was for ten days, and the medium contained thioglycollate, which is known to inactivate penicillin.

In view of this paucity of reliable information it seemed desirable to test an adequate number of strains by a uniform technique to determine their *in vitro* sensitivity to all available antibiotics. Such tests are usually, although not infallibly, a guide to therapeutic effect, and they should serve to indicate whether sensitivity is uniform or subject to strain variation.

Experimental

Strains of A. israeli.—Twelve strains were tested: six were personally isolated from cases of actinomycosis in this hospital (three of these are now in the National Collection of Type Cultures, their names and N.C.T.C. numbers being Burnett 8440, Hyder 8439, and Maslin 8441); three were obtained from the National Collection of Type Cultures (Bishop 8047, Burton 7504, and West 6831); and three were cultivated from granules in excised tonsils: these last had all the morphological and cultural characters of *A. israeli.*

Method.—Freshly made solutions of the antibiotics were added to ox heart extract peptone broth, *p*H 7.4, in bulk, and doubled dilutions prepared therefrom in bulk, each then being tubed in 5-ml. volumes. These tubes were inoculated with one drop (approximately 0.02 ml.) of a fine suspension of a fully grown culture, prepared by agitation when this sufficed to break up colonies, or if necessary by grinding the growth with the rounded end of a thick glass rod in a small tube. The size of the inoculum was not deliberately standardized in terms of opacity, but was nevertheless reasonably uniform, and no whole colonies or large fragments thereof were included; these have been shown by Holm (1948) to affect the result of such tests with penicillin. The cultures were incubated anaerobically with 5% CO_2 in McIntosh and Fildes jars for five days. Controls all gave profuse growth in this time in the form of a deposit of compact colonies or floccules.

Results.—The minimum completely inhibitory concentrations of each of five antibiotics for each strain are stated in the Table. Commonly one, and sometimes two, lower

Minimum Concentrations (μg./ml.) of Five Antibiotics Inhibiting Growth of 12 Strains of Actinomyces israeli. (Figures are Numbers of Strains Inhibited by Each Concentration)

	0·03	0·06	0·12	0·25	0·5	1	2	4	8	16	32	64
Penicillin*	2	3	6	1				1	3	4	2	2
Streptomycin								5	3			
Aureomycin						1	3	6				
Chloramphenicol ..						2	4					
Terramycin					1	4	5	1	1			

* Concentrations of this antibiotic are units/ml. (1 unit = 0·6 μg.).

dilutions reduced the amount of growth below that in the control. There are no extreme strain variations in sensitivity: penicillin is the most active and streptomycin the least, the three newer antibiotics occupying an intermediate position. Identical tests, not recorded here in detail, were made with five strains of *A. naeslundi* (Thompson and Lovestedt, 1951) cultivated from normal mouths, and gave closely similar results. Sensitivity to antibiotics is therefore apparently not a character distinguishing between pathogenic and non-pathogenic species in this as in some other genera—for example, *Bacillus.*

Discussion

So far as is known, only one of the patients from whom these strains were cultivated had previously been treated with penicillin, and neither this nor any other strain

displays any unusual resistance to the drug. Since 1944 I have similarly examined 18 other strains and found the minimum inhibitory concentration of penicillin for them to vary only between 0.02 and 0.14 unit/ml. On the other hand, unsuccessful treatment may be accompanied by an increase in resistance: the inhibitory concentrations for two of these strains rose from 0.03 unit/ml. in each case to 0.2 and >0.5 unit/ml. In such patients an alternative treatment may be indicated.

These findings and items of clinical evidence already cited suggest that any of the four newer antibiotics in general use may be effective, although it might perhaps be unwise to rely on streptomycin unless the strain were proved by test to be in the more favourable part of the range of sensitivity to this antibiotic. It should be noted that the *p*H change produced by an atmosphere containing added CO_2 is unfavourable to streptomycin, and allowance should be made for this in interpreting these results. Penicillin and streptomycin have been shown to act synergically on other species, and might with possible advantage be used together in a patient needing vigorous treatment. Of the three newer antibiotics terramycin is rather more active than aureomycin or chloramphenicol as judged by mean inhibitory concentrations.

The results with these three drugs should also be viewed in the light of their relative stabilities. Chloramphenicol and terramycin are both stable; aureomycin is so unstable in an alkaline medium that it loses the greater part of its activity in ordinary broth at 37° C. during the first day of incubation. (This process may have been somewhat retarded in the present tests by the addition of CO_2.) The observed result of any test with it consequently varies with the period of incubation, and becomes progressively more unfavourable as this is prolonged. The slow growth of *A. israeli*, necessitating incubation for five days, presents conditions highly unfavourable to the action of aureomycin, and it was not anticipated before these tests were made that it would perform so well: indeed, it seemed that some other form of test might have to be devised to obtain a true assessment of its activity. It would be more appropriate, for instance, to add further antibiotic to the cultures daily in order to restore the original concentration. If this were done and conditions *in vivo* during therapy thus more closely imitated (without invalidating comparison with the action of a more stable antibiotic) it might prove that the maintained concentration of aureomycin required to inhibit the growth of *A. israeli* is considerably lower than that observed in the present tests. The uniformly successful results of reported clinical trials also suggest a high degree of activity.

Summary

The mean minimum inhibitory concentrations (μg./ml.) of five antibiotics for twelve strains of *Actinomyces israeli* were found to be: penicillin 0.06, streptomycin 23.7, aureomycin 4.2, chloramphenicol 2.8, and terramycin 2.2.

The greater part of the technical work connected with the isolation and maintenance of these strains and the performance of the tests was carried out by Miss Pamela M. Waterworth.

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THE DIAGNOSIS OF INTERSEX

BY

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The problem of the infant of doubtful sex is one which occasionally faces every clinician who concerns himself with children's work, and yet, in spite of the importance of a correct decision, it is still impossible to obtain guidance on this matter from the ordinary textbooks. Too often the decision is postponed to see which way the child will develop; the parents are left without guidance, and their natural anxiety is communicated to the child, who is thus in danger of becoming a psychological as well as a surgical problem.

The present communication, which is based upon experience at the Hospital for Sick Children, Great Ormond Street, and upon a review of the literature which has appeared since the publication in 1937 of H. H. Young's classical monograph, is concerned with the diagnosis and with the probable outcome in various types of intersexuality. A classification is employed which has as its basis the histological appearance of the gonads: intersex males and intersex females have respectively testicles and ovaries; true hermaphrodites have gonadal tissue of both types. But it is not claimed that the nature of the gonad infallibly indicates the "genetic" sex, nor is it intended here to advance any theory of causation.

Intersex Females (Female Pseudohermaphrodites)

(a) Intersex Females with Hyperplasia of the Adrenal Cortex

Most intersex females in whom an adequate investigation has been carried out have proved to be suffering from adrenal cortical overactivity, and it is now commonly supposed that adrenal hyperplasia during foetal life is responsible for the deformity. It is suggested that development of a genetic female proceeds normally during the early weeks, the ovaries differentiate, and under their influence the Müllerian ducts evolve in the usual manner, forming uterus and Fallopian tubes. Some time after the twelfth week, however, the virilizing hormones of the adrenal cortex deflect the normal line of development; the vagina does not push downwards, nor does the urogenital sinus widen out below it; the phallus grows more rapidly and the urethral folds tend to fuse. At birth, therefore, the infant is found to have a phallus approaching in size a penis, with some degree of hypospadias. Most commonly the urethral meatus is represented by a rather wide opening in the perineum, and the vaginal orifice lies just within (see Fig. 1). In lesser degrees the vagina opens externally through a narrow introitus; in the more severe, closure of the urethral folds has proceeded to a point which brings the urethral meatus well up the phallus, and the vagina can be discovered only by endoscopy. In the latter type the labio-scrotal folds may also fuse to form an empty but otherwise normal scrotum. One case is on record (Wilkins, 1950) in which a penis was fully formed with a terminal meatus.

The action of the adrenal cortical hyperplasia does not cease at birth and there is commonly a continuing virilization: somatic growth is accelerated, and ossifi-