

be useful in stopping a series of recurrent boils. Probably the solution would have been more effective than the ointment, although more extravagant. Penicillin applied externally appeared to have no effect whatever on the evolution of an individual boil.

Acne (3 Cases)

Case 1.—M., aged 23. Pustular acne of face, neck, chest, and back of 8 years' duration. Culture sterile. 15,000 units (sodium salt in lanette base, 400 units per g.) in 12 days (applied twice daily). Very slight improvement.

Case 2.—M., aged 22. Pustular acne of face, back, and chest. Duration 6 years. Organisms, *Staph. aureus*. 37,060 units (sodium salt in lanette base, 400 units per g.) in 34 daily applications to left side of back only. Improvement for first 10 days; thereafter i.s.q.

Case 3.—M., aged 40. Acne conglobata of 5 years' duration. Arms, chest, and back extensively affected. Organisms, *Staph. aureus*. 235,320 units in 29 days. Solution of calcium salt (1,000 units per c.cm.) was sprayed on 4 times daily. Cured. Gradual relapse began after 7 days.

Commentary

Since greasy creams are in any event unsuitable for application to cases of acne, and since treatment in Case 3 was temporarily successful, it might have been better to use solution in Cases 1 and 2 also. The method is, however, extravagant. The value of penicillin in this condition remains uncertain. The acne bacillus is resistant to penicillin, and the response of the disease to treatment will depend presumably on the extent to which staphylococci are accountable for the suppuration in each particular case.

Miscellaneous

The following results are recorded for the sake of completeness, but it is not possible to draw any conclusions of value from them. The ointment was used unless otherwise stated.

Two cases of generalized seborrhoeic dermatitis were treated; one failed to respond with ointment, but recovered temporarily when solution was sprayed on to affected areas; the other responded to ointment only in areas where secondary infection was marked. A third case, in which the disease was confined to the scalp, cleared up well when treated with ointment. (Staphylococcal secondary infection was present.) One small carbuncle which was discharging healed in 12 days under treatment with ointment. A case of Bockhart's impetigo accompanied by boils was improved, but the boils were hardly affected. A case of dermatitis repens (Crocker) failed to respond. Four cases of chronic ulceration of the lower limb of uncertain aetiology were all improved. Two cases of varicose ulceration were treated; one patient failed to respond, and the other, who was suffering from varicose eczema as well, was greatly benefited. One case of psoriasis, one case of herpes labialis, and one case of pemphigus vulgaris were unaffected.

Summary

The treatment of 75 cases of skin disease by local application of penicillin is described.

The drug is of obvious value in sycosis barbae, impetigo, and blepharitis. It is also effective in some cases of chronic eczema with secondary infection and in some cases of otitis externa.

REFERENCE

Florey, M. E., and Florey, H. W. (1943). *Lancet*, 1, 387.

The World's Children (published by the Save the Children Fund, 20, Gordon Square, W.C.1; 6d.) now reverts to its former character as a "journal of child care and protection considered from an international viewpoint." The more domestic news of the Fund, in whose interest the magazine is published, is relegated to an appended "Current Chronicle." International interests include an article on the United Nations Relief and Rehabilitation Administration, by Gladys Skelton. Sylvia Thompson expands her suggestion that the best war memorial would be the interchange of young people between the different countries to help to promote world understanding; and Lillian de Lissa, describing wartime care of children in the United States, stresses the eagerness of the Americans to profit by Great Britain's wartime experience in the nursery schools field. Elsa Castendyck discusses the causes of juvenile delinquency. The German system of food rationing is revealed in a table showing the provision of essential foods for various age groups of children. The effects of the war on the rising generation in Europe are seen through Swiss eyes by Dr. Georges Thélin of Geneva.

THE LABORATORY CONTROL OF PENICILLIN TREATMENT

BY

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The bacteriological laboratory has very heavy duties in connexion with the clinical use of penicillin, and where many cases are being treated it is strongly advisable that someone in the laboratory should be enabled to devote almost whole time to the necessary work. This work falls into three main categories: the preparation of solutions, etc., bacteriological diagnosis, and observation of the results of treatment by various *in vitro* tests.

The methods of preparing penicillin for clinical use employed in this and other present research centres are described in a forthcoming M.R.C. Memorandum, and cannot be given in detail here. Penicillin is supplied in the form of powder or tablets; solutions of various strengths have to be prepared from this, and sometimes diluted powders or creams. In the performance of this work two properties of penicillin should be constantly borne in mind. It is a very labile substance, liable easily to lose its activity under adverse conditions. It is also not an ordinary antiseptic, capable of destroying any bacteria with which it may become contaminated; on the contrary, there are many species quite unaffected by it which will not only remain alive in solutions of penicillin but may even decompose them, with consequent loss of activity. These two properties call for high standards of precaution in handling during preparation and for exceptional safeguards during subsequent use.

A refrigerator is the only suitable place to keep solutions and creams before and during use. We have also found it advisable that each patient under local treatment should have his own bottle of solution, owing to the danger of contamination in the ward: this has been known to occur by withdrawing the contents with a syringe and needle or tubing which has already been used for aspirating the lesion. Penicillin can easily become a vehicle of cross-infection, and surplus solution returned after use to the laboratory has occasionally been found grossly turbid owing to the presence of pus and living bacteria.

Initial Bacteriological Diagnosis

Penicillin is effective only against certain bacteria, and it is therefore necessary to know the nature of the infection before embarking on treatment with any confidence in its result. In characteristic conditions having only one usual microbial cause, such as impetigo and sycosis, this may appear superfluous in routine treatment, but even here it may be advantageous, since it is advisable to know not only the species of micro-organism concerned but the sensitivity of the particular strain to penicillin; in some species, particularly staphylococci, this is variable. An exact but time-consuming method is to isolate the responsible pathogen in pure culture and inoculate broth containing various low concentrations of penicillin to determine the least which will inhibit growth. A much more rapid method, quite satisfactory for most purposes, which furnishes both a species and a sensitivity diagnosis from one primary culture, is Fleming's agar cup method. One or two circular holes are punched in a blood agar plate with a sterile cork-borer, and, after inoculation and spreading, a drop of weak penicillin solution (10 units and 1 unit per c.cm.) is pipetted into each hole. Penicillin diffuses into the surrounding medium, where its concentration varies inversely with distance from the cup; the width of the zone surrounding the cup in which no colonies appear is thus a measure of the sensitivity of the organism. In mixed cultures two zones are often evident—an inner containing no growth, and an outer containing colonies of only one more resistant species. Both for an expeditious diagnosis and for dealing with many cases this method is invaluable. The following observations on the sensitivity of different species have been made by this and other methods.

Gram-positive Cocci.—As previously reported by others, we have found occasional strains of otherwise typical *Staph. aureus* abnormally resistant to penicillin. This was sometimes

a property of the original culture, but more often it developed during treatment, clearly owing to habituation; there was, corresponding failure of clinical progress. No resistant strain of *Strep. pyogenes* or of *Pneumococcus* has been encountered. The widely varying susceptibility originally reported by the Oxford workers for both *Pneumococcus* and *Strep. viridans* is not borne out by our experience; no really resistant strain of either has been found. *Strep. faecalis* (enterococcus), on the other hand, is always highly resistant.

Mouth Bacteria.—Observations made mainly in connexion with work described in an earlier paper (p. 517) by Mowlem have led to the conclusion that the great majority of the very numerous bacterial species found in the mouth are penicillin-sensitive. This is of far-reaching practical importance, since it means that infections derived from or connected with the mouth are in general susceptible to penicillin treatment, just as those connected with the bowel are insusceptible owing to the prevalence there of resistant species. The flora of the mouth is highly complex and has been imperfectly studied; the following observations are therefore perforce of an elementary nature. In plates, both aerobic and anaerobic, inoculated with material from osteomyelitis of the jaw, from infected compound fractures of the jaw, and from infected gums, or with saliva, the only organisms ever found markedly resistant to penicillin as judged by the cup method have been *Monilia albicans*, an occasional *Neisseria*, and *Haemophilus*. Whole groups of bacteria which are thus sensitive in doubtless varying but apparently adequate degrees are aerobic streptococci (including various types of *Strep. viridans*), anaerobic

Mixed Infections.—It has sometimes to be decided whether a mixed infection including sensitive and resistant bacteria is suitable for treatment. The latter are usually Gram-negative bacilli, and much appears to depend on whether they are active producers of penicillinase and may thus destroy the penicillin in the lesion. *Proteus* does not produce penicillinase, and combined infections by this organism and staphylococci have been treated successfully in this series; the cocci disappear, and although *Proteus* remains the condition improves, suggesting that the coccus has played the more important part in maintaining the infection. On the other hand, combined infection by a pyogenic coccus and a penicillinase-producing coliform bacillus may be quite unaffected; in one such case, the lesion being a cavity in which solution could be well retained, haemolytic streptococci persisted in undiminished numbers. That the reason for this was the destruction of penicillin by the coliform bacillus in the wound fluid was proved by repeatedly demonstrating that on withdrawal after 24 hours the penicillin content of this fluid was *nil*.

Examinations to Check the Results of Treatment

Swabs or other specimens have been obtained daily, and sometimes at longer intervals later, which were cultivated in order to follow the effect of treatment. When this is successful, immediately scantier growths are succeeded by total sterility. The results obtained in 60 miscellaneous cases treated locally are given in Table I. This includes only wounds (not skin diseases)—cases in which there was a simple infection by staphylococci or haemolytic streptococci, or both—and excludes

TABLE I.—Response to Local Treatment of Wounds

Nature of Lesion	No. of Cases	No. of Cases Infected with		Days before Negative Swab (No. of Cases)												No. of Cases developing Secondary Infection	
				Strep. pyogenes						Staph. aureus							
				Str. pyog.	St. aur.	1	2	3-4	5-7	8-14	Failures	1	2	3-4	5-7		
Superficial and accessible wounds (chiefly operations for plastic repair)	33	10	27	2	1	3	2	1	1	0	3	7	10	5	2	6	2
Sutured wounds treated through tubes	11	4	11	2	1	—	—	—	1	1	1	2	3	1	3	4	—
Sinuses	16	5	15	4	—	1	—	—	—	1	—	4	2	2	6	3	1
Totals	60	19	53	8	2	4	2	1	2	2	4	13	15	8	11	13	3

streptococci, anaerobic Gram-negative bacilli (*F. fusiformis* and many others unidentified), and various Gram-positive bacilli. The mixed and predominantly anaerobic flora often found in infections derived from the mouth is thus in general sensitive to penicillin, and the results of treatment are as would be expected from this.

Actinomyces.—According to the Oxford workers, *Actinomyces* is penicillin-sensitive and actinomycosis susceptible to treatment. We have examined 5 strains, determining their sensitivity by cultivation in a series of broth tubes containing falling concentrations of penicillin, an identical series being inoculated with the Oxford H strain of *Staph. aureus* as a control; the former series was incubated anaerobically for 10 days, during which time it is probable that some of the penicillin activity of the medium was lost. Two strains had approximately the same degree of sensitivity as the standard staphylococcus; they were obtained from cases of actinomycosis of the face, and both appear to have responded well to local treatment. Another, forming a much more compact and firm colony, was 8 times more resistant; the jaw lesion from which it was derived did not respond to local treatment with penicillin. A fourth strain, from a patient with thoracic actinomycosis who died of *Ps. pyocyanea* septicaemia during systemic penicillin treatment, was 4 times as resistant as the staphylococcus. The fifth strain grows normally only in concentrations tolerated by the staphylococcus, but sparsely and atypically in concentrations up to 16 times greater: this case (jaw) has not been treated. The sensitivity of *Actinomyces* evidently varies, possibly to a greater extent than observations by this method reveal.

We have had no occasion to examine the sensitivity of strains of other susceptible bacteria, such as the various *Clostridia*, *B. anthracis*, and *C. diphtheriae*.

a series of cases with extensive bone involvement, such as old compound fractures (see Robertson, p. 519), in which we now know this method of treatment to be useless.

The variability of effect seen in Table I probably depends largely on the structure of the lesion, and thus on the feasibility of permeating it with penicillin completely and maintaining this condition. *Strep. pyogenes*, as would be expected from its greater sensitivity, tends to disappear earlier than *Staph. aureus*. Secondary infection with *Ps. pyocyanea* or *Proteus* often developed during treatment in these and other cases, and aroused a suspicion in some minds that penicillin not only fails to inhibit the growth of these organisms but actually stimulates it. Experiments to determine whether this is so are not complete.

Estimation of the Penicillin Content of Exudates

Since therapeutic effect depends on maintaining an adequate concentration of penicillin in the lesion, it is useful to know whether this condition has been secured. If solution is being introduced into an abscess or other cavity, pus can be withdrawn before a further injection is made and its penicillin content determined. This is done by placing it in a cup in an agar plate heavily inoculated with the standard staphylococcus; growth is inhibited in a zone around the cup the width (radius) of which is a measure of penicillin concentration (concentrations of 100, 10, and 1 unit per c.cm. produce zones measuring 20, 12, and 3 mm. in width respectively).

Persistence is most regularly to be expected in closed cavities treated by aspiration and injection. This fact is illustrated by estimations of penicillin content in pus from empyemas so treated: in 14 specimens of pus withdrawn 2 days after the first injection the mean inhibition zone was 9 mm., and after 3 days (7 specimens) 7 mm. In one case pus giving a 6-mm.

zone was withdrawn 5 days after the last injection. Zones of 2 mm. have been observed after 9 and even 11 days, but their significance is doubtful, since pus containing no penicillin has been observed sometimes to produce this degree of inhibition.

Specimens of fluid from other lesions examined in this way total 192. Some of these were from abscesses treated by aspiration and injection with results comparable to those in empyema. The majority were withdrawn either from sinuses or from recent operation wounds sutured over a tube. Table II records the penicillin content in 106 such specimens; only those obtained one day after treatment are included, and others are excluded in which it appeared that penicillin was being destroyed by coliform bacilli.

TABLE II.—Zones of Inhibition produced by Fluids withdrawn One Day after Local Treatment with Penicillin Solution (usually 1,000 Units per c.cm.)

	No. of Cases giving Zones (in mm.) of					
	0	1-3	4-6	7-10	11-15	16-21
Sinuses ..	10	2	3	5	4	1
Wounds ..	18	11	5	5	10	32
Totals ..	28	13	8	10	14	33

Mechanical factors favouring retention again appear paramount in determining results. It is possible that the vascularity or other properties of the tissue bordering a cavity have also some influence, but clear evidence of this has not been obtained.

Estimations of Penicillin Content of Blood

During systemic treatment the penicillin content of the blood should be estimated. This is possible only by determining the bacteriostatic power of the serum either for the patient's own organism or for the standard staphylococcus. All methods for doing this depend on using the undiluted patient's serum and dilutions of it in normal serum as a culture medium; a small inoculum of staphylococci grows in serum in the form of colonies, which may be counted if such cultures are made on slides, the cover-slip being sealed with petroleum jelly. Inhibition of growth in any given dilution may be either complete (no colonies) or partial (fewer colonies than in a normal serum control).

Dosage in this series of cases was uniform, 120,000 units a day being given to adults and a correspondingly smaller dose, approximately related to body weight, in children. The penicillin content of 37 specimens of blood from 13 patients receiving penicillin by intravenous drip or other continuous method of administration is stated in Table III.

TABLE III.—Numbers of Serum Specimens Bacteriostatic to Oxford H Staphylococcus in Different Dilutions

Bacteriostasis:						
Nil in pure serum	1
Partial in pure serum	5
Complete in pure serum	11
Partial in 1 in 2 serum	3
Complete in 1 in 2 serum	7
.. " 1 in 4 serum	5
.. " 1 in 8 or over	5

These variations cannot be fully explained, although one cause of a high titre may certainly be retention due to renal inefficiency; 3 of the 5 specimens listed as "1 in 8 or over" came from the case referred to also by Morgan, Christie, and Roxburgh (p. 515), in which repeated estimations (not listed here), after administration was stopped, showed long delay in excretion. A concentration giving complete inhibition in pure serum is probably adequate for full therapeutic effect, and *in vitro* tests (to be published later) have shown that the rate of death of staphylococci in blood containing penicillin is not accelerated by increasing the penicillin concentration within the range of 0.1 unit to 10 units per c.cm.

When three-hourly intramuscular injections are given, the blood concentration is known to rise rapidly after each injection, then to fall progressively until the next. Specimens obtained immediately before a dose was due gave no inhibition at all in 6 and partial inhibition in pure serum only in 3. A much greater number of such estimations must form the

basis of any judgment on this method of treatment; but to gain an idea of whether dosage is adequate must clearly be more difficult, and on theoretical grounds the maintenance of a steady level by continuous administration seems to me more likely to achieve full therapeutic effect.

Comment

This condensed account of laboratory work on cases some of which are described by foregoing authors is no more than an interim report on a study still in progress. It is intended to present a picture of the nature and volume of work which is called for from the laboratory if penicillin treatment is to be directed intelligently and, still more, if its efficiency is to be improved. At the same time a few minor original observations are briefly reported which may be of some service to other workers.

The greater part of the technical work here described was carried out by Miss P. M. Waterworth. Her services and the equipment of the laboratory used were made available by the generosity of "Bundles for Britain," which organization provided a fund under the control of Mr. Rainsford Mowlem for laboratory work in connexion with his surgical unit.

Medical Memoranda

Reliability of the X-ray Diagnosis of Early Pulmonary Tuberculosis

The case cited by Dr. Sharpe in the *Journal* of Nov. 6, 1943 (p. 579) and the series of letters which followed have brought into prominence the important question as to how far x-ray films of the thorax can be relied upon in the early diagnosis of pulmonary tuberculosis. The following is a case in which a fatal miliary tuberculosis supervened shortly after the lungs were declared to be entirely free of evidence of infection by a reliable radiologist who has had great experience in reading radiographs. This x-ray examination was made while the patient was in hospital suffering from tuberculous meningitis.

An electric welder aged 21 was admitted to hospital complaining of severe headache. Clinical signs of meningitis developed, and laboratory findings confirmed the diagnosis. The radiograph of the chest taken while in hospital was negative. Death occurred on the thirteenth day after admission. Necropsy findings were: a typical Ghon lesion about 1 cm. in diameter lying immediately beneath the pleura in the upper part of the right lower lobe, the lesion involving a vessel of medium size; enlarged caseous glands at the root of the right lung; small caseous tubercles in the cerebral meninges, spleen, kidneys, and adrenals.

The questions which arise are how frequently cases are missed by the radiologist, and what type of lesion it is which fails to show up in the x-ray film. As to the first matter, Dr. Richards, radiologist to the Toronto General Hospital, has collected the statistics of active tuberculosis developing in enlisted men in Canada during the first two years of the war. He finds (*Amer. J. Roentgen.*, 1942, 47, 66) that 16 men who had negative x-ray films on enlistment developed active tuberculosis—a percentage of only 0.004. We are informed verbally by a urologist in charge of one district in Canada, however, that he has seen no fewer than four cases of tuberculous epididymitis in soldiers who must have passed the test of a chest radiograph. This means that a percentage which is not negligible is declared free from evidence of pulmonary tuberculosis after inspection by the radiologist.

LESIONS WHICH FAIL TO SHOW UP

As to the matter of the type of lesion which fails to show up in the x-ray film, we are inclined to believe that many localized cases of primary tuberculous infection are invisible in radiographs. For the sake of brevity we may refer to this primary tuberculous pulmonary lesion as the Ghon lesion. It is situated at the periphery of the lung immediately under the pleura, usually not at the apex, and it consists of a small area of caseous consolidation later surrounded by a rampart of fibrous tissue. Spreading from it is a chain of infected lymphatics terminating in a caseous gland in the hilum of the lung. It is a moot point whether this lesion—which is usually small, often less than 1 cm. in diameter—can be demonstrated by x rays, especially if it be placed laterally and so hidden behind the ribs. When it becomes the seat of calcareous deposit it is more easily seen.

As to the matter of appearances in x-ray films which are suggestive of tuberculosis but which are not tuberculous in origin, probably every radiologist has seen suspicious lesions which disappear after a short time. Virus pneumonia of an