Oct. 5. Lab. B: W.R. double positive, Kahn positive on Sept. 26 (W.R. + + ; K.T. +) ; W.R. and Kahn both negative on Oct. 5.

At this stage it was considered unjustifiable to withhold routine treatment with arsenic and bismuth. This was started on Oct. 5, and it was interesting to note that after the first 0.3 g. of N.A.B. there was mild general malaise and the temperature rose to 99° F. Further treatment has been uneventful. Kahn tests on Oct. 9 and 18 were both negative.

Comment.—Although the date of exposure to infection is not absolutely definite the expectation in this case would be that blood W.R. and Kahn tests would have been positive before routine treatment was instituted. A weak positive was obtained in one laboratory, but repeated controls at this and the other laboratory were all negative. Depending on the sensitivity of the antigen, this probably indicates that the penicillin had prevented an impending positive serum reaction.

Case II

Gnr. Y. was admitted to hospital on Aug. 28, 1944, with the following history: exposure to infection two weeks and three months previously; treated for fresh gonorrhoea with 27 g. of sulphapyridine in M.I. room from Aug. 21 to 28: urethral discharge persisted.

On admission he had a purulent urethral discharge with some oedema and induration in the region of the fraenum. Clinically this latter condition was suggestive of an intra-urethral chancre. A blood Kahn test on Aug. 28 was negative. A further course of sulphapyridine was given from Sept. 2 to 6. A urethral smear on Sept. 11 and succeeding days still showed intracellular gonococci, and the urine was hazy in both glasses. Perifraenal oedema and induration persisted, but over the period Sept. 2 to 14 nine dark-ground examinations of the urethral discharge were negative to *T. pallidum* despite the clinical appearances.

In view of the sulphapyridine-resistant gonorrhoea 100,000 units of penicillin were given on Sept. 18. A urethral smear on Sept. 20 showed no gonococci, but a slight watery discharge persisted until Oct. 2. Daily dark-ground examination of this was negative to *T. pallidum*.

He was discharged from hospital on Oct. 5. There was no urethral discharge, and the urine was clear in both glasses. The oedema and induration around the fraenal region had slowly subsided after penicillin therapy and had disappeared by the time the patient left the hospital.

Comment.—Although oedema as described above is occasionally present in acute gonorrhoea it usually does not persist so long even in sulpha-resistant cases. It is felt, then, that in all probability this was a case of intra-urethral chancre despite negative dark-ground findings. In view of this and the findings in Case I it is intended to prolong the period of post-hospital surveillance in this case, regular blood tests being carried out over a period of two years.

Discussion

The present dosage of penicillin used in the treatment of resistant gonorrhoea is 100,000 units, and a group of cases was recently reported in which 60,000 units were found adequate. These dosages, then, according to the presumptive evidence of reports on the treatment of syphilis, are quite inadequate to cure this disease, but on our own observations are perfectly adequate to effect the clinical cure of a primary lesion in itself. Obviously they will also very successfully mask any primary lesion which a patient with gonorrhoea may be incubating.

The following problems now arise: (1) What interval must elapse before such an inadequately treated case will develop a positive blood W.R. or systemic manifestations of syphilis? (2) On the analogy that an early syphilis inadequately treated with the arsenicals frequently develops precocious tertiary lesions, will this also hold true of penicillin? (3) On the same analogy, is not such a patient likely to remain infective during the assumed latent period? At present we have no evidence on these points.

What, then, is to be the routine in cases of sulpha-resistant gonorrhoea treated with penicillin? We know that a certain small but definite percentage of gonorrhoea cases are incubating syphilis, which may not show until the final blood test after three months' surveillance. The reasonable assumption now is that with the advent of penicillin these cases will be completely masked, according to the observations on the two cases quoted. Two alternatives appear to be open. Either the initial dosage must be sufficient to deal with a possible coexisting syphilis (2,500,000 units) or surveillance must be extended to cover a period of (say) two years after treatment to exclude the latent systemic spread of any possible coexisting syphilis to which the patient is exposed on the present routine. In our

present state of knowledge the first is obviously wasteful. The second appears to be the only fair method, not only to the patient himself but also to the community in general.

Having reviewed briefly the probable effect of penicillin in masking an early syphilis it would be well at this stage to think of the numerous patients who have had penicillin treatment for general infections. Large numbers of these have been battle casualties with heavily infected wounds. With our knowledge of conditions in the various theatres of war it is reasonable to assume that any one of these cases may have been incubating syphilis at the time penicillin treatment was instituted, and the dose, though large, may not have been the postulated curative dose. Will the patient's signs and symptoms too be masked until in a few years' time the poor unfortunate begins at an early age to suffer from anginal pain or his wife gives birth to a snuffling child?

This article is not intended to be destructive or pessimistic, however much it may appear so. Penicillin has more than proved its value as a life-saving measure and in many acute and chronic infections which previously would have led to marked general debility and prolonged hospitalization. To the venereologist it is the drug of choice in the treatment of gonorrhoea. There is no doubt that as further supplies become available it will, and must, be even more extensively used.

However, it is hoped that enough has been said here to emphasize the need for the most careful and prolonged surveillance of penicillin-treated gonorrhoea cases (and even in cases where venereal disease might be suspected in a patient treated for some other condition) until our present knowledge of the effect of the drug in syphilis is much further advanced.

Regarding the second group of cases mentioned, it cannot be too strongly advocated that, in future, routine blood tests of the whole population should be enforced by law, particularly before marriage.

Conclusion

By force of circumstances this article has been largely theoretical and referable to a very small percentage of cases. However, until we know in detail the response of early syphilis to small doses of penicillin both from the clinical point of view and from that of change in the serum reaction, the necessity for very careful assessment and prolonged surveillance of every case would appear obvious, particularly in view of the two cases described.

THE TEMPORARY CHARACTER OF "FASTNESS" OF STAPHYLOCOCCI TO PENICILLIN

BY

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It is generally assumed that micro-organisms which have been made resistant to bacteriostatic drugs or to antibiotic agents by continued subcultivation in increasing quantities of the antibacterial substance will remain "fast" after many subcultures in ordinary laboratory media. Fleming and Allison (1927) showed that *Micrococcus lysodeikticus* could be made resistant to lysozyme, and that after repeated subcultures extending over nine months in ordinary culture media this resistance was fully maintained. Similarly, bacterial resistance to sulphonamide drugs appears to be a permanent characteristic. McLeod and Daddi (1939) found that a "sulphapyridine-fast" strain of pneumococcus Type I remained resistant after 30 subcultures in broth.

Rake *et al.* (1944) subcultured pneumococcus Type III in penicillin 55 times, thereby raising the bacteriostatic dose of penicillin from 0.03 unit to 0.95 unit. After 32 subcultures of their resistant strain in ordinary blood broth the bacterio-

* Seconded for the duration of the war from the London County Council.

static dose of penicillin remained unchanged. Schmidt and Sesler (1943) made pneumococci resistant by passages through penicillin-treated mice; they then passed their resistant strain through 30 normal mice without any loss of resistance.

Spink *et al.* (1944) found that staphylococci which they had made resistant by 109 transfers in penicillin *in vitro* showed no loss of resistance after storage on veal-agar for three months in the refrigerator.

Methods

In the following experiments 100 c.cm. of broth was infected with 0.25 c.cm. of a broth culture of staphylococcus. A suitable dilution of penicillin was added to a portion of this infected broth, and a series of tubes was then set up containing the infected broth with falling dilutions of penicillin on a 20% range in a total volume of 1 c.cm. After 24 hours' incubation the last tube showing no growth was noted, and 0.25 c.cm. from the next tube, in which the staphylococci had grown out, was subcultured into 100 c.cm. of broth for the next test. This process was continued daily until the culture was highly resistant to penicillin; it was then subcultured daily in ordinary broth without any penicillin, and titrations were done at frequent intervals to determine the resistance of the strain to penicillin.

Staphylococcus Strain "Oxford H"

This is the standard strain of staphylococcus used for the assay of penicillin. Fig. 1 shows the results of subculture in

FIG. 1.—Showing increasing resistance on subculture in penicillin followed by diminishing resistance on subculture in broth. *Staphylococcus* "Oxford H."

penicillin and of subsequent subculture in ordinary broth. At the beginning of the experiment 0.06 unit of penicillin was sufficient to cause bacteriostasis. After 11 subcultures in penicillin 2 units were required to cause bacteriostasis. During the next two subcultures the maximum quantities of penicillin used—namely, 4 units and 10 units—failed to cause bacteriostasis, and at the fourteenth subculture 40 units of penicillin were required for bacteriostasis. After 27 subcultures the resistant staphylococci grew in 160 units but not in 200 units, and had therefore become 3,000 times more resistant to penicillin than the original culture. As noted by previous workers, the character of growth in broth and on agar had changed with increasing resistance. Growth in broth was comparatively scanty, and various colony forms were seen on solid media. Rake *et al.* found that three colony forms of their resistant strain varied in their degree of resistance to penicillin, those with the greatest morphological variation from the parent strain showing the greatest increase in resistance.

The highly resistant strain was now subcultured daily in tryptic digest broth without any penicillin, and the opacity of each subculture was noted by comparison with barium sulphate standards. During the first three subcultures the opacity was less than 1. After six subcultures, when the cocci grew in 50 units of penicillin but not in 100 units, the opacity had returned to normal, giving readings between 2 and 3, and it remained at this level until the end of the experiment. There was then a rapid fall in resistance to penicillin, and after 19 subcultures the cocci grew in 0.1 unit but not in 0.2 unit. The resistance to penicillin had therefore fallen in 19 subcultures from 3,000 times to little more than twice the original level. After 36 subcultures the bacteriostatic dose was 0.12 unit, and for the original untreated culture it was still 0.06 unit.

Slight variations in resistance to penicillin can be obtained without any exposure to penicillin. The "Oxford H" strain was found to grow in 0.05 unit of penicillin but not in 0.06 unit. It was then subcultured daily for about a month in horseflesh-infusion broth containing 2% of proteose peptone and in tryptic digest broth. The two cultures were then retested, and it was found that the infusion-broth culture still had a bacteriostatic dose of 0.06 unit, while the digest-broth culture had a bacteriostatic dose of 0.03 unit.

A resistant strain of "Oxford H" which had been subcultured 40 times in penicillin grew in 250 units but not in 300 units. After 21 subcultures in infusion broth it grew in 0.16 unit but not in 0.2 unit. When a highly resistant strain of staphylococcus "Oxford H" was subcultured on agar the fall in resistance was slower and less complete. A strain which grew in 320 units of penicillin per c.cm. but not in 400 units was subcultured daily on agar slopes. After 28 subcultures the cocci grew in 0.5 unit of penicillin per c.cm. but not in 0.6 unit.

Staphylococcus Strain "A"

Fig. 2 shows the results of a similar experiment with a different strain of staphylococcus. The original bacteriostatic dose of penicillin for this strain was 0.08 unit. After 21 subcultures in penicillin the bacteriostatic dose was 20 units, and after 10 subcultures in broth the organisms grew in 0.2 unit but not in 0.5 unit. After 36 subcultures the bacteriostatic dose was 0.2 unit, while the untreated culture

FIG. 2.—Showing increasing resistance on subculture in penicillin followed by diminishing resistance on subculture in broth. *Staphylococcus* strain "A."

grew in 0.06 unit but not in 0.08 unit. With this strain the bacteriostatic dose was increased 250 times by subcultivation in penicillin, and on transfer to broth it rapidly fell again to less than three times the dose required to inhibit the untreated culture. The first four subcultures in broth had an opacity of less than 1, the next four had opacities between 1 and 2, and subsequent subcultures were between 2 and 3. As in the previous experiment the cultures remained coagulase-positive throughout.

Discussion

It appears from these experiments that staphylococci which have become highly resistant to penicillin after subcultivation in increasing concentrations of the drug *in vitro* lose their resistance to such an extent on subcultivation in broth that they become sensitive to penicillin in concentrations which are easily attained in the human body. It does not seem unreasonable to suppose that staphylococci which have become resistant to penicillin *in vivo* might similarly revert to the penicillin-sensitive state on withdrawal of the drug. Indeed, it seems possible that the reversion might be more rapid *in vivo* than *in vitro*, since Spink *et al.* have shown that penicillin-resistant staphylococci are much more susceptible to the bactericidal action of human blood than normal staphylococci which are sensitive to penicillin.

It seems to be a general rule that organisms which have become "fast" remain in that condition for an unlimited number of generations, and it is well known that organisms such as *Streptococcus pyogenes* and *Gonococcus* which have become resistant to the sulphonamides retain their resistance to the drug through many generations, and that cross-infections with these resistant strains breed true. Organisms other than staphylococci have not been tested in the present work, but Rake *et al.* have shown that pneumococci retain their resistance

to penicillin on subcultivation in laboratory media. It may be that penicillin-resistant staphylococci are an exception to the general rule, and that, consequently, resistant strains of this organism will not appear in clinical practice after more prolonged use of the drug.

Summary

Two strains of coagulase-positive staphylococci were made penicillin-"fast" by cultivation in increasing quantities of penicillin. They were then subcultured daily in broth without penicillin and, contrary to expectations, a rapid fall in resistance to penicillin was noted. It therefore appears that "fastness" of staphylococci to penicillin is not a permanent characteristic.

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WEIL'S DISEASE IN NORMANDY: ITS TREATMENT WITH PENICILLIN

BY

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In view of the experiences of the last war Weil's disease was not unforeseen in the present campaign in North-West Europe. Sporadic cases have occurred, and the opportunity has been seized of treating cases with penicillin. Such results as have been obtained are presented now, as no further cases have been seen for over two months.

History of Outbreak

Weil's disease is spread by infected rats who pass the spirochaetes in their urine; the organisms can live for some time in stagnant streams, in wells, and in the zoogloea-like substance on the walls of wells and sewers. It has been impossible to make detailed studies of the epidemiology of the disease, nor are these necessary. The source of the infection was almost certainly water which men used for washing. Drinking-water was supplied from water-carts or was sterilized in some way, but the supply was not sufficient for other uses; the men were forced to draw water from wells or streams for washing, shaving, and brushing their teeth. They bathed when they could in any available stream. On some sectors they lived in damp rat-infested ditches. As cases were few it was decided that the risk was worth taking, as the alternative—even if it could have been enforced—would have been that the men remained dirty or unrefreshed by bathing during the dreary and often very hot period on the Normandy beach-head.

Up to date (December, 1944) cases have been met with only in Normandy, and only from the middle of July to the end of September. It is surprising that conditions in the Low Countries have not produced further infections: there is plenty of water, both in canals and in the fields, but the weather has been unattractive for the bather, and organized bathing and ablution facilities are much better. I saw the first recognized case on July 18 with Major C. L. Davidson in a C.C.S. near Caen. The patient had had a febrile illness with severe muscle pains and a leucocytosis; on the tenth day he had a very faint icteric tint in his conjunctivae, but was convalescent. We discussed the possibility of Weil's disease, and a few days later we learnt that the agglutination test was positive. The beach-head was so small then that it was easy to tell all the physicians at C.C.S.s and hospitals to watch for it: its presence made common or garden jaundice an attractive clinical problem. Cases cropped up in many units, and when the outbreak ceased at the end of September I had been informed of 39. It is difficult to compute the extent of the outbreak; my figures must be incomplete, and cases must have been missed, especially those without jaundice. Of the 39 cases only two did not have jaundice, whereas the number should have equalled those with jaundice. I would make an informed guess that about 100 cases occurred altogether.

Some cases were very severe, and supplies of antileptospiral serum at first were scanty; it seemed possible that the leptospira might be penicillin-sensitive, and Col. J. S. K. Boyd, Deputy Director of Pathology, ascertained in a telephone conversation with the War Office that it was moderately sensitive. It was at once decided to investigate the matter. In only one case—the only fatality in the penicillin series—was antileptospiral serum given.

Clinical Features

These will not be recited at length—they are well dealt with in the standard textbooks. Those of us who were little familiar with the disease were struck by its unusual features: a brisk febrile illness with severe muscle pains as the leading symptoms, associated with a leucocytosis and complicated by signs of gross renal damage. In the presence of an outbreak this picture is highly suggestive even if jaundice does not follow. In all but two of our cases jaundice developed on or after the fifth day; in sharp contrast to "infective hepatitis," the temperature did not subside when jaundice appeared; it usually increased, and the urine still showed the signs of renal damage. In most cases there was some haemorrhagic incident—conjunctival ecchymosis, epistaxis, haemoptysis, haematemeses, purpura.

The disease if at all severe ran a longish course, the temperature was maintained for perhaps two weeks, and there were often febrile relapses later. The patients were very debilitated, but as they could not remain in the theatre of war we did not find out how long convalescence was going to take, or whether some would develop a type of cirrhosis within a few months.

Of the special types described the anginal form was rare: there were three examples of the meningeal form, but only one had an abnormal cerebrospinal fluid; lung involvement with haemoptysis, watery sputum, and abnormal signs, both physical and radiological, was not uncommon.

Laboratory Findings.—Spirochaetes were found in the blood in several cases, and in the urine in quite a number—both by dark-ground illumination. Animal inoculation was possible only when a resourceful pathologist was able to procure a guinea-pig by local purchase. In most of the cases positive agglutination tests were obtained by sending blood to the U.K. I am satisfied that the errors in diagnosis of Weil's disease have been negligible.

Penicillin Treatment

The dosage recommended was 40,000 units 3-hourly, with a total of about a million units. The average amount given was 1,125,000 units—some by continuous intramuscular drip, some by intramuscular injection—and there was a certain variation in dosage.

Our difficulties in assessing the results have been great. The disease rapidly inflicts severe damage on liver and kidneys, and to be successful any treatment must be given early enough to anticipate this; in practice this means that treatment must be started before the diagnosis has been firmly established—perhaps at a time when a diagnosis is impossible to make. We were dealing with a disease with which few of us were familiar, and no case was treated in the pre-icteric stage when there was the best chance of rapid resolution. Even if penicillin should prove to be a specific remedy it seems doubtful how often really early treatment will be practicable, unless there is so big an epidemic that everyone becomes very skilled in early diagnosis.

We have been unable to find any satisfactory measurement of progress. The numbers we had were too small to warrant any statistical approach—I have avoided any deduction from the deaths occurring in the treated and the untreated. With an elaborate laboratory service it might be possible to estimate the rate of disappearance of spirochaetes from blood or urine—this would need experimental animals, which we lacked. The most promising indication of progress was the effect of the drug on the temperature and on the number of febrile relapses. As is only too common in medicine, we were left with a clinical impression; this demands a nicety of judgment and an intellectual honesty which the observer may indeed possess but which a critic could be forgiven for doubting.

Most of the severe cases received penicillin and recovered; all observers claimed a dramatic improvement in 36 hours, which was usually reflected in the temperature chart, but with