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THE ACTION OF PENICILLIN ON BACTERIA

BY

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Sulphonamides had been in general use for five years before the nature of their action on bacteria was discovered. The history of penicillin is developing on similar lines: so remarkable were its properties as demonstrated by *in vitro* and animal experiment, and so encouraging to clinical trial, that the treatment of human disease was embarked on at an early stage, and has engrossed almost all attention. The far-reaching success of these efforts does not diminish the need for a clearer understanding of how penicillin acts on bacteria: such knowledge may indeed enable clinical treatment to be directed even more effectively.

In the first full description by Florey and his colleagues (Abraham *et al.* 1941) observations on the persistent respiration and survival of staphylococci exposed to penicillin are mentioned in support of the view that its action is bacteriostatic rather than bactericidal. By performing viable counts on bacteria exposed to the action of penicillin Hobby, Meyer, and Chaffee (1942) and Rammelkamp and Keefer (1943) showed that a great diminution in the number of living cells occurs, often ending in extinction, although a small proportion of survivors may persist. Similar experiments by Rantz and Kirby (1944) led them to state unequivocally that penicillin is bactericidal. Bigger (1944b) has reached the same conclusion, but qualifies it by advancing the hypothesis that this lethal action is exerted only against bacteria which are about to divide. The same idea has been put forward with similar supporting evidence by Hobby and Dawson (1944) and by Miller and Foster (1944).

Up to now no author appears to have examined penicillin by the methods usually applied to ordinary disinfectants. It is true that penicillin is far from being ordinary, but, on the other hand, if it is a disinfectant, as some of these findings suggest, an accurate knowledge of the influence on its action of the various factors which are known to control chemical disinfection generally might well be helpful. This was the aim of some of the following experiments.

Method.—The only organism used was *Staphylococcus aureus* (Oxford H strain), of which such an amount of a 24-hour broth culture was added to the test mixture as to give a concentration of from 25,000 to about 5,000,000 viable cocci per ml. The medium (usually broth), the concentration of penicillin (added as a distilled-water solution in a small volume), and the temperature were varied as stated later. Survivors were counted at intervals in pour agar plates from 1 in 10 to 1 in 10,000 dilutions, penicillinase made by Ungar's (1944) method being added if necessary.

Bactericidal Action

It has been shown by others, and is evident from the following experiments, that penicillin causes the death of bacteria under favourable conditions fairly rapidly. I was not at first inclined forthwith to accept this as evidence of true killing, because bacteria kept at 37° C.—a necessary condition for the rapid penicillin effect—and merely prevented from multiplying, whether by a mechanism involving deprivation of nutriment or otherwise, will certainly not survive long. It might appear simple to settle this point by employing a non-nutrient medium, but in my hands the death rate at 37° C. of washed staphylococci in different batches of Ringer's solution made with glass-distilled water varied inexplicably, and although usually accelerated by penicillin, was once (by a different sample) clearly retarded. To distinguish between bactericidal and bacteriostatic action also demands a definition of these terms; which is to be used of a substance that inhibits an essential metabolic process and thus causes ultimate death by starvation? The distinction, in terms of effect, can only be a matter of time, and later observations have convinced me that the penicillin effect is far too rapid in some circumstances to be regarded as anything but bactericidal.

The Effect of Concentration

An increase in concentration accelerates the action of all common disinfectants. The reverse is often true of penicillin: in the experiment illustrated in Fig. 1 death was much more

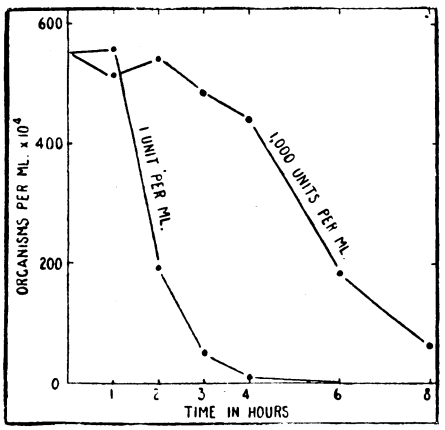

FIG. 1.—Numbers of surviving *Staph. aureus* in broth at 37° C. containing penicillin (TRC10) in concentrations of 1 unit and 1,000 units per ml.

rapid in the presence of only 1 unit per ml. than with 1,000 units. This result is an example of those obtained in many experiments, three of which are expressed, for the sake of brevity, in a different form in the following Table. The LT50 (Withell, 1942) is the time required to kill 50% of the inoculum, and, since the survivor curve in the presence of penicillin in any given medium is of constant shape, this value fairly reflects the rate of disinfection.

LT50 in Minutes for 3 Different Penicillins in 4 Concentrations in Broth at 37° C.

Penicillin	Potency (Units per mg.)	Concentration of Penicillin (Units per ml.)			
		1	10	100	1,000
TRC8	30	105	111	258	195
TRC10	100	106	118	152	318
"Pure"	1,360	100	91	89	95

The first two samples were of early manufacture and contained a high proportion of impurities: both are most active in the lowest concentration—1 unit per ml.—and least active in one case in that of 1,000 units and in the other of 100. "Pure" penicillin, on the other hand (actually of about 85% purity), disinfects at an almost constant rate regardless of concentration within this range: the differences observed are within

the limits of error of the method. It seems probable that above a certain necessary minimum the rate of disinfection by pure penicillin is unaffected by increase of concentration. On the other hand, something—presumably impurities—actually retards disinfection when the concentration of impure material is increased. That this does not apply only to samples of very low potency was shown by a similar experiment with a recent commercial product (Pfizer) of high potency (about 1,050 units per mg.). In concentrations of 0.1; 1, 10, 100, and 1,000 units per ml. there were less than 4% of survivors after 2 hours, but in 10,000 units per ml. there were 34%.

The effect of smaller variations in a lower range was also tested. In the following experiment each concentration is one-quarter of the preceding:

LT50 in Minutes for 2 Penicillins in 5 Concentrations in Broth at 37° C.

Penicillin	Concentration of Penicillin in Units per ml.				
	10	2·5	0·62	0·15	0·039
TRC10	92	118	118	148	>480
"Pure"	110	102	114	130	>480

There is in either case no significant difference between the rates of disinfection in the three higher concentrations: retardation is slight in 0.15 unit per ml. and marked in 0.039. A similar experiment in human blood (inactivated at 50° C. for 15 minutes), using TRC25 (360 units per mg.) in concentrations of 10, 5, 2, 1, 0.5, 0.2, and 0.1 unit per ml., gave values for LT50 of 58, 70, 75, 88, 108, 140, and 174 minutes respectively. Although these figures suggest that raising the concentration accelerates disinfection, there is less difference at a later stage: at 4 hours the percentage of survivors in all concentrations was under 3, showing that in this medium disinfection is accelerated only in its early stages by increase in concentration.

The Effect of Temperature

The action of all chemical disinfectants is accelerated by increase in temperature, and penicillin is no exception (Fig. 2).

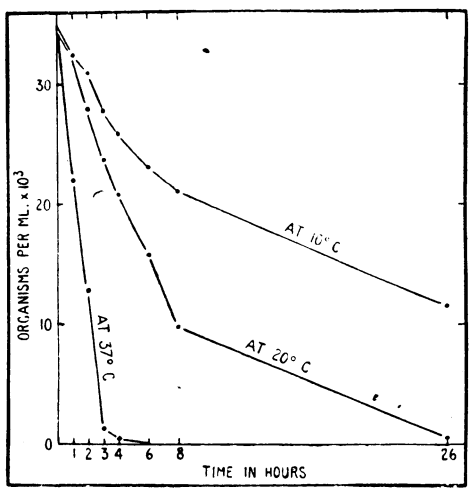

FIG. 2.—Numbers of surviving *Staph. aureus* in broth containing 10 units per ml. of penicillin at 10°, 20°, and 37° C.

The degree of this acceleration (i.e., the temperature coefficient) varies among different classes of disinfectant, and appears to be by no means so extreme for penicillin that it can be used to support the argument that this drug has a peculiar type of action. Bigger (1944b) believes that penicillin kills only dividing cells, and cites its inactivity at low temperatures in support of this; it is, in fact, slowly bactericidal not only at 10° C., as shown in Fig. 2, but at 4° C. This was demonstrated in another experiment, in which a 50% reduction in the viable count was observed in 2 days and 100% in 14. Disinfection by penicillin was also further accelerated as compared with that at 37° C. by raising the temperature to 42°. This is characteristic of an ordinary disinfectant, and not what would be expected according to Bigger's hypothesis, since at this temperature growth ceases. Further observations of the action of penicillin at this or even higher temperatures might be instructive.

The Effect of *p*H

Penicillin, although rapidly destroyed by strong acids, is said to be stable between *p*H 7.0 and 5.0. That it is not equally active throughout this range of acidity is shown in Fig. 3.

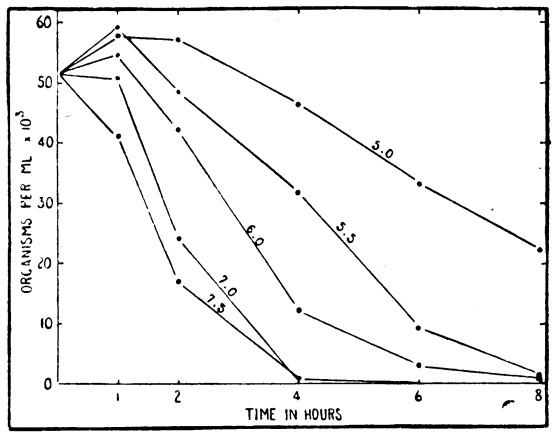

FIG. 3.—Numbers of surviving *Staph. aureus* in broth adjusted to *p*H 7.5, 7.0, 6.5, 6.0, 5.5, and 5.0, and containing 10 units of penicillin per ml. at 37° C. (The curve at *p*H 6.5 is almost identical with that at 7.0, and is omitted. Slow multiplication occurred in control broth at *p*H 5.0 with no penicillin.)

which illustrates the rate of disinfection by 10 units per ml. of penicillin in broth adjusted to six different reactions by the addition of HCl (all were diluted to the same extent by making up to constant volume with distilled water). This experiment was suggested by an anomalous result in an attempt to confirm the observation of Abraham *et al.* (1941) that penicillin acts as well in pus as in blood, serum, or broth. The pus used contained only an anaerobic streptococcus; the standard staphylococcus was added to it. Disinfection proved to be much retarded relative to that in control media (broth, serum, and blood), and a possible cause for this was found when the *p*H of the pus was discovered to be 5.2. A portion was brought to 7.6 and the experiment repeated with broth: at 5.2 the LT50 was still over 4 hours; at 7.6 it was under 2. A low *p*H in an inflammatory exudate may therefore impair the action of penicillin: this factor is worthy of study as a possible cause of failure in treatment.

The Effect of Reproductive Activity

The suggestion recently made by various authors that penicillin kills only dividing cells prompted several experiments, some of which support this conclusion in part, while others do not. Their results are here stated briefly:

1. Action in Diluted Broth.—According to Bigger, the viable count of staphylococci in 1 in 800 broth remains stationary, the medium supporting life but not growth, and penicillin in this medium is inactive. When the LT50 for 10 units of penicillin per ml. in undiluted broth at 37° C. was 103 minutes, it was 208 minutes in 1 in 800 broth: the population remained stationary in a 1 in 800 broth control during this period, but increased subsequently. In another experiment with a different broth, in which very little late growth occurred, the LT50 in 1 in 800 medium was 335 minutes. This medium therefore does reduce the rate of disinfection; it by no means prevents it.

2. Presence of Another Bacteriostatic Agent.—Bigger found that boric acid, which is bacteriostatic, prevented the bactericidal action of penicillin. I have tried two other such agents. Proflavine in low concentrations is bacteriostatic for staphylococci and only slowly bactericidal. The LT50 of 1 in 90,000 proflavine in broth at 37° C. was 215 minutes, that of 10 units of penicillin per ml. 92 minutes, and that of both combined 165 minutes. Proflavine therefore does reduce the activity of penicillin, but this may possibly be due to chemical incompatibility. The ideal purely bacteriostatic agent appears to be a sulphonamide: the results of an identical experiment using

sulphathiazole are given in Fig. 4. Sulphathiazole in a concentration which almost completely prevents growth somewhat reduces the rate of disinfection by penicillin.

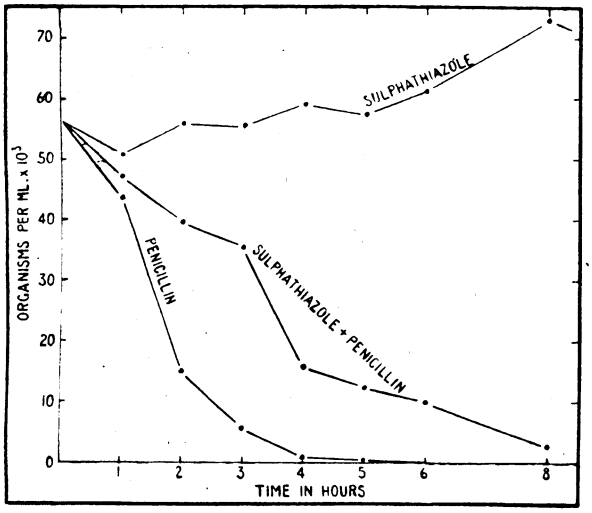

FIG. 4.—Numbers of surviving *Staph. aureus* in broth at 37° C. containing (1) 0.2% sulphathiazole; (2) 10 units penicillin per ml.; (3) both of the drugs.

3. Age of Culture.—If reproductive activity is an important factor there should be a marked difference in the action of penicillin on old and that on young cultures. In this there would be nothing unique: young cultures are more susceptible to disinfectants generally than old, and this is recognized in the stipulation that a culture of prescribed age shall be used for any standard test. Nevertheless it seemed worth while to study this factor, and the behaviour of a culture 10 days old (for 9 of which it had been on the bench) was compared with that of one 5 hours old (i.e., in the logarithmic phase of growth). Previous viable counts on such cultures made it possible to add approximately equal numbers of living cells from each to flasks of broth containing 5 units of penicillin per ml. at 37° C. As shown in Fig. 5, the viable count in the young

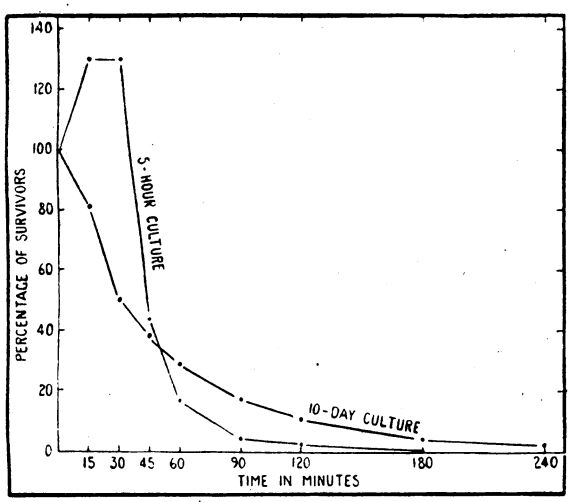

FIG. 5.—Numbers of surviving *Staph. aureus* in broth at 37° C. containing 5 units of penicillin per ml., the organism being derived from a 5-hour-old broth culture and a 10-day-old broth culture respectively.

culture after a slight initial rise fell rapidly; that of the old fell continuously and rather less rapidly; but the rate of disinfection is much the same in both, and lends little support to the idea that a cell is vulnerable only when dividing. Curves similar but for the absence of an initial rise in the young culture were obtained with 1 in 300 phenol controls; the greater resistance of a small proportion of old cells is clearly evident in both. Reproductive activity therefore affects susceptibility to penicillin only in the same way as it affects susceptibility to phenol.

(In this experiment the penicillin broth and the broth for diluting the cultures were brought to 37° C. before the experiment began: this doubtless accounts for the rapid death rate. In other experiments—perhaps unfortunately—the test mixtures were made at room temperature and placed in the incubator or water bath at zero time. They should have reached the required temperature within a few minutes, but Bigger's observations on the effect of cooling suggest that this brief period at a lower temperature may have unexpected effects.)

Discussion

These experiments show that penicillin is fairly rapidly lethal to susceptible bacteria. Beyond that they throw no direct light on the nature of its action. The only other lesson to be learned from them is that further studies of the intimate nature of this action must clearly be pursued with pure reagents. All commercial penicillins tested were less active in high than in low concentrations, and impurities which presumably cause this and other anomalies offer a serious obstacle to such a study. It will indeed be necessary to consider whether pure penicillin itself is a single substance of unvarying composition and uniform action.

On the other hand, some conclusions can rightly be drawn which have a bearing on the clinical use of penicillin as now available. The foremost of these is that nothing is likely to be gained by using high concentrations, particularly for local treatment. Failure of clinical response is usually considered to call for an increased dose, and there is a pardonable belief that the greater the dose the more sure the effect. True as this may be of other therapeutic agents, it is even the reverse of the truth when penicillin is concerned: a concentration of 1 unit per ml. is not only just as effective as one of 1,000 units, but often more so. The only good reason for using strong solutions in local treatment is to ensure that loss by escape, dilution, or absorption shall not permit the concentration to fall below the minimum level for full effect, which may be taken as about 0.1 unit per ml. The factors controlling this loss vary almost infinitely, from good retention in a closed space such as the theca or pleura to the impossibility of retention and the dilution by copious exudate which characterize some open wounds. The optimum strength of solution must therefore vary, but the standard probably need not exceed 250 units per ml.: this was the strength originally employed with satisfactory results for instillation into gunshot wounds after secondary suture by Florey and others in North Africa. That this policy has an additional advantage in economy needs no emphasis.

These experiments also have some practical bearing in relation to Bigger's proposal that systemic penicillin treatment should be intermittent. His argument is that penicillin kills only dividing cells, and that a very small proportion of dormant non-dividing cells which he terms "persisters" are liable to survive: these must be permitted to grow by interrupting treatment if sterilization is to be complete. Of the experiments described here, those using diluted broth and combinations of penicillin with bacteriostatic agents give some support to the idea of a special action on dividing cells. It should be observed in this connexion that Bigger (1944a) has previously recommended combined treatment with sulphathiazole and penicillin: if, as it appears, the action of sulphathiazole as a bacteriostatic agent interferes with that of penicillin, it must detract from rather than add to therapeutic effect. His two ideas of synergic action and bacteriostatic interference are in fact plainly incompatible.

On the other hand, studies of the effect of temperature and particularly of reproductive activity on susceptibility to penicillin demonstrate behaviour which seems to differ in no qualitative sense from that of other disinfectants. While much remains unknown or doubtful, I submit that the evidence for Bigger's hypothesis is inadequate to support so drastic a change in therapeutic policy as he advocates. Clinical experience supports this attitude; the reason for therapeutic failure is to be found not in any property of the micro-organism but in the morbid anatomy of the lesion. Treatment fails in patients with inaccessible foci of infection, such as an area of necrosis in bone, an endocardial vegetation, or an undetected abscess; even those with the severest infections but without such foci regularly recover.

Summary

Penicillin has a bactericidal action.

This action proceeds at a constant rate regardless of concentration within wide limits if pure penicillin is used: impure samples are more effective in low than in high concentrations.

This action is accelerated by increase in temperature throughout the range 4° to 42° C.

The action of penicillin is progressively impaired by an increase in the acidity of the medium between *p*H 7.0 and 5.0.

The behaviour of penicillin in diluted broth and in the presence of bacteriostatic agents supports the hypothesis that it acts only on dividing cells; the effect of temperature and the almost uniform susceptibility of cells from both old and very young cultures are against it.

I am indebted to the Penicillin Clinical Trials Committee of the Medical Research Council for most of the penicillin used, and to I.C.I. (Pharmaceuticals) Ltd. for the gift of 183 mg. of nearly pure penicillin. This work could not have been done without the assiduous technical assistance which was given by Miss P. M. Waterworth. For her services and for the equipment of the laboratory in which the work was done I am indebted to a fund, under the control of Mr. Rainsford Mowlem, which was generously provided by the United States organization "Bundles for Britain."

REFERENCES

Abraham, E. P., Gardner, A. D., Chain, E., Heatley, N. G., Fletcher, G. M., Jennings, M. A., and Florey, H. W. (1941). *Lancet*, **2**, 177.
Bigger, J. W. (1944a). Ibid., **2**, 142.
—— (1944b). Ibid., **2**, 497.
Hobby, Gladys L., and Dawson, M. H. (1944). *Proc. Soc. exp. Biol.*, N.Y., **56**, 178.
—— Meyer, K., and Chaffee, Eleanor (1942). Ibid., **50**, 281.
Miller, C. P., and Foster, Alice Z. (1944). Ibid., **56**, 205.
Rammelkamp, C. H., and Keefer, C. S. (1943). *J. clin. Invest.*, **22**, 649.
Rantz, L. A., and Kirby, W. M. M. (1944). *J. Immunol.*, **48**, 335.
Ungar, J. (1944). *Nature*, **154**, 236.
Withell, E. R. (1942). *J. Hyg.*, Camb., **42**, 339.

PENICILLIN IN GONORRHOEA AND SYPHILIS

WITH NOTES ON TWO CASES OF DUAL INFECTION

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In recent years the widespread use of the sulphonamide group of drugs in the treatment of venereal diseases has had fairly satisfactory results. These drugs have been employed successfully in the treatment of gonorrhoea, soft sore, and other types of non-specific genital ulceration, and lymphogranuloma inguinale. However, from the clinical point of view certain difficulties were early encountered. These were due to the simple fact that a patient presenting the appearances of one venereal disease may well be incubating one or more of the others. Sulphanilamide itself, while almost uniformly successful in clearing an uncomplicated Ducrey infection, is not nearly so effective in the treatment of gonorrhoea. In fact, many cases of the latter infection treated with sulphanilamide developed afterwards a persistent mucoid or muco-purulent discharge due to such complications as anterior folliculitis or a posterior spread. Later a similar effect was seen in certain cases treated with inadequate or "self-administered" doses of sulphapyridine. These cases were extremely difficult to clear up and required prolonged hospital treatment.

The incubation periods of chancroid and gonorrhoea are roughly the same, and in addition a patient may have had more than one recent exposure to infection. Thus a man admitted to hospital a few days after his last exposure to infection and presenting a clinical chancroid might at the same time be incubating gonorrhoea. It became obvious, then, that in such a case the administration of sulphanilamide, while clearing the Ducrey infection, would in all probability mask the signs of the incubating gonorrhoea. In the early days this not infrequently happened. The patient was discharged from hospital with a healed sore and returned in a week or ten days with a persistent urethral discharge showing many pus cells, some secondary organisms, but no gonococci. There was no history of further exposure to infection. This type of case was peculiarly resistant to treatment, and generally one or other of the commoner complications of gonorrhoea was found. This difficulty was obviated by a routine which forbade the use of sulphanilamide in "sore cases" until at least 14 days after the last admitted exposure to infection.

These facts, when realized, are in the main a matter of clinical interest and do not present any serious problem or administrative difficulty. They serve, however, as an introduction to the theme of this short article, which is intended to point out the similar but much more serious difficulties at present attendant on the treatment of sulphapyridine-resistant gonorrhoea with penicillin.

Penicillin is now coming into general use in the treatment of resistant gonorrhoea cases, in which it has been found almost uniformly successful. In the first place, then, it will not be remiss to discuss what we know of its effect on the spirochaete of syphilis, more particularly as many—one might even say most—of the gonorrhoea cases so far treated have been within the incubation period of syphilis—that is, less than three months after the last exposure to infection.

The first observation to be made is on the report from the U.S.A. on 50 cases of early syphilis treated with penicillin, the dose used being 2,500,000 units. The details are not available here. So far as can be gathered, however, *Treponema pallidum* disappeared early from primary lesions, which healed rapidly, and the blood has remained Wassermann-negative for the present period of observation; while in early sero-positive cases there was a gradual reversal of the blood Wassermann to negative. This is as far as the story goes. It is a hopeful report, but we have no means of assessing what will be the ultimate outcome of these cases—i.e., whether this dosage is curative. In this connexion it would be well to remember that penicillin administered intramuscularly or intravenously does not pass into the spinal fluid unless perhaps in minute quantity. Therefore, since invasion of the central nervous system by *T. pallidum* is common in early syphilis, one consideration of vital importance is the possibility that C.N.S. involvement may show an increased incidence.

The second observation is on two cases of sulpha-resistant gonorrhoea recently under our care.

Case I

Pte. X. entered hospital on Sept. 11, 1944. Definite dates of exposure to infection were difficult to obtain accurately (language difficulty). Though he said that the last exposure was approximately seven weeks before admission, the clinical findings suggested a more recent one.

On examination the patient was found to have a purulent urethral discharge and a clinical syphilitic chancre in the coronal sulcus. He stated that the sore had been present for four weeks and the urethral discharge for a shorter period. A urethral smear showed many intracellular gonococci, and the urine was hazy in both glasses. Dark-ground examination showed the sore to be positive to *T. pallidum*. Routine sulphapyridine—27 g. over 5 days—was given, and anti-syphilitic treatment was withheld until the course was completed. A blood Kahn test on Sept. 12 was negative. On Sept. 19 a urethral smear still showed intracellular gonococci, and there was a muco-purulent urethral discharge with hazy urine in both glasses. On dark-ground examination the sore was again positive to *T. pallidum*. It was therefore decided to start penicillin treatment for the gonorrhoea and use the opportunity offered to observe the action of this drug on the primary syphilitic lesion.

On Sept. 19, 100,000 units of penicillin were administered intramuscularly in doses of 20,000 units three-hourly. Dark-ground examination of the sore was carried out before each injection of penicillin. The following observations were made: (1) The sore was positive to *T. pallidum* before the first, second, and third injections of penicillin but was negative thereafter—that is, after 60,000 units. (2) A mild general reaction was noted and the temperature rose to 100° F. six hours after the first injection. This reaction corresponded in time to the usual Herxheimer reaction found after initial arsenic in a primary case. Twenty other patients treated with the same batch of penicillin had no reaction. (3) The next day—Sept. 20—the sore was still negative to *T. pallidum* and considerably cleaner. Urethral discharge had ceased, and urine was clear in both glasses. (4) Daily examination showed the sore to be negative to *T. pallidum* on each succeeding day until Oct. 3—i.e., 14 days after starting penicillin treatment, and at this time had almost completely healed. (Only saline dressings had been applied.) (5) The following Wassermann and Kahn results were obtained at two different laboratories:—Lab. A: Kahn test negative on Sept. 21, 24, 29, and