ORIGINAL ARTICLES

FURTHER OBSERVATIONS ON PENICILLIN

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THE work on penicillin briefly reported by Chain and others (1940) is here presented in greater detail, and its further development to the stage of human therapy is described.

Growth of Penicillin-producing Mould

The mould will grow and produce penicillin on a variety of different media, but that used by Clutterbuck, Lovell and Raistrick (1932) is easy to prepare and gives as high a yield of penicillin as others containing peptone, horse-muscle digests, &c. This modified Czapek-Dox medium consists of: $NaNO_3$ 3 g., KH_2PO_4 1 g., KCl 0·5 g., $MgSO_4.7H_2O$ 0·5 g., $FeSO_4.7H_2O$ 0·01 g., glucose 40 g., with water to 1 litre. Oxford tap-water has proved as good as distilled water for this purpose. Yeast-extract has usually been added to speed up the growth of the mould (details later).

The medium, sterilised by autoclaving, is sown with a spore suspension made by shaking up sterile water in a screw-capped bottle containing a slope on which the mould has grown and spored freely. Twenty-four hours after sowing a very delicate fluffy gauze-like growth can be seen with difficulty on the bottom of the vessel (at 24° C.). The growth becomes more voluminous during the next day and on the 3rd day, if the liquid layer is not more than 1 cm. thick, this reaches the surface of the medium and throws up dry white mycelium, usually in isolated foci, particularly around the sides of the vessel. Usually by the 4th or 5th day the whole surface of the medium is covered by dry mycelium, which about 24 hours after it appears begins to turn bluish green. The colour is first seen at the centre of each focus of white mycelium, but it rapidly spreads outwards and by the 6th or 7th day the growth consists of a continuous, compact, often corrugated, dark greenish-blue felt whose upper surface cannot be wetted by water. The under surface is of course freely wetted, and is brownish yellow and slimy. If on the 1st or 2nd day the culture-vessel is disturbed or rocked the gauze-like growth tends to conglomerate into ropes and balls, and those parts of the surface of the medium which do not lie over these will only be covered several days later by secondary growths; for this reason once they have been sown the vessels should not be touched for some days. Droplets, usually but not invariably yellow, may or may not appear on the surface of the mould, but the mould is never wetted by them. As incubation is prolonged the colour of the mycelium becomes more faded and grey.

The changes in the appearance of the mould are accompanied by changes in the pH of the medium, which starts between 6 and 7 and may fall as low as 3 by the 3rd day—usually as the dry mycelium is forming. It then rises, reaching 5 as the greenish-blue colour appears. At this stage a faint yellow colour can be seen in the medium and traces of penicillin can be detected. The pH continues to rise and the titre of penicillin and the yellow colour both increase rapidly. As the mycelium becomes more faded the pH rises more and more slowly; it seldom exceeds 8·8. Penicillin production is usually at its maximum at about pH 7, and may stay almost constant for some days or may fall again rapidly. The pH is perhaps the most useful gauge of the state of the growth, apart from an assay for antibacterial activity. The rate of development may be greater or less than that described, depending largely on the depth of the medium. A systematic study of the factors influencing penicillin-production was begun, but it could not be completed owing to the very numerous and often interdependent variables, and to the fact that the assay-method then in use could only detect large differences of titre. The following conclusions, however, could be drawn:

1. Penicillin production seems to take place over a wide range of oxygen tension. (The mould will not grow anaerobically.)
2. The mould grows satisfactorily at 24° C. At lower temperatures growth is delayed and as harvesting of the medium is carried out in the incubator higher temperatures have not been studied, 24° C. being about the upper limit of comfort. Fleming (1929) in his original description stated that the mould would not grow at 37° C. and this has been confirmed.
3. Crude attempts to change the pH of the medium or to maintain it at a constant value have not resulted in a noticeable increase in yield of penicillin, nor has the incorporation of ten times the normal amount of phosphate buffer.
4. The medium should not have a depth greater than 1·5–2 cm. If deeper than 2 cm. diffusion is visibly inadequate, for two distinct layers can be seen in it, the upper being yellow, the lower colourless.
5. When the medium is fit to be harvested it can be drawn off from under the mycelium and replaced with fresh medium in which more penicillin will form in about half the time required for the initial production. The medium can be changed several times in this way; with one batch it was changed 14 times.
6. The mould must be grown and the medium harvested and replaced under strictly sterile conditions since penicillin is destroyed by certain bacteria (Abraham and Chain 1940).
7. The addition of yeast-extract (Gladstone and Fildes 1940) accelerates the growth of the mould but does not affect the yield of penicillin. In large-scale growth we have always used yeast-extract; in starting a batch the medium is made up to contain 10% of it, but the medium used for replacement contains only 2½%. The accelerating effect of the yeast is not impaired by prolonged autoclaving at a high temperature. Marmite or malt extract have no effect on the rate of growth or on the yield of penicillin.
8. The yield is not greatly affected either by doubling or by halving the strength of the medium.
9. If the sodium nitrate is replaced by ammonium lactate the pH of the medium falls to below 3 and development almost ceases. On adjusting the pH to about 6·5 development proceeds and the medium ultimately becomes very alkaline. Very little penicillin is produced.
10. The sodium nitrate can be wholly or partly replaced by peptone or by peptic and tryptic digests of muscle, and sucrose or maltose can be used in place of glucose, without materially altering the yield of penicillin.
11. Occasionally a batch produces little or no penicillin. Although this can generally be traced to bacterial contamination in some instances contamination could not be proved, and the suspicion arose that penicillin production might be a variable and easily disturbed function of the mould. To test this, pure-line cultures were made from single cells of two batches, one of which had given a yield of penicillin and the other none. Five pure lines of the former and nine of the latter were inoculated in triplicate into bottles of medium and the penicillin titre was followed. No difference could be detected between any of the lines, proving that the mould had not lost its power to form penicillin. The possibility that the failure had been due to contamination by another mould was investigated, but no evidence of this could be found.

Method of Assay

The serial dilution method used by Fleming (1929), which measures the lowest concentration of penicillin that will prevent growth of the test-organism in broth, is laborious and can only be applied to sterile material. This at once reduces its usefulness for chemical investigations, for the material to be tested would have to be sterilised by filtration and it seems that under some conditions penicillin may be partially adsorbed on Seitz filter pads. The method of estimation finally adopted is

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as follows. Ordinary nutrient agar plates are seeded with the test organism—*Staphylococcus aureus* has been used in this work—by allowing a broth culture of the organism to flow over the surface of the agar and draining off the excess broth. The plates are then dried for an hour in the incubator at 37° C. in a special rack which supports the lid of the Petri dish half an inch above the lower part. When dry the seeded plates are removed from the incubator and can be kept in the refrigerator for one or two days. Cylinders made from short lengths of glass tubing, the dimensions of which will be seen from the inset of fig. 1, are placed on the agar. The lower edge of the cylinder is carefully ground level and has an internal bevel so that the thin edge tends to sink into the agar and make a water- and bacteria-tight seal. Vitreous porcelain cylinders of the same size and shape, coloured at the non-bevelled end to facilitate their orientation, have also proved satisfactory. The cylinders are filled with the fluid to be tested, and the plates, resting on a block of wood, are incubated for 12–16 hours at 37° C. (If placed directly on the warm incubator shelves moisture may condense on the lids of the Petri dishes and drop on the agar, thus obscuring the results.) By the end of incubation most of the fluid in the cylinders has disappeared and each cylinder is surrounded by a circular zone where no bacterial growth has occurred. The diameter of the zone depends on the concentration of the penicillin, the type of relation being shown in fig. 1.

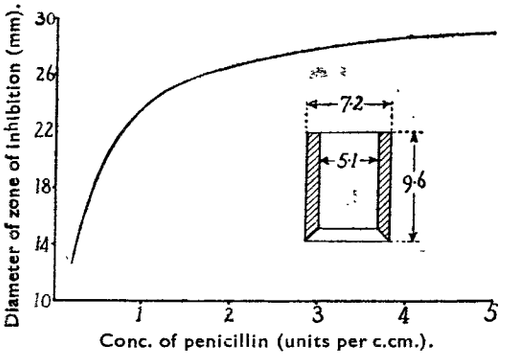


FIG. 1—Relation between assay value and concentration of penicillin in solution being tested. *Inset.*—Median vertical section through assay cylinder; dimensions in mm.

The possibilities and limitations of this method of assay have not yet been fully worked out but the following points may be noted.

1. The diameter in mm. of the zone of inhibition (which we have called the "assay value") is only slightly smaller (1–2 mm.) when the cylinder is half filled than when it is fully filled.

2. The assay value is unaffected by the pH of the fluid being tested, provided it is not strongly buffered and lies within the range pH 5–8·5.

3. No inhibition is produced by a saturated aqueous solution of ether or by water containing free droplets of chloroform.

4. Diffusion of penicillin seems to be practically complete in 2–3 hours, and assay values after 14 hours of incubation are only very slightly smaller (0·5–1 mm.) than after a further 8 hours at 37° C.

5. Provided the plate is not jarred the fluid cannot escape from the cylinders, and even if the fluid is not sterile the contaminating bacteria are confined to the inside of the cylinder.

6. The assay value is not affected by the thickness of the agar provided it is between the limits of 3 to 5 mm.

7. The assay value varies slightly with different batches of plates and with the density of bacterial population at the beginning of incubation. For this reason uneven seeding of the plates must be avoided.

8. Sometimes the clear zone of inhibition is surrounded by a halo of partial inhibition, which varies from a faint ghost to almost complete inhibition. So far no explanation for, or means of controlling, this phenomenon has been discovered.

9. When the antibacterial activity of blood is to be assayed plasma or serum must be used, since red cells tend to form a layer immediately on top of the agar which seriously impedes the diffusion of penicillin and leads to low values.

High accuracy cannot be claimed for this method of assay, but if it is done in triplicate (preferably on three different plates), and if the unknown solution is diluted so as to give an assay value of not more than 25 mm. (before the curve has flattened out), the error is probably not greater than ± 25% and may be considerably less. We have no evidence that under suitable conditions this method is inferior in accuracy to the serial dilution method and it is certainly many times quicker. In addition, less than 0·25 ml. of fluid is required for each test.

Unit of antibacterial activity.—When day-to-day comparisons have to be made rather large errors may be introduced for the reasons mentioned in note 7 above, unless a standard is set up on each plate to which all the assay values may be related. We have been using as such a standard a partially purified solution of purely arbitrary strength. It is made up in dilute phosphate-buffer, is saturated with ether and is kept in the ice-chest. It gives an average assay value of 24 mm. and as far as can be seen its activity has not altered during the three months in which it has been in use. We have adopted as the arbitrary unit (until penicillin is obtained chemically pure) *that amount of penicillin which when dissolved in 1 c.cm. of water gives the same inhibition as this standard.* The material used in the human therapeutic tests usually contained 40–50 of these units per mg.

Large-scale Production of Penicillin

Culture vessels and sowing.—After many types of containers had been tried a satisfactory ceramic vessel was eventually designed,[1] the shape and dimensions of which can be seen in fig. 2. The vessels are glazed only on the inside; this both reduces the cost and renders them easier to handle and less liable to slide when stacked one on top of the other. The inset of fig. 2 shows a convenient way in which they can be stacked for autoclaving, sowing and so on; each plug is well separated from the other but no bench space is lost, and should the medium boil in the autoclave the plugs are unlikely to be wetted. One litre of medium fills the vessels to a depth of about 1·7 cm. When a batch of vessels is first set up the medium (containing 10% of yeast-extract) is sterilised in the vessels, which are then inoculated with a few drops of a spore-suspension[2] and incubated at 24° C. Apart from an occasional test the vessels are not touched until the medium is ready to be harvested.

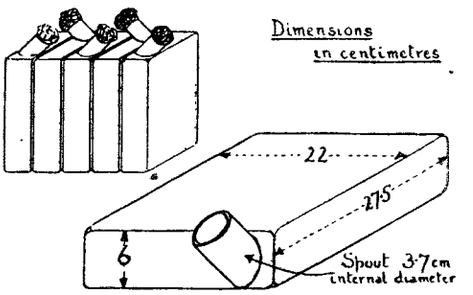


FIG. 2—Earthenware culture vessel. *Above.*—Vessels stacked for autoclaving.

Arrangements for withdrawing and replacing medium.—The penicillin-containing fluid is withdrawn from the vessels and replaced with fresh culture-medium. To carry out these two processes without introducing contamination is far from easy and needs thorough bacteriological precautions. In order to hasten the production of penicillin we attempted to reduce these precautions to a minimum, and at first obtained satisfactory results; but lately contamination has become so frequent that substantial modifications have had to be introduced, some of which are still on trial and therefore cannot usefully be described.

The withdrawal of culture-medium is done by suction, the medium being replaced with sterile air. In the original method the refilling was done by simple pouring, but safer methods (not yet finally determined) have now been introduced, and precautions at all stages are being tightened up.

The operations can conveniently be carried out on the "changing-trolley" (fig. 3), made by replacing the top of a small all-metal table by a wooden plank A, to which are hinged the four wooden slabs B on which the vessels are placed. The raised rim C is prolonged into a handle D, by which the slab (and the vessel on it) can be tilted forward. The slab is not hinged square with the edge of A, but at a slight angle to it, so that on depressing the handle D the fluid in the vessel drains not merely to the front edge but to the corner nearest the spout. The medium is drawn off by suction with the help of a special pistol-shaped holder E, carrying two detachable sterilised tubes F and G. F is plugged with cotton-wool before sterilising, and through it is blown into the vessel a current of (sterile) air at a somewhat faster rate than

1. Made for us by J. Macintyre and Co., who also made the porcelain assay cylinders.
2. The strains of penicillium used in this work have been obtained from Prof. Alexander Fleming of St. Mary's Hospital, London.

the fluid is drawn out by tube G. Suction and pressure are controlled by the single trigger H of the "pistol." A fresh tube G is used for every batch of four vessels and F is changed after every two batches. Both tubes are sterilised wrapped in paper and only one end of the tube is unwrapped when being

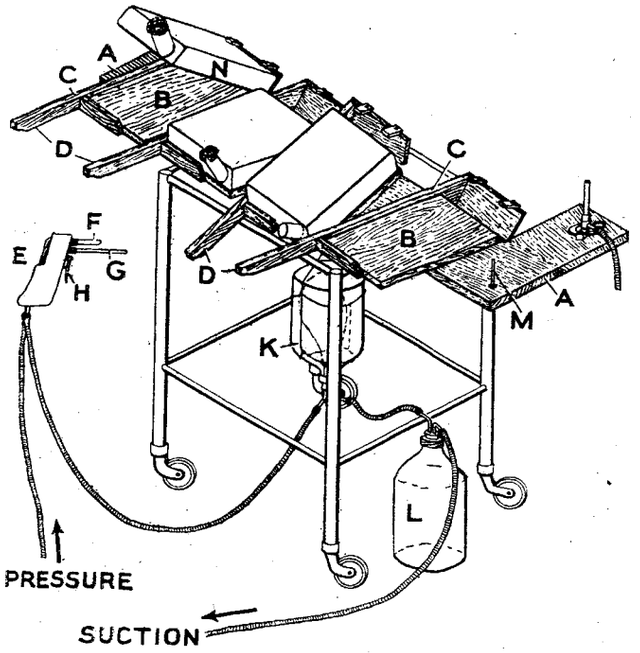


FIG. 3—Changing trolley.

fitted into the pistol; the tubes are unsheathed from the remaining paper wrappings immediately before use. From the pistol the harvested medium passes to the inverted bottle K, in which it is filtered through a silk bag. The filtered liquid than passes to the collecting bottle L which is changed after every batch of four vessels. When not in use the pistol is hung on the pin M. For refilling the vessels are tilted at about 45°. A fitting on each wooden slab enables the vessel to be wedged in this position (as shown by vessel N in fig. 3), so that both hands are free to manipulate plug and bottle of medium.

EXTRACTION FROM CULTURE MEDIUM

Principle.—Penicillin can be extracted by ether, amyl acetate and certain other organic solvents from an aqueous solution whose pH has been adjusted to 2. From the organic solvent the penicillin may be re-extracted by shaking with phosphate-buffer or with water the pH of which is kept at 6–7. Penicillin is quickly destroyed at pH 2 at room temperature, so the first extraction must be carried out rapidly or at a low temperature. Once it has been extracted into solvent the penicillin is stable for some days.

Laboratory large-scale extraction.—It was planned to produce a weekly output of about 500 litres of the crude medium containing penicillin, the working up of which by hand would obviously have been out of the question. A continuous countercurrent extraction apparatus was devised, an adequate description of which cannot be included in this paper. The crude penicillin, having been filtered and acidified, is passed through special jets which break it up into droplets of uniform size. These are allowed to fall through a column of amyl acetate, to which the penicillin is given up. The crude solution is acidified immediately before entering the jets, so that the aqueous solution is at a pH at which penicillin is unstable for only a few seconds before it has been extracted. As the crude solution is passed through a cooling coil surrounded by circulating tap-water probably very little destruction of penicillin takes place. Phosphoric acid is used for the acidification as the pK of the first stage of dissociation is approximately 2; it will therefore act as its own buffer and inaccurate correlation of the rates of flow of the acid and of the crude solution will not cause big fluctuations in the final pH. Fresh solvent is continuously fed in to the bottom of the column, from the top of which an equal amount of penicillin-rich solvent is collected for further working up. This solvent has 1/10–1 5 of the volume of the crude solution from which the penicillin has been extracted, and many impurities, notably those forming emulsions, have been eliminated.

The preparation used in the mouse-experiments previously reported (Chain et al 1940) was purified only to the stage of recovering the sodium salt from a first ether extract (ether was at that time used instead of amyl acetate). Though strongly active this product probably had less than 1/10 of the activity of our present "therapeutic penicillin." A batch intended for injection into man was first tested on mice. A mouse was little affected by the intravenous injection of 10 mg. in 0·3 c.cm. of water but became very ill after receiving 20 mg., though it recovered in about 1½ hours. When given intravenously to an afebrile human subject 100 mg. of the same material caused a shivering attack with a rise of temperature in about an hour. Trial on other subjects gave the same result and left no doubt that a pyrogenic substance was present in the preparation. The pyrogen can be assayed by the method used by Lees and Levvy (1940) in which five rabbits of uniform weight (a little over 2 kg.) are kept at a constant temperature (67° F.). On the day of the test they are deprived of food and the rectal temperature is taken hourly. Twenty milligrammes of the substance used in the mouse experiments dissolved in 1 c.cm. of distilled water, injected intravenously, gave a rise of about 2° F. in each rabbit, but "middle fraction" from the adsorption column (see below) had no pyrogenic effect. The further purification here described secures a pyrogen-free product suitable for intravenous use.

Further purification.—Batches of 3 litres each of the penicillin-containing solvent as delivered from the extraction-apparatus are extracted with five successive amounts of 300 ml. each of water, using baryta to adjust the pH to 6·5–7. The five watery solutions have already been used for former extractions and are used in order of decreasing concentration of penicillin. The first extract, which is the strongest, is set aside for further working up, but a sixth extract is made with fresh water so that the number of watery solutions remains the same. The amyl acetate, which may still contain a small amount of penicillin, is recirculated in the extraction apparatus. In the course of time pigment accumulates in the solvent and is periodically removed by extraction with 1% sodium hydroxide followed by thorough washing.

The strongest aqueous extract is partially decolorised by shaking with about 8% of animal charcoal and filtering. The charcoal residue is washed twice, the washings being put through the extraction apparatus or used in other ways. The partially decolorised solution is cooled, acidified and extracted into successive amounts of ether; the strongest of the ether extracts is then passed through an adsorption column of Brockmann alumina. The spent ether contains solid matter but no penicillin, which remains behind in the column. Though the chromatograms vary, four main zones can be seen, their boundaries overlapping to some extent. These are, starting from the top:

1. A dark brownish-orange layer whose depth is inversely proportional to the amount of charcoal used for the decolorisation and which may be absent altogether. This layer contains some penicillin.
2. A light yellow layer containing most of the penicillin but none of the pyrogen.
3. An orange layer which contains some penicillin and some or all of the pyrogen.
4. A brownish or reddish-violet layer which contains practically no penicillin. The violet pigment disappears on exposure to light.

The column is washed by passing successive small amounts of ether through it and is then divided into the four fractions, of which the fourth is discarded. The others are eluted with successive amounts of M/15 phosphate buffer (pH 7·2), from the strongest of which the penicillin is again extracted into ether, while the more dilute solutions are used for the elutions of the next batch. Finally the penicillin is extracted back into water using sodium hydroxide to adjust the pH. (As the solution is not buffered the greatest care must be taken in adding the alkali, for penicillin is rapidly destroyed in alkaline solution.)

The "non-pyrogenic" or "therapeutic" fraction, which contains perhaps 80% of the penicillin put through the column, is extracted into pyrogen-free water, all glassware having been rinsed with the latter. It is a deep reddish-orange fluid, yellow in dilute solution, with a faint but characteristic smell and a bitter taste.

Yields and losses.—The crude medium, as harvested, usually contains between 1 and 2 units per c.cm. and the dried purified therapeutic material has an activity of 40–50 units per mg. A hundred litres of the medium, containing, say, 150,000 units, gives a yield of about 1 g. of therapeutic material containing, say, 45,000 units, so that about a third of the penicillin present is actually extracted in a form suitable for intravenous injection. There are many stages in the preparation at which the loss may occur and as far as possible these have been checked individually, but owing to overlapping of different batches during the working up (e.g., in serial extraction) and insufficient accuracy in the assay method, it has been difficult to analyse the losses accurately. No big loss, however, occurs at any one stage.

Storage and dispensing.—The strong aqueous extract saturated with ether is quite stable. It is stored either as it is in the refrigerator, or is dried by the lyophilic method and kept in a desiccator, as the voluminous yellow powder is hygroscopic. A solution of the sodium salt of penicillin in water kept in the ice-chest for 3 months did not lose any activity. The barium salt kept dry at room temperature in the desiccator retained its activity for at least 8 months. In dispensing the material for intravenous injection it is assumed that the ether-containing solution is sterile, but most of the ether must be removed by suction before use. The ether-free solution is stored in the ice-chest in measured doses.

BACTERIOSTATIC ACTION OF PENICILLIN

Using purified penicillin more than a thousand times stronger than the crude material employed by Fleming in 1929, we have been able to obtain more precise information about the bacterial species he examined and also about a number of other important pathogenic organisms.

Method.—To 4·5 c.cm. quantities of fluid culture medium 0·5 c.cm. of graded dilutions of a strong, filtered penicillin solution were added. Each tube of a series was then inoculated with a standard drop (0·04 c.cm.) of a 24 hours fluid culture of the microbe under investigation. In the case of the robust organisms, such as *Staphylococcus* or *Bacterium*, the culture was usually diluted 1 in 100 or 1 in 1000 before use. All tests were done in duplicate. Complete inhibition was shown by the absence of turbidity after 24 hours at 37° C., the control culture showing good growth; partial inhibition was recorded when the growth was clearly less than that of the control.

Results.—In table I the bacterial species are arranged as far as possible in their order of sensitivity, but both the order and the actual figures must be taken with reserve. As the work progressed the penicillin was continually becoming purer and stronger, so that some of the figures obtained in the early stages may well be too low. Moreover, there has sometimes seemed to be some loss in potency of penicillin solutions when sterilised by Seitz-filtration, a point that needs further investigation. Finally, only a few species have been tested in more than one medium and with a minimal inoculum, both of which factors influence the observed titre.

Certain species, such as pneumococci and *Strep. viridans*, cannot be put in a single place in the order of sensitiveness because they show great strain differences, and it will be noted that in the pneumococci these cut across the type distinctions. Maclean, Rogers and Fleming (1939) have recorded a similar variability in sensitiveness to sulphapyridine of individual strains of the pneumococcal types. It seems likely that an experimental comparison of the effects of the two inhibitory agents on a wide range of pneumococci would throw light on their modes of action.

The testing of *Myc. tuberculosis* was not easy. Owing to the imperfect stability of penicillin at 37° C. and the slow growth of the microbe it was inadmissible merely to incorporate the inhibitor with the medium at the outset. The microbe was therefore cultivated in glycerol-broth, and penicillin solution was added every 2 days in such a way as to maintain at least the stated concentration for 14 days. The microbe grew well in all dilutions. A similar experiment had already been done by Dr. R. L. Vollum, with similar results.

It should be noted that complete, or even partial inhibition of macroscopic growth after 24 hours of incubation is not the most delicate criterion of antibacterial action. In somewhat higher dilutions the growth is retarded, and, as has already been recorded (Gardner 1940), a microscopical effect on the bacteria, indicative of defective fission, may often be seen far beyond the macroscopically inhibitory dilution. We cannot say whether the virulence of the bacteria is thereby reduced; but if it is the effect may be of therapeutic importance. For example, in one experiment with *S. typhi* (*Bact. typhosum*), whereas the last visible, partial inhibition was at 1/10,000, an elongation of the cells was microscopically detectable up to 1/60,000, a concentration which may be a therapeutic possibility.

Adaptation of Staph. aureus to penicillin.—In order to find an answer to the questions whether bacteria will

TABLE I—DILUTIONS OF PENICILLIN AT WHICH VARIOUS INHIBITORY EFFECTS HAVE BEEN OBSERVED

Bacterial species	No. of strains	Dilutions at which inhibitory effects were observed			Notes
		Complete	Partial	None	
*N. gonorrhœæ**	6	2,000,000	>2,000,000	>2,000,000	—
N. meningitidis	1	1,000,000	2,000,000	4,000,000	—
Staph. aureus	4	1,000,000	2,000,000	4,000,000	—
Strep. pyogenes	3	1,000,000	2,000,000	4,000,000	—
B. anthracis	1	1,000,000	2,000,000	4,000,000	—
A. bovis (hominis)	1	1,000,000	2,000,000	4,000,000	Deep glucose-agar shake cultures.
Cl. tetani †	1	1,000,000	..	..	Limit not observed.
Cl. welchii	1	1,500,000	..	..	,,
Cl. septique	1	300,000	1,500,000	7,500,000	Fivefold dilutions used.
Cl. œdematiens	1	300,000	..	1,500,000	Inoculum of spores.
Strep. viridans‡	2	625,000	..	3,125,000	But see other strains below.
Pneumococcus ‡	6	250,000	500,000	1,000,000	3 of type 1; one each of types 3, 7 and 9. Some complete at 500,000.
C. diphtheriæ (mitis)	1	125,000	..	625,000	Intermediate dilutions not tested.
,, *(gravis)*	1	32,000	64,000	128,000	—
S. gärtneri	1	20,000	40,000	80,000	—
S. typhi	2	10,000	30,000	90,000	Higher of two results shown.
Pneumococcus ‡	3	9000	..	27,000	Types 1, 7 and 19.
Anaerobic streptococcus ‡	1	4000	8000	16,000	—
Proteus	3	4000	32,000	60,000	Best strain shown. Others 4 times less sensitive.
Strep. viridans ‡	1	4000	8000	16,000	—
Past. pestis	2	1000	100,000	500,000	Partial inhibition between 1000 and 100,000
S. typhimurium	1	<1000	8000	16,000	Partial 1000–8000
S. paratyphi B	2	<1000	5000	10,000	Partial 1000–5000.
Bact. dysenteriæ Shiga	1	2000	4000	8000	—
Br. abortus	1	2000	4000	8000	—
Br. melitensis	1	<1000	2500	10,000	—
Anaerobic streptococcus	1	<4000	<4000	4000	—
V. cholerœ	1	<1000	1000	2000	—
Bact. coli	5	<1000	<1000	1000	One strain showed complete 200, none 400.
Bact. friedländeri	1	<1000	<1000	1000	—
Ps. pyocyanea	2	<1000	<1000	1000	—
Myco. tuberculosis	1	<1000	<1000	1000	See text.
L. icterohæmorrhagiæ	1	<3600	<3600	3600	Lower dilutions not tested.

* Another strain was only inhibited up to 32,000. † Grown in Lemco broth. In beef broth complete inhibition only reached 100,000.
‡ In *Pneumococcus, Strep. viridans* and anaerobic streptococci different strains appear at different levels in the table.

acclimatise themselves to inhibitory concentrations of penicillin, and if so whether they do so by producing the penicillin-destroying enzyme penicillinase, which has been demonstrated in certain saprophytes and commensals (Abraham and Chain 1940), a strain of *Staph. aureus* was cultivated for some months in broth in the presence of increasing quantities of comparatively crude penicillin. Even after a few daily subcultures there was evidence of increased resistance, the coccus showing growth in at least twice the previously inhibitory concentration. In about nine weeks, with subcultivation every few days, an approximately 30-fold adaptation was reached, and after a further 7 weeks the microbe was able to multiply in a concentration of penicillin a thousand times greater than that which inhibited the parent strain in a parallel test—while the parent was almost completely inhibited by 1 in a million, the adapted strain grew quite well in 1 in 1000.

Contrary to expectation the main general effect of adaptation to penicillin was a reduction both in the velocity of growth in ordinary media and of enzyme activities. The adapted strain was repeatedly tested in comparison with the parent strain on a range of fermentable substances, and it was found that although after 10–14 days of incubation both strains showed the same reactions (acid in glucose, lactose, maltose, mannitol and saccharose, no change in dulcitol; liquefaction of gelatin; acid and coagulation in milk) the adapted strain showed a considerable lag, taking 2 days instead of 1 to acidify lactose, 4 days instead of 1 for mannitol, and 11 days instead of 1 to coagulate milk. A reduction of growth-velocity in nutrient broth and agar was also seen and hæmolysis on blood-agar plates was much delayed. Both strains however gave a good coagulase reaction.

Both about the middle (9 weeks) and at the end of the experiment just described attempts were made to discover in the adapted strain a penicillin-destroying enzyme not present in the parent coccus. The adapted staphylococcus was grown on nutrient agar in two batches of 20 rolled Winchester bottles, in order to obtain a sufficient amount of bacterial bodies to be used in the bacterial crushing mill of Booth and Green (1938). The rather poor growth was washed off after 3 days, centrifuged, washed with water, centrifuged again and made up with water to a pasty consistency. It was then ground in the crushing mill for 3 hours. After the crushing, which was very satisfactory, the extract was centrifuged at 7000 revs. for 20 minutes; 1 c.cm. of the extract was then incubated with 1 mg. of penicillin for 5 hours at 37° C. There was no sign of loss of penicillin-activity after this period. There is therefore no evidence that the adaptation of the staphylococcus to penicillin depends on the production of a penicillin-destroying enzyme by the organism.

In an earlier experiment, aimed at discovering whether the resistance of insusceptible bacteria is due to their power of destroying penicillin, *Bact. coli* was cultivated in broth containing the strongest non-inhibitory concentration of penicillin for this organism (1/400). Incubation overnight produced a rich growth of the bacterium. The culture was then Seitz-filtered and the filtrate was tested in serial dilutions on *Staph. aureus*. Complete inhibition occurred up to 1/500,000, which was the full titre for the batch of penicillin used. It could therefore be concluded that the growth of *Bact. coli* had not caused any destruction of the penicillin.

EFFECTS OF SERUM, BLOOD, PUS, TISSUE AUTOLYSATES AND PEPTONES

In view of the inhibitory effect known to be exerted by products of tissue autolysis on the action of the sulphonamides, it was natural to look for a similar effect on penicillin. None, however, was found.

Serum.—To exclude any such action on the part of blood-serum, penicillin was diluted with human serum and tested on the staphylococcus by a modification of the assay method described above.

The modified method consists of making shake cultures of the microbe, in this case *Staph. aureus*, in narrow glucose-agar tubes, solidifying and then running 0·6 c.cm. of the penicillin or other solution on the top. After 24 hours' incubation at 37° C. a zone of clear medium is seen below the fluid and above the zone of growth. A small inoculum of cocci gave the best results—e.g., one standard drop of a 24-hours broth culture diluted 1/100 added to 5 c.cm. of melted glucose agar.

When penicillin diluted 1/10,000 with serum was thus tested in comparison with the same dilution in distilled water, both in duplicate, the depth of the zones of inhibition measured in millimetres:

In serum	(1) 23–24	(2) 22
In water	(1) 22	(2) 23–24

Serum, therefore, in no way interferes with the action and diffusibility of the active substance.

Whole blood.—One or two experiments were done in slide-cells to test the action of whole blood, and of these it is enough to say that in one such experiment a penicillin dilution of 1 in 2 million in human blood inhibited a dose of *Strep. pyogenes* which, when plated, produced about 50,000 colonies per c.cm. of inoculated blood. Other experiments agreed in demonstrating that there was no antagonistic effect.

Pus.—With regard to pus, the following experiment failed to show any antagonistic action.

Some very thick pus from a *Staph. aureus* abscess was incubated for 3 days at 37° C. to "thin" it; after centrifugalisation the supernatant fluid was diluted 1 in 2 with nutrient broth and filtered through a Seitz filter. To 2·25 c.cm. of this 50% pus-broth 0·25 c.cm. of a solution of 0·2 mg. of (impure) penicillin per c.cm. was added, making a penicillin dilution of 1/50,000. As a control, 2·5 c.cm. of the pus broth without penicillin was taken. Both these were inoculated with one standard drop (0·04 c.cm.) of a 1/1000 dilution of a 24-hour broth culture of *Staph. aureus*. The number of cocci in the inoculum was probably of the order of 40,000. After 24 hours' incubation at 37° C. the control tube showed a rich growth of staphylococcus and the penicillin tube showed none. Platings from this tube and cultivation of 20, 5 and 1 drops in 5 c.cm. broth tubes showed no growth, indicating that at least most of the cocci had been killed, since the amount of penicillin carried over with 1 drop (1 in over 6 million) was insufficient to inhibit growth.

A similar experiment was done with a sample of urine loaded with pus due to infection with *Bact. coli* and *Strep. fæcalis*. In this case several dilutions of penicillin were made in the filtered 50% pus-broth—1/50,000, 1/150,000, 1/450,000 and 1/1,350,000. The inoculum of *Staph. aureus* was the same as in the previous experiment. After due incubation the two stronger concentrations of penicillin showed complete inhibition of staphylococcal growth, while in the two weaker ones, as in the control of 50% pus-broth alone, full growth occurred. Since the full antistaphylococcal titre of the sample of penicillin used was only about 1/250,000, the experiment gives no evidence of any antagonism by the pus.

Tissue autolysates.—The possibly antagonistic effect of tissue autolysates was tested as follows.

A liver taken aseptically from a rat was minced in the homogeniser of Potter and Elvehjem (1936) and allowed to autolyse in the homogeniser tube for 4 weeks at 37° C. There was no gas evolution during the incubation and no putrefactive smell at the end of the experiment. The liver suspension had partly liquified and when it was centrifuged about 2 c.cm. of a clear, dark yellow solution were obtained. This fluid (0·9 c.cm.) was incubated at 37° C. for 16 hours with a penicillin solution in water (0·1 c.cm.) containing 1 mg. of the crude material. A control solution of 1 mg. of penicillin in 1 c.cm. of water was incubated for the same period. When after incubation the solutions were assayed, it was found that the liver autolysate had not destroyed the bacteriostatic power of the penicillin.

Peptones are equally devoid of antagonistic action; in fact in the usual assay method agar containing peptone is used as the culture medium for the staphylococcus.

CHARACTERISTICS OF ANTIBACTERIAL ACTION

The bacteriostatic power of penicillin against streptococci and staphylococci is as great as, or greater than, that of the most powerful antiseptics known, such as the heavy metal compounds, the acridine derivatives, &c. Yet penicillin is no antiseptic; it exerts no direct immediate bactericidal action. This was borne out by two observations. First, the oxygen uptake of staphylococcal suspensions was not inhibited to any measurable degree by the addition of penicillin to a final concentration of 1/1000, over a period of 3 hours. Secondly, after incubation at 37° C. for 24 hours a staphylococcal suspension in broth containing 1/1000 penicillin grew

large numbers of colonies on subculture. The only trace of lethal effect that we observed was an acceleration of the death-rate of small numbers of streptococci in Ringer's fluid containing 1/50,000 penicillin, compared with the rate in pure Ringer's fluid.

In its mode of action, penicillin resembles the bacteriostatic sulphonamide drugs, but on comparing their actions one becomes aware of the following important and significant differences.

1. The bacteriostatic power of penicillin against streptococci and staphylococci is much greater than that of the sulphonamides, even when these substances are tested under optimal conditions (small inoculum, peptone-free media, &c.). Saturated watery solutions of sulphapyridine and sulphathiazole show no complete inhibition on an assay plate, whereas penicillin at 1/500,000 gives an appreciable clear zone.

2. The action of penicillin on streptococci and staphylococci, unlike that of the sulphonamides, is only influenced to a minor extent by the number of bacteria to be inhibited. Even when the culture media are inoculated with several millions of staphylococci or streptococci per c.cm. of medium, the multiplication of these organisms may be completely inhibited by penicillin in a concentration as low as 1/1,000,000. With smaller inocula the inhibition will occur in even higher dilutions. This property of penicillin is of great importance for the treatment of heavily infected wounds, on which the sulphonamide drugs seem to have but little beneficial action.

3. The bacteriostatic power of penicillin against streptococci and staphylococci is not antagonised to any appreciable degree by hydrolytic protein breakdown products or products of tissue autolysis or pus, substances which annul completely the bacteriostatic action in vitro of the sulphonamide drugs. This is again of great importance in the treatment of suppurating wounds and makes possible the successful treatment of infections in which abundant production of pus takes place.

EFFECT OF PENICILLIN ON CELLS

LEUCOCYTES

There is little doubt that substances which are not bactericidal but only bacteriostatic depend to a considerable extent for their curative properties on the activity of the leucocytes which deal with the organisms while the latter are prevented from multiplying. In tests on leucocytes "therapeutic penicillin" has been compared with some other bacteriostatic substances which are at present engaging attention both for general and local use.

Technique.—We are indebted to Dr. C. G. Paine of The Jessop Hospital, Sheffield, for sending details of his technique for examining leucocytes. His solution has been used, though his method for examining the leucocytes has been altered.

Human leucocytes survive well in a solution containing urea 0·3 g., glucose 1 g., $NaHCO_3$ 1·61 g., $NaH_2PO_4.H_2O$ 0·425 g., NaCl 4·95 g., KCl 0·625 g., $MgCl_2$ 0·24 g., $CaCl_2$ 0·31 g. in a litre of distilled water, with 10% of serum added at the time of the experiment. The test substance was dissolved in the salt solution and diluted serially, each dilution allowing for the addition of the serum. The control consisted of salt solution and serum. The leucocytes came from the same donor as the serum. A drop of blood was allowed to clot on a coverslip and the coverslip incubated at 37° C. for about 30 min. in a Petri dish containing moist blotting-paper. The clot was picked off, the red blood-cells washed away with warm salt solution and the coverslip reversed over a well-slide containing the test solution. Many leucocytes stuck to the coverslip and could be examined under all powers of the microscope. The slide and microscope stage were kept at 36°–37° C. in a thermostatically controlled box.

Penicillin, the flavines and the sulphonamides were obtained as dry powders and dissolved in the salt solution. Hypochlorite was obtained as the concentrated stock solution of "electrolytic sodium hypochlorite" dispensed for clinical use, which contains 1% NaOCl. Dilutions are given in terms of this solution, not of hypochlorite. Albucid was provided as a 30% solution of the sodium salt (solvent not stated); this solution was diluted as usual, and the concentrations given are those of the pure substance. To make concentrated solutions of sulphapyridine and sulphathiazole the substance was shaken with the salt solution for 2 or 3 hours at 37° C. and the excess centrifuged off.

The pH of the salt solution and of the final preparations was checked with brom-thymol-blue. Albucid was found to be slightly acid even when buffered by serum. Each substance was tested more than once; the results were always consistent. A wide range of dilutions was used in most experiments but only the significant results are reported.

Results.—Penicillin at a dilution of 1/100 killed leucocytes immediately; at 1/250 more than 50% lived for 4 hours—the longest time for which they were watched—but the survivors were sluggish towards the end. The preparation at 1/500 was indistinguishable from the control.

Proflavine (2 : 8-diaminoacridine hemisulphate) killed immediately at 1/20,000 and within 2 hours at 1/50,000. In 1/100,000 the leucocytes were sluggish after 2 hours, but in 1/200,000 they were active for at least the same length of time. In the lower dilutions the leucocytes became stained yellow immediately, at 1/100,000 by the end of an hour and at 1/200,000 by the end of 2 hours. Leucocytes were killed immediately by 2 : 7-diaminoacridine monohydrochloride at 1/10,000 but at 1/20,000 they were active. Albert, Francis, Garrod and Linnell (1938) found leucocytes fully active in both these substances at 1/8400 after 1 hour. They mixed whole blood with the test solution on the coverslip and lowered a slide over to form a thin film. Though the method was not the same as ours it is difficult to see where the discrepancy arises, unless the large amount of serum present inactivated the proflavine.

The stock solution of hypochlorite diluted to 1/100 killed immediately. After 2 hours the leucocytes in 1/500 were motile but sluggish compared with 1/1000, which equalled the control in activity.

In 1/500 sulphanilamide after 2 hours, leucocytes showed only feeble pseudopod formation and no movement. At 1/1000 their activity was less than the control, though all were alive. Albucid gave a similar result at 1/500 and 1/1000; 1/100 killed immediately and at 1/10,000 the leucocytes remained active for 2½ hours, the longest time for which they were watched. Sulphapyridine and sulphathiazole, being relatively insoluble, were used in saturated solution. At dilutions of 1/1·1 and 1/2 of the saturated solution neither inhibited immediately. After 2 hours some of the leucocytes were dead in both the sulphapyridine preparations; in the sulphathiazole preparations all were alive though some were inactive at 1/1·1.

It appears therefore that the flavines are more toxic than was previously supposed (this finding is in agreement with recent tissue-culture studies) and are not suitable for repeated use in high concentration on such lesions as burns. The hypochlorite solution, which is being used in 5% strength for washing burns, is also very lethal to leucocytes, though it may possibly be inactivated at the surface of the tissue. The sulphonamides in general have little inhibitory effect on the leucocytes, which strengthens their value for local application. Penicillin is less toxic to leucocytes in vitro than the sulphonamides and should make an admirable local application for infected tissue surfaces. Its great solubility might lead to rapid removal by the bloodstream, but only further tests can show whether this is so. Continuous irrigation or repeated application would in any case overcome the difficulty.

The fact that penicillin at 1/500 does not appear to embarrass leucocyte activity in vitro contrasts with its complete bacteriostatic effect on staphylococci and streptococci in vitro at dilutions of at least 1/1,000,000. It is clear that leucocytes will remain completely active in any concentration of penicillin likely to be reached after intravenous injection.

TISSUE CULTURE

Complementary to these experiments on leucocytes are those done on tissue cultures by Dr. P. B. Medawar of the Zoology Department, who has kindly allowed us to include his results here.

Technique.—The method employed had been devised for the determination of the inhibitory power of drugs (Medawar 1940, Jacoby, Medawar and Willmer 1941). It takes advantage of the fact that tissues freshly explanted into a medium of blood-plasma diluted with saline when incubated at body temperature show a characteristic power of resistance to growth-inhibition which depends on the source and cellular composition

of the tissue and the embryonic age. The method determines by serial dilutions the drug-concentration *just sufficient* to suppress all outgrowth of fibroblasts from fragments of the ventricle of the 10-day-old chick embryo when incubated for 24 hours at 37° C. The drug is dissolved in an equal mixture of fowl plasma and Ringer and the dilutions prepared from it form a series each member of which is nine-tenths of the strength of its predecessor. At each level of the scale five or ten explants are grown in separate chambers each containing 0·1 ml. of the medium. Inspection of the culture series after 24 hours makes it possible to determine the threshold concentration of the drug for total growth-inhibition under the conditions described.

Results.—The threshold concentration for penicillin under the conditions described is 1/800. Growth at half this concentration (1/1600) is feeble and is arrested after the 24th hour of incubation. The cells are small and vacuolated and undergo retrogressive changes leading to fragmentation and death if contact with the drug is prolonged. The lowest concentration so far examined by this method, 1/6000, is still detectably toxic to fibroblasts.

If the culture series used in the experiment described above is maintained for 48 hours and each explant is then washed for an hour in Ringer and re-cultivated into a normal medium, it is possible to give an estimate of the minimum lethal concentration of the drug—i.e., the concentration which the standard explants are just able to withstand for 48 hours without undergoing changes which are irreversible by further cultivation. For penicillin this concentration is well below 1/1200 and rather higher than 1/1600. Explants re-cultivated after 48 hours' subjection to 1/1600 penicillin are in fact capable of resuming normal growth and activity after 3 or 4 additional passages in a normal medium.

It is interesting to contrast these two figures for penicillin—1/800 and 1/1600 for threshold point and minimum lethal concentration respectively—with those for proflavine under similar conditions. The corresponding values are 1/120,000 and 1/250,000.

The action of penicillin on epithelial tissues (10-day chick embryo intestine and lung, late rat-embryo kidney) can be investigated by growing small fragments on the surfaces of a series of coagula containing falling concentrations of the drug. Epithelial tissues proliferate freely for 48 hours on the surface of coagula containing 1/2000 penicillin. Degenerate growth with maceration of epithelial sheets can be obtained in concentrations as high as 1/1000. Penicillin has no power of differential inhibition for fibroblasts and epithelia; from a specified

TABLE II—EFFECTS OF PENICILLIN ON HEN'S BLOOD MACROPHAGES IN VITRO DURING A SINGLE GROWTH CYCLE

Final conc. of penicillin	Latent period	Mitotic activity	Cell morphology	Remarks
1/10,000 1/2000	Slightly prolonged	Strong	Unchanged	No after-effects
1/500	Prolonged	Reduced	Little change; slight tendency to round off	Quick recovery in fresh serum
1/200		Complete inhibition (time of observation 42 hours)	Cells "grow" smaller; rounding off, clumping	Morphological recovery in fresh serum is rapid; mitotic activity also resumed but not immediately

source, fibroblasts and epithelia either grow together under the influence of the drug, or neither grow.

The tests described were designed only to indicate the order of magnitude of the concentrations of penicillin which are significantly toxic to various types of living cell. These concentrations may be said to be not less than 1/5000. Significance can be attached to tissue-culture experiments with drugs only when they exert no secondary action, such as reaction with serum, high osmotic pressure or surface activity, which obscure an estimate of their specific toxicity. Penicillin exerts no appreciable secondary reaction of this kind. The preparations used in the experiments were "therapeutic penicillin" and also an even more highly purified sample. Their actions in vitro proved to be identical, and the figures quoted may be taken to refer to either.

Dr. F. Jacoby, of the physiology department, Birmingham University, has tested the effects of penicillin on hen's macrophages cultivated in vitro. His results are presented in table II and fig. 4.

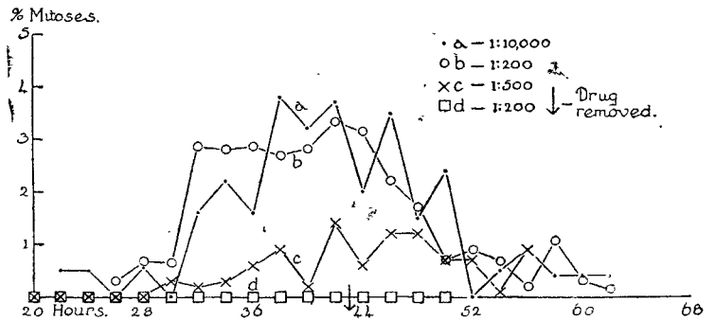


FIG. 4—Effect of penicillin in various dilutions on hen's macrophages cultivated in vitro.

It thus appears that penicillin is even less toxic to hen's macrophages than to fibroblasts and epithelium.

LOCAL APPLICATION

The effect of local application has been tried on the relatively delicate tissue of the central nervous system. In two rabbits under Nembutal anæsthesia a needle was inserted into the cisternal space and when a few drops of clear cerebrospinal fluid had escaped a burette containing 1/1000 penicillin in normal saline was attached to the needle. A slow intravenous injection of 11 c.cm. of 25% sodium chloride was then made, which caused rapid absorption of cerebrospinal fluid into the blood-stream with consequent indrawing of fluid from the burette into the cisternal space. Weed (1922) showed that under the influence of this powerful osmosis the fluid introduced passed along the perivascular sheaths and even into the perineuronal spaces of the cerebral cortex. In this instance 2 c.cm. of penicillin solution was drawn into the space in 23 minutes in one rabbit and 1·5 c.cm. in 32 minutes in the other. After the cisternal needle had been withdrawn and the anæsthetic had passed off neither rabbit showed the slightest functional disturbance. Both were killed 6 days later, when histological examination of the brain by Dr. Dorothy Russell showed that there was no meningitis or damage to the marginal glia and that the choroid plexuses and ependyma were everywhere normal. Dr. Russell investigated the effect on the brain in 3 other rabbits by methods she had previously described (Russell and Falconer 1941) and again no lesions attributable to the penicillin were discovered. These results, taken in conjunction with those on leucocytes and on tissue cultures, strongly support the view that local application of quite strong solutions should prove innocuous to tissue cells.

ABSORPTION AND EXCRETION OF PENICILLIN

It has previously been shown that penicillin is absorbed after subcutaneous injection in mice, and from the intestine in rats, and that antibacterial activity appears in the urine secreted subsequently. Absorption and excretion have now been further investigated on rabbits, cats and man, always using the sodium salt of penicillin. The blood and urine levels have been compared after intravenous, subcutaneous and intestinal administration, often on the same animal. As the substance loses activity quickly in acid solution it was not considered worth while at this stage to study the results of direct administration into the stomach, except for two tentative observations on normal subjects and in Case 6. Absorption from the gastro-intestinal tract has mainly been studied by the direct introduction of penicillin into the duodenum through a small fistula. In man a duodenal tube was used. The standard dose in all experiments on the rabbit and cat was 400 units. Unless otherwise stated the urine was collected by catheter, the bladder was washed out and the washings were added to the urine for assay. When necessary a short ether anæsthetic was given to facilitate the injection and the collection of the urine.

In rabbits.—Rabbit 1 received two doses by each route. After duodenal administration no detectable activity was

observed in the blood after ½ hr. (one experiment) or 1 hr. (2 experiments); 14% and 5·3% respectively of the active substance were excreted in the urine. Similarly after intravenous injection there was no activity in the blood at 1 hr. 20 min. and 1 hr. 50 min. in one experiment; or at ½ hr., 1 hr. and 1½ hr. in another; the yields in the urine were greater, however, being 44% and 24%. Subcutaneous injection produced a trace of activity in the blood in the two samples taken—at ½ hr. in one experiment and 1 hr. in the other; 40% and 14·5% were recovered from the urine.

One rabbit after a duodenal injection showed no activity in the blood at 1 and 2 hr. and none in the urine, but since the urine was passed naturally in a metabolism cage during the night activity may have been lost by bacterial action, in spite of the presence of chloroform.

In a third rabbit the standard dose was injected into the intestine in an acute experiment. On the supposition that penicillin might be absorbed from the gut and then inactivated in the liver, samples were drawn from the portal vein and the vena cava at 10, and later 20, minute intervals for 1½ hr. In no blood sample could activity be detected, but the urine at the end of 2½ hr. contained 12% of the injected penicillin. Bile from a cannula in the common duct (the cystic duct having been tied) consistently showed a trace of activity up to 2½ hr., the end of the experiment. In a fourth rabbit, also under nembutal, the penicillin was injected intravenously and blood taken from the ear vein. There was no activity in 4 half-hourly blood samples and a trace in the first bile sample only, but the urine samples were active. A repetition of this experiment on a fifth rabbit gave essentially the same result. The rabbit, therefore, gives a good return in the urine after intravenous injection (20–50%), but a return of less than 20% after administration into the intestine. Penicillin can almost never be detected in the blood at ½ hr. by whatever route it is given. There is some excretion in the bile. Because activity cannot be shown in the blood these experiments do nothing to decide whether the greater "loss" which appears to be associated with intestinal administration occurs before, during or after absorption.

Cats.—Cat 1 was equivalent to rabbit 1, receiving the standard dose of penicillin by three routes. After intestinal injection a trace was detectable in the blood at ½ and 1 hr. (2 experiments) and at 1½ hr. (1 experiment) and the urine contained 60% of the active substance (1 experiment). Similarly after intravenous and subcutaneous injection activity was observed in the blood at ½ and 1½ hr., and the urine showed 33% and 52% of the activity respectively. In the blood of cat 2, taken half hourly for 3 hours after a duodenal injection, activity could not be detected in the first sample but was seen in all the rest, rising to the end of the second hour and then falling again.

Cat 3 was anæsthetised with nembutal and the bile-duct cannulated, the cystic duct having been tied. Penicillin was injected into the duodenum. Activity could just be detected in the portal and vena caval blood in samples taken every 15 min. for ¾ hr. The bile had no activity in the first 15 min., a little in the second and a high titre was maintained during the second hour, at the end of which the experiment was stopped. A similar experiment on cat 4 showed activity in the systemic blood for 3½ hr., with a trace until 4½ hr., and in the bile and urine throughout the 6 hours of the experiment. Excretion in the bile reached its peak at 3½ hr. and then fell away; in the urine it showed a small decline after the 4th hour.

Cat 5 was decerebrated and prepared in a similar way and here the injection was given intravenously. Penicillin was excreted by the liver for the whole 6 hours of the experiment (only a trace in the last hour) but since little bile was secreted the total amount was small. Half of the active substance was excreted in the urine in the 6 hours. In cat 6, anæsthetised with chloralose, the investigation was extended by producing a flow of saliva and tears with pilocarpine, and of pancreatic juice with secretin. Penicillin given intravenously was excreted in high concentration in the bile for the first three hours, slightly in the fourth hour and not at all in the fifth. It was secreted also in the saliva, but here the concentration was lower than in blood collected at the same time. It was present in the blood for 1½ hr. Tears and pancreatic juice showed no activity. Half of the penicillin was excreted in the urine in the 5 hours of the experiment. Cat 7 prepared in the same way gave a similar result, but the experiment was stopped at the end of an hour to collect the cerebrospinal fluid, which proved to be inactive. Controls showed that bile alone was inactive on the assay plate and that pancreatic juice did not destroy penicillin activity.

These experiments show that the cat differs from the rabbit in maintaining an antibacterial concentration of penicillin in the blood for at least 1½ hr. after subcutaneous or intravenous injection and at least 3 hr. after intestinal administration. It differs also—and the two facts are no doubt connected—in excreting in the urine about 50% of the penicillin, even when injected by the intestinal route.

In man.—Subcutaneous injection has not been used in man, but from preliminary observations and from the treated cases a good deal has been learned about the blood-levels and urinary excretion reached after both intravenous and intestinal administration. Trials with ascending doses of "therapeutic penicillin" showed that a single intravenous injection of 200 mg. could be given without ill effect. This is the largest single dose which has been given. The inhibitory power of the blood was followed in one subject at frequent intervals from 5 min. until 2 hr. 5 min. after the injection. The high initial value gradually fell to a just discernible trace in the last sample. Urinary excretion was still occurring at 6 hr. 15 min. and there was a trace of inhibition in a sample of urine collected from the 6th to the 14th hour. After a dose of 100 mg. inhibition was just detectable in the blood at the end of an hour. After repeated 100 mg. doses a trace of inhibition was similarly found in the blood one hour after an injection (case 3, who had had five initial hourly doses of 200 mg.). The best experiments from the point of view of estimating the urinary recovery after intravenous injection were those on patients No. 3 and 5, whose urine output was accurately measured during the course of treatment. In case 3 about 50% of the total active substance injected was present in the urine and 30% was actually recovered in purified form (the rest being lost in the re-extraction process). In case 5, in which penicillin dissolved in saline was administered continuously, 68% of the antibacterial activity reappeared in the urine and 54% was recovered for further use.

For duodenal administration a tube was passed and its location in the duodenum confirmed by fluoroscopy. Sixteen thousand units (about 400 mg. of the solid material) dissolved in 25 c.cm. of water were injected down the tube, followed by 60 c.cm. more of water. After this injection the blood inhibited bacterial growth for 3 hr. and urinary excretion continued for 6 hr. The inhibitory level in the blood was steadier and persisted for a longer time than after intravenous injection. The dose was twice the maximum so far given intravenously and it is quite possible that larger doses will always be needed for treatment by the intestinal route. Nevertheless the indications are that this is a possible way of administering penicillin.

A contrast thus emerges between the behaviour towards penicillin of the rabbit on the one hand and of the cat and man on the other. After giving 400 units intravenously to a rabbit no activity could be detected in the blood at the end of half an hour, while in the cat after the same dose the blood was active for at least 1½ hr. Similarly after duodenal introduction, there was no activity in the blood and little in the urine of the rabbit, whereas in the cat activity could be detected in the blood and there was a considerable excretion in the urine. Man appears to be in both instances more like the cat than the rabbit. Apparently the rabbit has a more effective mechanism for the inactivation of penicillin than either of the other two species.

By whatever route penicillin is administered not all the active substance appears in the urine. Where this "loss" occurs is not yet clear. Incubation for 3 hr. at 37° C. of penicillin with blood and with slices of liver, kidney, spleen, brain, muscle, lymph-gland, lung and intestine from the rabbit caused no detectable destruction. Nor was there any diminution of activity after incubation with rabbit-bile, though there was perhaps a slight fall with cat-bile. It is impossible to say whether the material recovered from the urine has been changed in any way during its passage through the body. It has a high antibacterial titre and has been used again for injection into patients without ill effect. It is of interest to note that material partially purified but containing pyrogen is freed from that impurity by passage through the body.

THERAPEUTIC TRIAL OF PENICILLIN

Methods of administration.—To avoid the uncertainties of intestinal absorption, the first cases were treated by penicillin given intravenously. Since it is rapidly eliminated by the kidneys and probably partially

inactivated in the body, it was clear that frequent doses would be necessary. At the outset it was felt that intermittent administration might give the best diffusion into the tissues as a result of the repeated temporary raising of the concentration of the drug in the blood above the highest level that could be attained by continuous administration of the same total quantity. To facilitate frequent injections a cannula was tied or a needle inserted into a vein and connected to a slow drip apparatus (Marriott and Kekwick 1940) which delivered a steady flow of 500 c.cm. of 1·05% sodium citrate and 0·8% sodium chloride in 24 hr. Each dose of penicillin was dissolved in about 2 c.cm. of non-pyrogenic water, injected into the rubber tubing of the drip apparatus and flushed in with a little of the citrate-saline solution. This method is admittedly inconvenient owing to the well-known difficulties of maintaining an intravenous infusion for more than two or three days, but it was thought that if the therapeutic efficacy of penicillin could be established by this method other ways of giving the drug could then be explored.

In case 5 the effect of running in a continuous supply of penicillin solution was tried. At first a dilution of 1/1000 in normal saline was used, and as this was found to be no more harmful to the vein than normal saline alone the concentration was raised to 1/500. The vein tolerated this concentration well. Higher concentrations have not been tried. It is probable that this method of intravenous medication is the simplest and most useful.

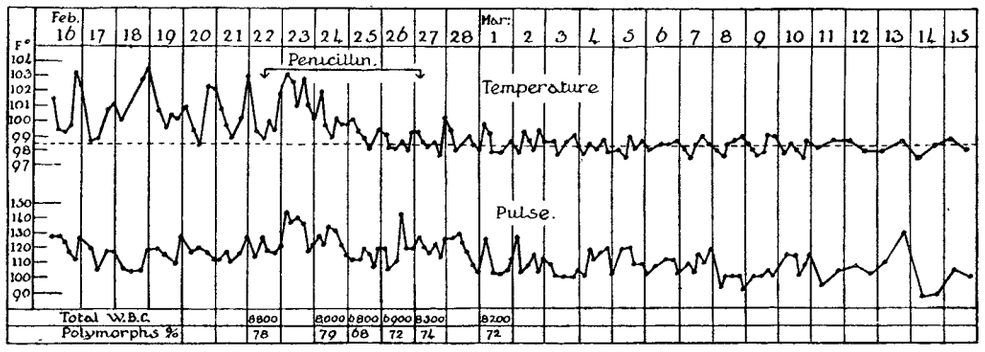


FIG. 5—Chart and white-cell counts in case 2.

Administration by the mouth is complicated by the fact that penicillin is rapidly destroyed by acid and therefore serious losses are to be expected in the stomach. It may however be possible to carry the penicillin through the stomach by raising the pH of the gastric contents. Experiments with such substances as magnesium trisilicate or the recently described aluminium phosphate (Fauley et al. 1941) are clearly required. In case 6 a strongly antibacterial concentration of the drug was maintained in the urine for 7 days by giving it by mouth together with sodium bicarbonate. In this case however we were aiming at keeping up an antibacterial concentration only in the urinary tract, and not in the blood. The possibility of administration in capsules which will pass through the stomach has not been overlooked, but some tentative experiments with salol-coated capsules were discouraging. Moreover, Cook and LaWall (1936) stated that only 10% of such capsules passed through the stomach satisfactorily; we have therefore not thought it worth while to entrust a scarce and valuable substance to such unreliable vehicles. Rectal administration was tried in one subject, but very little active substance was recovered in the urine and it was afterwards found that fæces inactivate penicillin, probably by bacterial action. It was thought undesirable to introduce very strong solutions of penicillin subcutaneously or intramuscularly owing to uncertainty about the local effects. More dilute solutions, for example 1 in 500, which have been shown to be innocuous to leucocytes, would involve the use of excessive amounts of fluid. In case 1 the injection of 100 mg. in 2 c.cm. of water intramuscularly caused some tenderness, though this cleared up quickly.

INTRAVENOUS ADMINISTRATION IN STAPHYLOCOCCAL AND STREPTOCOCCAL INFECTIONS

CASE 1.—Policeman, aged 43. Admitted Oct. 12, 1940. Suppuration of face, scalp and both orbits, starting from a sore at the corner of the mouth a month earlier. Primary infection *Staph. aureus*; secondary, *Strep. pyogenes*. Sulphapyridine 19 g. given from Dec. 12 to 19; no improvement; drug-rash. Jan. 19: incision of multiple abscesses on face and scalp. Osteomyelitis of right humeral head, proved by X rays, showed on Jan. 31, 1941, after 3 weeks of pain; a resulting arm-abscess, incised, gave *Staph. aureus* pus. General infection of left eye; cornea perforated Jan. 21. Eye eviscerated Feb. 3. Blood-transfusion 2 pints Feb. 9. Fever intermittent all this time, 98°–101° F. Very ill and emaciated; tongue heavily furred. Feb. 11: right eye bulging and conjunctival chemosis, orbit incised, pus gave *Staph. aureus* and *Strep. pyogenes*.

Feb. 12: all incisions suppurating, in scalp, face, both orbits, and right arm. Lungs involved, with purulent expectoration containing both the pyogenic cocci. Hb 36%; red cells 1,800,000. Blood-culture sterile. Penicillin 200 mg. given intravenously; then 100 mg. 3-hourly, intravenous except for two intramuscular doses. Slight rigor after first dose, otherwise no reactions. Striking improvement after total of 800 mg. penicillin in 24 hours. Cessation of scalp-discharge, diminution of right-eye suppuration and conjunctivitis. Arm discharge seemed less. Blood-tests just before injections for penicillin: 7.30 A.M. faint trace; 11.30 A.M. none. Feb. 13: penicillin 100 mg. intravenously 4-hourly. Feb. 14: condition much the same. Blood-transfusion 3 pints. Penicillin 100 mg. 2-hourly by injection into transfusion tube; total 1 g. in 24 hours. Feb. 15: 1 pint blood transfused and penicillin 100 mg. given 3-hourly, most of which had been recovered from previous urine. Feb. 16: much improvement; Hb. 74%. Right eye almost normal. Some discharge still from left eye and arm. Shortage of penicillin interrupted treatment from noon to 6 P.M.; then drip-infusion of sodium chloride and citrate, penicillin 200 mg. being injected into drip, then 100 mg. 3-hourly; no reaction. Feb. 17: penicillin supply exhausted. Total administered, 4·4 g. in 5 days. Patient felt much improved; no fever; appetite much better; resolution of infections in face, scalp and right orbit; still coughing; sputum contained *Strep. pyogenes* and *N. catarrhalis*. Left orbit and right humerus still suppurating. Blood-urea 30 mg. per 100 c.cm., urine normal. Condition stationary for 10 days, then deteriorated, especially lungs. March 15: died. Autopsy showed typical picture of staphylococcal pyæmia with multiple abscesses.

White counts.—Feb. 12, 20,000 (polymorphs 88%); 13th, 19,000; 14th, 11,200; 15th, 16,800; 18th, 8400; 19th, 7600; 20th, 7600 (polymorphs 84%); 25th, 8000; March 5, 11,000 (polymorphs 88%).

The attempt to treat this forlorn case was chiefly valuable in that it showed that penicillin could be given over a period of 5 days without significant toxic effect. There was a fall in the total white count, but both granular and agranular cells were equally affected. Apart from this effect, which has not been seen in subsequent cases, and the slight rigor due to a pyrogenic impurity in the penicillin, no contra-indications to its use were observed. Assessment of its effect was difficult since a blood-transfusion was given at the same time, and later experience showed that the dose of penicillin employed was too small, and the period of administration too short. None the less the superficial sepsis responded well, and did not relapse after the penicillin was stopped.

CASE 2.—Boy, aged 15. Admitted Dec. 29, 1940, for slipped right femoral epiphysis. Open reduction with insertion of Smith-Petersen pin Jan. 24, 1941. Severe post-operative hæmorrhage followed by infection of wound with hæmolytic streptococcus (group A, type 13); positive blood-culture. Wound reopened and patient given Proseptasine from Jan. 25 to Feb. 3, two courses of 5 g. sulphapyridine soluble intravenously in 30 hours on Feb. 7th and 9th, and two blood-transfusions, but swinging temperature (99°–103° F.) continued (see fig. 5).

Feb. 22: looked ill, pale and wasted; two granulating areas over right hip, discharging sero-pus. Hb. 62%; red cells 3,000,000; white cells 8800 (polymorphs 78%). Blood-culture sterile. Blood-urea 32 mg. per 100 c.cm. Urine normal. At noon 100 mg. penicillin injected into intravenous drip-infusion of citrate-saline; repeated 2-hourly for 8 hours. After interval of 3 hours given another 100 mg. producing

slight rigor; then 75 mg. 3-hourly. Feb. 24: general condition better, discharge less. By 9 P.M. had received 1·4 g. penicillin; dose raised to 100 mg. 3-hourly, using a purer penicillin; no reaction. Feb. 27: penicillin stopped; total 3·4 g. Three blood-examinations just before injections had only once shown slight antibacterial activity. Feeling better; local condition unchanged. Blood-urea 29 mg. per 100 c.cm. Urine normal. White cells 8300 (polymorphs 74%). Feb. 25: blood-transfusion 2 pints. Feb. 26: plaster spica applied. After 3–4 weeks of almost normal temperature pin removed. Again developed swinging temperature as before penicillin treatment.

Several different samples of penicillin were used in this case. The first five doses had been recovered from the urine of case 1 and caused no reaction. The next samples, which caused some shivers, were "third fraction," and although they had been passed through an absorption column again they still contained the pyrogen. The later doses were "second fraction" (our present "therapeutic penicillin"). Here there was a local infection by a hæmolytic streptococcus which had proved resistant to sulphanilamide in large doses and to moderate doses of sulphapyridine. Penicillin therapy was followed by a great improvement in the patient's general condition, in spite of the dose being insufficient to maintain a detectable concentration of penicillin continuously in the blood.

CASE 3.—Labourer, aged 48, of poor physique. Admitted May 2, 1941. Carbuncle 4 in. across over left scapula for 5 days; now discharging; pus grew pure *Staph. aureus*. History of chronic bronchial and nasal catarrh for 4 months. Left axillary adenitis. Hb. 106%; red cells 4,970,000; white cells 23,000. Blood-urea 31 mg. per 100 c.cm. Urine normal.

May 3: penicillin 200 mg. hourly for 5 doses by injection into intravenous drip as in case 2; then 100 mg. hourly. No reaction. Antibacterial activity continuously maintained in blood. May 7: carbuncle much improved; slight tenderness, only slight serous discharge. Dose dropped to 100 mg. 2-hourly and then 3-hourly. May 10: carbuncle almost completely resolved; no axillary adenitis; bronchial and nasal catarrh cleared; 100 mg. penicillin 6-hourly for 4 doses and then stopped. May 11: Hb. 90%; red cells 4,970,000; white cells 15,800. Blood-urea 37 mg. per 100 c.cm. Urine normal. Temperature, which had been swinging 97°–101° F. now normal and remained so. Local treatment had consisted in kaolin poultice for first 48 hours, sodium sulphate dressing for next 48 hours and subsequently dry dressing. May 15: discharged. Seen as outpatient on May 19 when skin over carbuncle almost normal and patient generally well; right ulnar neuritis which cleared quickly, possibly from splinting arm for several days during infusion.

White counts.—May 3, 23,000 (polymorphs 87%); 5th, 16,400; 6th, 15,200; 7th, 7800; 8th, 9200; 9th, 19,200; 10th, 19,800; 11th, 15,800.

In this case the supply of penicillin was sufficient to enable a detectable concentration of penicillin to be maintained continuously in the blood for the first 4 days. There was no toxic effect from the larger dosage. The result was a rapid resolution of the carbuncle without its discharging and without scar formation. In addition the chronic nasal and bronchial catarrh were cleared. The white count was temporarily depressed on the 4th and 5th days but rose again in spite of the drug being continued.

CASE 4.—Boy, aged 4½ years. Admitted May 13, 1941. Cavernous-sinus thrombosis from septic spots on left eyelid and face following measles 5 weeks before. Had received 30 g. sulphapyridine in 14 days before admission. Semi-comatose, incontinent of urine and fæces. Gross œdema both eyelids (fig. 6a), especially left, with bilateral proptosis. Complete bilateral external ophthalmoplegia and 2 dioptres of papillœdema; neck rigidity; bilateral Kernig's sign and extensor plantar responses. Moist sounds both bases. Liver edge two finger-breadths below costal margin. Blood-culture sterile. Lumbar puncture gave a faintly yellow cloudy fluid under high pressure (see table III).

May 13: intravenous infusion of citrate saline at 10 c.cm. an hour (rate maintained with slight variations for 9 days, the site of infusion being changed 4 times). Penicillin injected into infusion; dose 100 mg. hourly for two doses, 50 mg. hourly for four doses, then 25 mg. hourly. May 14: pus from incision made into left eyelid and swab from nose grew *Staph. aureus*. X ray: opacity of left antrum, ethmoids clear. May 15: blood sample an hour after dose of penicillin showed no antibacterial activity; dose increased to 50 mg. hourly. General improvement. May 16: obviously better; swelling of eyelids largely subsided. Blood taken just before injection showed trace of antibacterial activity. May 19:

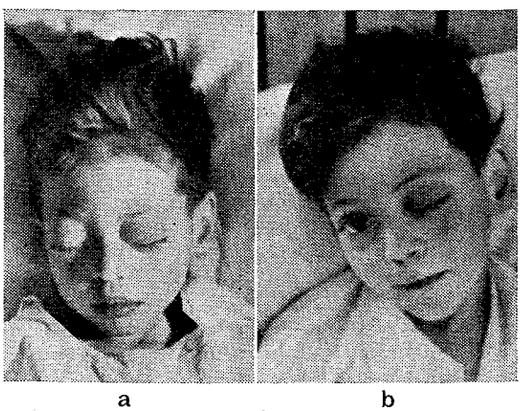
TABLE III—CEREBROSPINAL FLUID OF CASE 4

Date	Pressure	Protein (mg. per 100c.cm.)	Red cells per c.mm.	White cells per c.mm.	Culture
May 13*	Raised	110	v. few	109	*Staph. aureus*
,, 14	Normal	100	v. few	372	*Staph. aureus*
,, 19	Normal	60	v. few	110	*Staph. aur.* and *alb.*
,, 22	Normal	95	v. few	45	Sterile
,, 27*	Raised	120	14,600	56	Sterile
,, 28	Raised	140	21,800	276	Sterile
,, 29	Raised	95	12,500	920	Sterile
,, 30	Raised	90	840	2120	Sterile

* Cell-count done after fluid had stood for several hours.

general and local condition vastly improved (see fig. 6b); bilateral 6th nerve palsy and extensor plantar responses remained. Penicillin reduced to 50 mg. 3-hourly. Small corneal ulcer left eye treated with penicillin 1 in 5000, which caused no discomfort. May 22: improvement maintained, patient talking and playing with toys. Chest clinically normal. Slight pyrexia still thought to be due to pyrogen in penicillin or to reaction from thromboses in veins used for injections (see fig. 7). Penicillin stopped. May 26: progress good. Temperature normal. General condition excellent. Eye movements returning. X ray of sinuses: only slight clouding left antrum; chest; patch of consolidation left apex and small ring shadow right mid-zone. These thought to be embolic signs but general condition so good that no further penicillin needed. May 27: 1 A.M. vomited and had general convulsions. Lumbar puncture gave uniformly blood-stained fluid under high pressure. Became comatose with neck rigidity, positive Kernig's sign and spastic limbs. May 28: temperature began to rise again. May 29: appearance much as on admission. Penicillin 2 g. given in next 36 hours, but died May 31.

Autopsy (Dr. A. H. T. Robb-Smith).—Brain showed no thrombosis of main venous sinuses; adhesions and old hæmorrhage in hypophysial region. Considerable old and recent hæmorrhage in region of pons and cerebellum due to rupture of aneurysm on left vertebral artery. Cavernous-sinus region and left orbit occupied by œdematous granulation tissue; left carotid artery partially occluded by thrombus in its cavernous course and completely occluded in its bony course. Both lungs showed scattered abscess cavities, larger ones being air-containing cysts lined by yellowish membrane; smaller ones containing yellowish material not exactly resembling pus. Other organs not remarkable.



a b

FIG. 6—Case 4: (*a*) at beginning of treatment; (*b*) on 11th day of treatment.

Histologically granulation tissue is essentially similar whether in lung abscesses, orbital tissues or cavernous regions (fig. 8). There is a small central area of necrosis sometimes containing a few gram-positive cocci; around this is an œdematous exudate with lipoid-containing histiocytes; surrounding this is a granulation tissue formed largely of histiocytes containing lipoid and blood-pigment, lymphocytes and plasma cells with a very occasional neutrophil

leucocyte; this tissue is well vascularised and there is some fibroblastic proliferation, greatest in the periphery. In the cavernous region some of the veins contain organising thrombus; the left carotid and vertebral arteries show organising thrombi which do not appear to be infected, but as there are large breaks in the media and elastica of the walls of both these vessels it must be presumed that they are the late results of an acute arteritis probably of bacterial origin. The other organs show no significant change.

The autopsy showed that the infection in the cavernous sinus, orbits and in the lungs had been almost entirely overcome, and that healing processes were well advanced. Death was due to the ruptured mycotic aneurysm and not to a recrudescence of the infection. Before this vascular accident the patient had been restored from a moribund condition to apparent convalescence. No toxic effects from the penicillin were noticed.

CASE 5.—Boy, aged 14½. Admitted May 6, 1941. Staphylococcal septicæmia with early osteomyelitis of left femur following fall 6 days before. Blood-culture grew *Staph. aureus*. Given sulphathiazole 64 g. between May 7 and 12 with little benefit. May 17: left hip-joint explored, no abscess found, joint contained little sterile fluid with polymorphs in it. May 22: blood and albumin in urine. Embolic pustular rash. Extension by skin traction applied to left leg. May 30: X rays showed osteitis left femoral neck with involvement of joint and destruction of alveolar walls. Discharge from wound grew *Staph. aureus*.

June 6: still extremely ill, with temperature rising to 101° F., much pain in left hip and thigh; urine still contained blood and albumin. Hb. 74%; red cells 4,290,000. Blood-urea 23 mg. per 100 c.cm. Intravenous infusion of 500 mg.

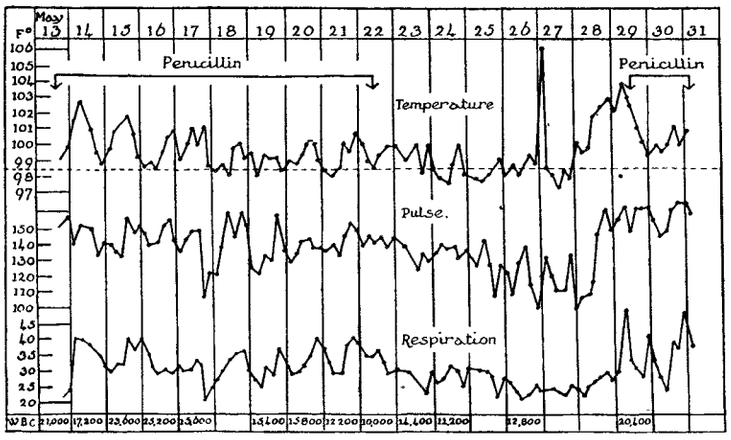


FIG. 7—Chart and white-cell counts in case 4.

penicillin in 540 c.cm. normal saline started at 45 c.cm. an hour. Infusion continued for 90 hours during which time 3·5 g. penicillin given. No reaction. Daily tests showed constant bacteriostatic concentration in blood. General condition much improved. By June 10 had lost almost all pain and tenderness. Cannula transferred to another vein and infusion continued at half rate with double strength of penicillin (1 g. in 500 c.cm.). On June 11 and 13 sudden rises of temperature without apparent cause; traced to use of penicillin prepared 2 months previously and containing pyrogenic impurity. June 14: cannula transferred to another vein and penicillin recovered from boy's urine used. No further rise of temperature. Steady improvement. On June 16 continuous administration stopped. Given another 50 mg. 3-hourly for 12 doses and then 4-hourly for 12 more doses. Stopped June 20; total dosage 17·2 g. Urine then normal. Blood-urea 29 mg. per 100 c.cm. June 21: Hb. 64%; red cells 3,750,000. June 23: radiograms of hip showed no extension of destructive process; boy felt quite well. July 9: extension removed. July 11: had been afebrile for 3½ weeks. No pain in leg. Passive hip movements, flexion 80°. Other movements almost full.

White counts.—June 6, 9400; 8th, 9700; 9th, 11,800; 10th, 13,800; 11th, 11,400; 12th, 7500; 13th, 9400; 14th 16,400; 15th, 10,400; 16th, 8400; 17th, 7600; 18th, 6200; 20th, 11,800; 21st, 12,400.

This was a case of staphylococcal septicæmia, localising in the left hip-joint, which had been uninfluenced by large doses of sulphathiazole in the early stages. When penicillin was started the boy was extremely ill, in great pain, and had an active nephritis. As in the other cases there was a striking improvement in the general condition during the period of therapy. All pain in the region of the left hip disappeared, the nephritis cleared and his temperature fell to normal. At the time of writing it had remained normal for 3½ weeks and there was good function in the hip.

ORAL ADMINISTRATION IN URINARY INFECTION

CASE 6.—Boy, aged 6 months. Admitted April 8, 1941. Ill a fortnight with diarrhœa and vomiting, melæna and generalised convulsions. Dehydrated, few petechiæ in skin. Red

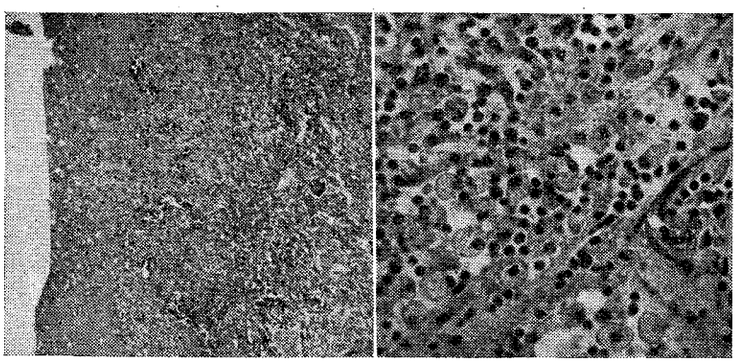


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FIG. 8—(*a*) Low-power view (×40). Wall of "abscess cavity" in the lung showing the œdema fluid rich in lipoid-containing histiocytes, and the wall made up of a vascular granulation tissue in which there are large numbers of lipoid-containing histiocytes, lymphocytes and plasma-cells and only a few neutrophil leucocytes. At the extreme right of the section the normal lung parenchyma can be seen.

(*b*) High-power view (× 180). Granulation tissue from the left orbital region showing the characteristics of the cellular reaction, a mixture of lipoid-containing histiocytes, lymphocytes and plasma-cells, and a moderate fibroblastic proliferation.

cells 1,560,000; white cells 28,000 (atypical lymphocytes 50%, many primitive cells). Blood-urea 60 mg. per 100 c.cm. Urine contained albumin and pus; grew *Staph. aureus* and *Bact. coli*. Sulphapyridine tried but stopped because polymorphs fell to 400 per c.mm. After fortnight's course of ammonium mandelate urinary infection remained. June 3: urine grew pure *Staph. aureus*. Hb. 62%; red cells 3,200,000; white cells 5000 (polymorphs 600). Blood-urea 66 mg. per 100 c.cm. June 5: attempts to pass duodenal tube failed, so penicillin given by mouth; 20 mg. with 2 g. sodium bicarbonate given hourly for 3 doses and then 3-hourly. No toxic signs. Vomited once, which was not unusual. All specimens of urine contained strongly bacteriostatic concentration of penicillin. June 7: urine contained no pus cells; penicillin reduced to 10 mg. 3-hourly. Vomited 2–3 times daily. Steady general improvement. June 9: polymorphs up to 1000 per c.mm.; blood-urea 46 mg. per 100 c.cm. June 12: penicillin stopped. June 17: blood-urea 39 mg. per 100 c.cm.; Hb. 70%; white cells 4000 (polymorphs 440). June 19: urine sterile though still containing heavy cloud of albumin and occasional casts and red cells. General condition continued to improve. June 26: urine still free from *Staph. aureus*.

This case demonstrates that it is possible to maintain a bacteriostatic concentration of penicillin in the urine with small doses given by mouth. Although the diagnosis was obscure there was no doubt of the urinary infection by *Staph. aureus*, and the administration of penicillin overcame this infection.

LOCAL APPLICATION TO THE EYE

In case 4 a 1/5000 solution of penicillin in normal saline was applied to a corneal ulcer. It caused no irritation and the condition of the eye improved. The local application of penicillin in other eye infections was therefore tried on patients in the Oxford Eye Hospital.

CASE 7.—Married woman, aged 32. Had a corneal ulcer of the left eye 4 months previously, treated successively by instillation of Collosal Argentum, boracic lotion and mercurochrome, and ultraviolet light. In 6 weeks ulcer resolved completely, but 3 weeks later similar ulcer developed in right eye. Treated on similar lines, for 6 weeks as outpatient and then for fortnight as inpatient, but without improvement. May 26, 1941: infiltrating ulcers in inner and upper quadrants of limbus, gross injection of conjunctiva and considerable corneal opacity. Swab from eye grew *Staph. aureus*.

May 30: treatment with penicillin begun; 1/5000 solution in normal saline dropped into eye hourly by day and 2-hourly

by night. After 2 days little progress had been made and continuous application considered necessary. Modification of Bunyan-Stannard bag (Bunyan 1940) made to fit eye and 1/5000 solution penicillin run into it. New bag applied each day, and remained full for about 8 hours, after which began to leak and needed refilling occasionally. After one day of continuous application less injection of conjunctiva and patient free from pain for first time. Treatment given for 4 days; on second day concentration of penicillin raised to 1/1000 but this caused slight irritation and for last 2 days solution of 1/2500 used. By end of fourth day eye greatly improved; conjunctiva only slightly injected and corneal opacity almost disappeared; deeper ulcer remained, but considerably smaller. Treatment continued with hourly drops of 1/2500 penicillin and after one day strength increased to 1/500 "therapeutic penicillin," which caused no discomfort. June 9 (8 days after bath had first been applied): ulcer no longer stained with fluorescein, but still slight injection. Treatment continued with drops of 1/500 penicillin, 2-hourly by day, 4-hourly by night, but further improvement slow. June 20: patient discharged, ulcer healed, leaving only slight residual infiltration of conjunctiva, which had cleared a week later.

CASE 8.—Girl, aged 19. Burnt her face with cigarette end 6 weeks before admission; burn became infected and from this a septic rash spread over face. Week before admission left eye became red, swollen and painful. On admission on June 9, 1941, many small crusted impetigo-like spots on both sides of face. Left conjunctiva inflamed and showed thick mesh of fine vessels; cornea clear, lids slightly swollen. Eye-bath as in case 7 applied and filled with 1/2500 penicillin. Next day less injection and eye not painful. After 3 days eye almost normal; treatment continued with drops of 1/500 penicillin 2-hourly by day and 4-hourly by night. June 13: eye normal. On and after June 10 ointment consisting of 1/500 penicillin in hydrated lanoline applied to rash on face twice daily. When patient was discharged on June 15 rash had healed completely with only slight residual erythema.

While the treatment of these 2 cases was in progress, some 1/2500 penicillin solution was given to 2 outpatients who were told to apply drops of the solution hourly by day.

CASE 9.—Man, aged 24. Foreign body in left eye May 28, 1941; developed acute conjunctivitis treated with boracic acid and zinc lotion, collosol argentum, and painting lids with silver nitrate. Only slight improvement by June 7, when given penicillin drops; in next 2 days rapid improvement, with complete relief of pain. Slight residual conjunctival infiltration, so treatment continued until June 19, when healing complete.

CASE 10.—Woman, aged 20. Developed acute mucopurulent conjunctivitis on June 5, 1941; treated for 2 days with boracic acid and zinc lotion and by painting lids with silver nitrate. Penicillin drops given on June 7, and 5 days later the eye was almost normal.

In these 4 eye cases, 2 of which had proved resistant to routine therapeutic measures, penicillin application resulted in rapid relief from pain and resolution of the inflammation. In none was there any ill effect.

Discussion

From experiments in vivo and in vitro much evidence has now been assembled that penicillin combines to a striking degree two most desirable qualities of a chemotherapeutic agent—low toxicity to tissue cells and powerful bacteriostatic action. Its capacity to prevent multiplication covers a wide range of bacterial species, including some of the most common and destructive organisms with which man may be infected, and this bacteriostatic action is in no way interfered with by body fluids or pus, and only to a limited extent by very large numbers of organisms.

The crucial test, however, of the efficacy of any chemotherapeutic agent is whether it can favourably influence a natural infection. In spite of the difficulties of large-scale production in the laboratory, we have collected enough material to try penicillin in a few cases. Cases 1, 2, 4, 5 and 6 had previously been treated with various forms of chemotherapy and some also by surgery. They were, in fact, patients for whom no further treatment likely to be of benefit could be proposed. Case 3 had had no previous treatment. In all patients the temperature fell and the general and local condition improved. Where there was a recrudescence of infection it could in each case be attributed to insufficient administration of penicillin. The only toxic effect of any importance so far seen was due to a pyrogenic impurity, which could be removed; and it is worth noting that an improvement in the spirits and appetite of the patient during treatment was remarked on in all the cases. Where local application has been tried, it has given equally promising results.

Methods of administration and dosage have necessarily been tentative, but the many points which still need investigation await an adequate supply of penicillin. Enough evidence, we consider, has now been assembled to show that penicillin is a new and effective type of chemotherapeutic agent, and possesses some properties unknown in any antibacterial substance hitherto described.

Summary

A description is given of the cultural and other conditions required for the effective production of penicillin by *Penicillium notatum*; and of methods of small-scale mass production and assay of material suitable for therapeutic use in man.

Observations on the absorption and excretion of the drug have been made on the rabbit, the cat and man. The material is excreted in high concentration in the urine of all three species. There is a high concentration in cats' and less in rabbits' bile (that of man has not yet been investigated).

Evidence is produced of the low toxicity of the substance when applied directly to body tissues.

No toxic effects attributable to "therapeutic penicillin" injected intravenously have so far been observed, though crude penicillin products contain a pyrogenic substance which can be removed by suitable treatment.

It is shown that the growth in vitro of many pathogenic bacteria is prevented by purified penicillin at a dilution of one in a million or more, and that others possess diminishing degrees of sensitiveness, some being quite resistant.

Proof is given of the inability of blood, pus and tissue derivatives to prevent the action of penicillin.

Adaptation of *Staph. aureus* to high concentrations of the substance has been demonstrated, and this has been shown not to be due to the development of a penicillin-destroying enzyme.

A comparison is made between the antibacterial activity of penicillin and the sulphonamides, and reasons are adduced why penicillin can be expected to operate when the sulphonamides are ineffective.

During the course of some therapeutic trials in human infections it has proved possible to secure and maintain a bacteriostatic concentration of penicillin in the blood without causing any toxic symptoms. After intravenous administration a large proportion of the active substance can be recovered from the urine and used again.

Pencillin was given intravenously to five patients with staphylococcal and streptococcal infections and by mouth to one baby with a persistent staphylococcal urinary infection. It was also applied locally to four cases of eye infection. In all these cases a favourable therapeutic response was obtained.

The work was planned and started by E. Chain and H. W. Florey. The chemical and biochemical part of the work was carried out in the main by E. Chain and E. P. Abraham. N. G. Heatley devised the assay method and developed and supervised the production of penicillin. M. A. Jennings and H. W. Florey have conducted the biological tests except those especially mentioned in the text. A. D. Gardner has conducted the bacteriological investigations and made some special observations on the growth of the mould. C. M. Fletcher (of the Nuffield Department of Medicine) has been in charge of the administration to man. The successful conduct of the work to its present stage has only been made possible by the closest collaboration of all concerned. We wish to thank the physicians and surgeons who placed their cases at our disposal.

We wish also to acknowledge the work of the following technicians, without whose efforts adequate supplies of penicillin could not have been produced: D. Callow, R. Callow, E. Cooke, S. A. Cresswell, G. Glister, C. Inayat, J. Kent, M. Lancaster, P. McKegney, E. Vincent.

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References at foot of opposite page

DRY AFTER-TREATMENT OF INFECTED HANDS

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To soak infected hands in hot baths and apply fomentations "as hot as can be borne" are methods of preoperative and postoperative treatment which are so ingrained in the medical and nursing professions that it will take time for them to be supplanted. Let us review the disadvantages of the "wet" method:—

(*a*) It is extremely painful. I still remember dreading the time coming round for my own fomentations, which were applied at two-hourly intervals by a conscientious sister who believed in fomentations being really hot.

(*b*) General swelling and tenderness of the hand, due to the hot applications, mask localised swelling and tenderness due to extension of the infected process; consequently the accurate diagnosis of the location of pus is often delayed.

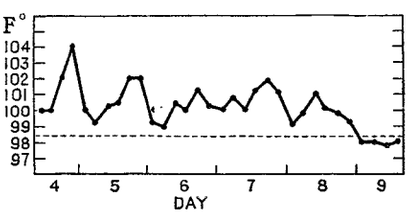
(*c*) The hot-water treatment produces scalding, which, in addition to causing pain, predisposes to superficial inflammation and even paronychia of uninfected digits.[1]

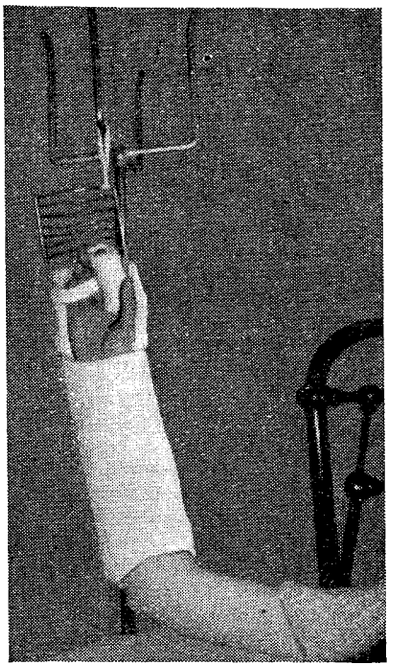
(*d*) Sodden, swollen tissues tend to narrow exits through which pus can escape.

TECHNIQUE OF THE DRY METHOD

During the past three or four years I have been increasingly impressed by the manifold advantages of the dry method of treating seriously infected hands. The best way of expounding its principles will be to quote the case of a surgical staff-nurse with suppurative tenosynovitis of the index finger. As I have suffered from suppurative tenosynovitis myself, and as this patient was a trained observer, it was possible to contrast and compare the wet and dry treatments with more than ordinary enlightenment.

I was called to see the nurse four days after the symptoms and signs had begun. She had scratched the back of her index finger while doing a dressing; the facts that the original lesion was on the dorsum of the finger and that the patient could flex the finger without much pain had been the cause of diagnostic difficulty to several observers. (How often the all-important diagnosis of suppurative tenosynovitis is not made because a patient can flex the finger; it is far more significant if the infected digit cannot be completely extended by voluntary effort.) By the time I saw this patient she was gravely ill. She had received sulphonamide therapy and the usual baths and fomentations, but the temperature had continued to rise, and the toxic symptoms were so great that septicæmia was feared. Her temperature was 104° F. and pulse-rate 140. By palpation with a burnt match-stalk the point of maximal tenderness could be defined clearly over the flexor surface of the head of the second metacarpal. Under Evipan anæsthesia the tendon sheath and the corresponding lumbrical space, both of which were found to be distended with pus, were drained, and a wisp of Vaseline gauze was tucked into the proximal end of the sheath. A piece of Gamgee tissue was wrapped round the forearm, and a length of Cramer wire was bandaged firmly to the dorsal aspect of the forearm. A suitable piece of Sorbo sponge was placed between the Cramer wire and the dorsum of the hand and fingers, and the incised index finger was encased in a Viscopaste bandage which did not fix it to the splint except at the base of the finger, where it was carried round the splint and the palm. The sound fingers were bandaged lightly to the splint, in order to extend them. The patient having been transferred to bed, the arm was elevated by suspending the end of the Cramer wire from an irrigator stand. The nurses were instructed that if the patient was comfortable and the temperature falling the dressing should not be disturbed, neither should the position of the arm be altered for 48 hours. On the third day she complained of aching in the shoulder; this was eased somewhat by adjusting the pillows, but she asked to have the arm lowered. This was done, and the dressing was changed and the viscopaste bandage reapplied. After about an hour the hand began to throb, and the patient was glad to have it elevated again, for in the raised position the pain and throbbing vanished. The report on the pus was now to hand; it was a pure growth of *Staphylococcus aureus*. Each day the flexed, uninfected fingers were bandaged back a little more to the Cramer wire, until they were extended completely. The patient, whose general condition had improved greatly by the ninth day, was encouraged to move the fingers frequently under the turn of bandage.





While this treatment is essentially dry, dryness is not a fetish. About the fourth day the vaseline gauze drain is removed and the hand is put into an antiseptic bath for five minutes and washed. If there are any sloughs the wound is irrigated; the hand is then dried and the splint reapplied. Whether the vaseline gauze drain is replaced or not depends on circumstances; in this case it was removed, and the arm was kept elevated for 20 days. For 1½ hours each day it was lowered, and during this period the bandage was undone and the patient performed finger exercises under infrared rays. On the 21st day she was allowed up. The wound had healed and movement of all the uninfected fingers and the thumb was perfect.

In nearly all the cases in which I have employed dry after-treatment for seriously infected hands the infecting organism has been a streptococcus. As sulphonamide therapy came into general use about the same time that I changed from the wet to the dry treatment, it might be argued that it was the sulphonamide which was playing the major part in the improved results. I have no wish to detract from the great benefits which have accrued from sulphonamide therapy in this branch of surgery, but the case reported here demonstrates that, with adequate drainage, dry after-treatment and elevation are in themselves an advance; I think it unlikely that in this instance sulphonamide therapy played any part.

In my experience, the dry after-treatment has proved uniformly satisfactory. T. B. Mouat, whose experience is greater than mine, informs me that both he and Sister Wray, of Sheffield Royal Infirmary, find that in a small percentage of cases progress is unsatisfactory. In such cases Sister Wray changes to what are known as "rotation" dressings for about 48 hours. The hand remains immobilised, but every 4 hours the following moist dressings are applied in turn: eusol, normal strength;

1. Kennon, R. *Lancet*, 1929, **1**, 167; *Brit. med. J.* 1934, **2**, 1189.

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