spaces full. Impaired resonance R. axilla and R. base. X ray: no abnormality.

CASE 27.—Blast. Fracture R. hip. No chest symptoms. Fullness of lower chest. Diminished air-entry both bases. X ray: no abnormality.

DISCUSSION

Symptoms relating to the chest.—Cough and expectoration were noticed by 6 patients, and in 1 of these there was shortness of breath and general restlessness. The symptoms appeared between the second and fifth days. In no instance were they noticed on the day of the bombing. No-one complained of pain in the chest or of spitting bloodstained sputum.

Physical signs.—In 16 cases there were abnormal physical signs of a recognised character. In only 3 of these were the signs marked. In 15 cases the chest seemed fuller than normal, especially in the lower parts (fig. 1). In 1 case the intercostal spaces seemed to share this fullness. Diminished diaphragmatic movement was present in 10 cases, in 2 affecting the right side, in 4 the left side, and in 4 both sides. There was no tenderness to suggest fracture of the ribs and no evidence of this was found in the radiograms; there was no surgical emphysema. There was generalised hyper-resonance in 1 case. In 3 cases there was impaired resonance either at one or both bases. Loud harsh breath-sounds were present in 1 case. In 7 patients the breath-sounds were faint and almost inaudible. Added sounds were heard in 6 patients—i.e., coarse or fine crepitations at one or both bases and in 2 cases diffusely scattered rhonchi. In no case was a pleural friction sound heard.

One patient (case 23) was clearly the subject of old chest trouble as confirmed by X-ray examination, and the findings were discounted. Case 18 had patchy areas of dullness at both bases, especially the right, with fine crepitations and diminished air-entry resembling a bronchopneumonia. This was confirmed on X-ray examination. Another (13) had signs of collapse in the left lower lobe also confirmed by X ray.

Radiological findings.—X-ray examination presented difficulties, for the patients had to be examined in bed, on account of their injuries, and they could not have their position in bed adjusted as required. Standardisation of position, therefore, could not be attained and allowance had to be made for this in scrutinising the radiograms. Abnormal radiological appearances were found in 14 out of the 27 cases. Of these, 2 showed obvious changes; 1 case (13) had a fairly dense area in the lower left lung field due to consolidation of part of the lung, probably the lower lobe (fig. 2), while case 18 showed the congestive changes of a bronchopneumonia (fig. 3). The remainder showed changes similar to an early pleurisy—a diminution of rib-expansion on the affected side, together with a slight loss of translucency (figs. 4 and 5). In nearly all the cases this appearance was on the left side.

SUMMARY AND CONCLUSIONS

1. A series of 27 patients who were under treatment for burns or other injuries resulting from the bursting of high-explosive bombs at close quarters are reviewed with special regard to the state of their chests. In only 2 patients was the question of exposure to blast doubtful, in the remainder severe blast had been experienced.

2. Only 6 patients complained of symptoms related to the chest; 16 showed some abnormal physical signs and 14 showed abnormal radiological appearances.

3. Evidence of serious or gross pathological changes in the chest was absent in all but 2 cases; 1 of these had the signs of collapse of a lobe of the lung, the other had the signs of a patchy consolidation of the bronchopneumonic type.

4. The relative importance of the three factors to which the patients were exposed—blast, burns, and immersion—in relation to the chest conditions is impossible to assess. It will be difficult to find cases in which there are no external injuries. Immersion may have played an important part in case 18, for example, but only 3 suffered this experience and the other 2 showed neither symptoms nor signs of chest involvement. Burns were extensive though superficial, but in case 17 with burns which involved almost the whole skin of the chest, there was no X-ray evidence of chest involvement. Physical examination was impossible in this case.

5. Attention is specially drawn to:

(*a*) The relative disproportion in frequency between the symptoms and physical signs in the cases studied. This may be due to the fact that all were the subject of serious injuries which would tend to direct their attention away from the chest. Moreover, they were all confined to bed. Chest complications may arise after explosion blast without definite warning symptoms and this should encourage the performance of routine examinations even in those who are apparently unaffected by the blast.

(*b*) The physical signs, judging from what we have seen, that may be expected are: diminished movement of the diaphragm; fullness of the chest giving it an emphysematous appearance; and impairment of resonance at one or both bases, with or without crepitations.

(*c*) The frequency of a "blown-up" or ballooned appearance of the chest, especially at the lower costal margins after such injuries. This may be related to the posture the patients adopted in bed, or to the presence of an already existing emphysema, though this seems unlikely since they were all young men and in good health before being injured. This appearance was often associated with diminished movement of the diaphragm. It may be that some true traumatic emphysema results in these cases.

(*d*) The radiological appearances of a diminution of rib-expansion, together with slight loss of translucency, particularly on the left side. This is an appearance that theoretically should be produced by a pleura slightly thickened by bruising and "bruised pleura" may be the pathological condition present. The explanation of the frequency of this appearance on the left side may be that this was the side exposed to the blast, but we have been unable to confirm this point.

PENICILLIN AS A CHEMOTHERAPEUTIC AGENT

BY

E. CHAIN, PH.D. CAMB.
H. W. FLOREY, M.B. ADELAIDE,
A. D. GARDNER, D.M. OXFD, F.R.C.S.
N. G. HEATLEY, PH.D. CAMB.
M. A. JENNINGS, B.M. OXFD,
J. ORR-EWING, B.M. OXFD,
A. G. SANDERS, M.B. LOND.

(*From the Sir William Dunn School of Pathology, Oxford*)

IN recent years interest in chemotherapeutic effects has been almost exclusively focused on the sulphonamides and their derivatives. There are, however, other possibilities, notably those connected with naturally occurring substances. It has been known for a long time that a number of bacteria and moulds inhibit the growth of pathogenic micro-organisms. Little, however, has been done to purify or to determine the properties of any of these substances. The antibacterial substances produced by *Pseudomonas pyocyanea* have been investigated in some detail, but without the isolation of any purified product of therapeutic value.

Recently, Dubos and collaborators (1939, 1940) have published interesting studies on the acquired bacterial antagonism of a soil bacterium which have led to the isolation from its culture medium of bactericidal substances active against a number of gram-positive micro-organisms.[1] Pneumococcal infections in mice were successfully treated with one of these substances, which, however, proved to be highly toxic to mice (Hotchkiss and Dubos 1940) and dogs (McLeod et al. 1940).

Following the work on lysozyme in this laboratory it occurred to two of us (E. C. and H. W. F.) that it would be profitable to conduct a systematic investigation of the chemical and biological properties of the antibacterial

1. See *Lancet*, 1940, 1, 1172.

RESULTS OF THERAPEUTIC TESTS ON MICE INFECTED WITH *Strep. pyogenes*, *Staph. aureus* AND *Cl. septique*

Expt.	—	Dose of infecting culture (c.cm.)	Interval before starting treatment (hrs.)	Duration of treatment	Single dose (mg.)	Total dose (mg.)	No. of mice	Survivors at end of— hours 6	12	24	days 2	3	4	5	6	7	8	9	10
Strep. pyogenes—Lancefield, Gp. A.																			
1	Controls	0·5	..	..	..	..	25	..	15	9	8	6	..	5	..	4	..	..	4[1]
	Treated	0·5	1	12 hrs.	2	10·0	50	..	..	..	49	42	..	34	30	28	..	26	25
2	Controls	0·5[2]	..	..	..	..	25	24	3	0[3]	..	..	..	..	..	..	..	..	0
	Treated	0·5	2	45 hrs.	0·5	7·5	25	24	..	..	..	..	..	..	..	..	..	..	24
Staph. aureus[4]																			
1	Controls	1·0	..	..	..	..	24	21	1	0[3]	..	..	..	..	..	..	..	..	0
	Treated	1·0	1	55 hrs.	0·5	9·0	25	25	12	..	11	..	10	..	..	..	..	..	8
2	Controls	0·2[2]	..	..	..	..	24	23	15	5	0	..	..	..	..	..	..	..	0
	Treated	0·2	1	4 days	0·5	11·5	24	..	23	22	..	..	21	..	..	..	..	..	21
Cl. septique																			
1	Controls	see text	..	..	..	..	25	..	21	0[5]	..	..	..	..	..	..	..	..	0
	Treated	..	1	10 days	0·5	19	25	..	..	24	21	..	18	..	..	..	..	..	18
		..	1	10 ,,	1·0	38	25	..	..	..	..	..	24	..	..	..	..	..	24

1. A control mouse which was killed by mistake at 24 hrs. is counted as a survivor. Heart-blood culture strongly positive.
2. Between experiments 1 and 2 the virulence of the organism was raised by passage.
3. Controls all dead within 16 hrs.
4. A bovine strain exceptionally virulent to mice kindly supplied by Dr. H. J. Parish of the Wellcome Laboratories.
5. Controls all dead within 17 hrs.

substances produced by bacteria and moulds. This investigation was begun with a study of a substance with promising antibacterial properties, produced by a mould and described by Fleming (1929). The present preliminary report is the result of a coöperative investigation on the chemical, pharmacological and chemotherapeutic properties of this substance.

Fleming noted that a mould produced a substance which inhibited the growth, in particular, of staphylococci, streptococci, gonococci, meningococci and *Corynebacterium diphtheriæ*, but not of *Bacillus coli*, *Hæmophilus influenzæ*, *Salmonella typhi*, *P. pyocyanea*, *Bacillus proteus* or *Vibrio choleræ*. He suggested its use as an inhibitor in the isolation of certain types of bacteria, especially *H. influenzæ*. He also noted that the injection into animals of broth containing the substance, which he called " penicillin," was no more toxic than plain broth, and he suggested that the substance might be a useful antiseptic for application to infected wounds. The mould is believed to be closely related to *Penicillium notatum*. Clutterbuck, Lovell and Raistrick (1932) grew the mould in a medium containing inorganic salts only and isolated a pigment—chrysogenin—which had no antibacterial action. Their culture media contained penicillin but this was not isolated. Reid (1935) reported work on the inhibitory substance produced by Fleming's mould. He did not isolate it but noted some of its properties.

During the last year methods have been devised here for obtaining a considerable yield of penicillin, and for rapid assay of its inhibitory power. From the culture medium a brown powder has been obtained which is freely soluble in water. It and its solution are stable for a considerable time and though it is not a pure substance, its anti-bacterial activity is very great. Full details will, it is hoped, be published later.

EFFECTS ON NORMAL ANIMALS

Various tests were done on mice, rats and cats. There is some œdema at the site of subcutaneous injection of strong solutions (e.g. 10 mg. in 0·3 c.cm.). This may well be due to the hypertonicity of the solution. No sloughing of skin or suggestion of serious damage has ever been encountered even with the strongest solutions or after repeated injections into the same area.

Intravenous injections showed that the penicillin preparation was only slightly, if at all, toxic for mice. An intravenous injection of as much as 10 mg. (dissolved in 0·3 c.cm. distilled water) of the preparation we have used for the curative experiments did not produce any observable toxic reactions in a 23 g. mouse. It was subsequently found that 10 mg. of a preparation having twice the penicillin content of the above was apparently innocuous to a 20 g. mouse.

Subcutaneous injections of 10 mg. into two rats at 3-hourly intervals for 56 hours did not cause any obvious change in their behaviour. They were perhaps slightly less lively than normal rats but they continued to eat their food. Their blood showed a fall of total leucocytes after 24 hours, but after 48 hours the count had risen again to about the original total. There was, however, a relative decrease in the number of polymorphs, but the normal number was restored 24 hours after stopping the administration of the substance. One of these two rats was killed for histological examination; there was some evidence that the tubule cells of the kidney were damaged. The other has remained perfectly well, and its weight increased from 76 to 110 g. in 23 days. It is to be noted that these rats received, weight for weight, about five times the dose of penicillin used in the curative experiments in mice. No evidence of toxic effects was obtained from the treated mice, which received penicillin for many days.

Other pharmacological effects.—On the blood-pressure, heart-beat and respiration of cats no effects have been observed after intravenous injection of 40 mg.—enough to bring up the concentration in the blood just after injection to 1/5000. *Perfusion* of the isolated cat's heart, with Ringer-Locke solution containing 1/5000 penicillin produced progressive slowing during 15 minutes and at the end of that time the heart looked as though it would stop beating; however, it was quickly revived by perfusing with Ringer-Locke solution alone. The same depressant action was seen at 1/10,000 dilution but the effect was less than at 1/5000. Solutions are absorbed from the intestine in the rat without causing any observable damage to the mucosa. They are also readily absorbed after subcutaneous injection and the substance can be detected in the blood. It is excreted by the kidneys, the urine becoming bright yellow. At least 40–50 % appears in the urine in a still active form. *Human leucocytes* remain active in a 1/1000 solution for at least 3 hours.

It must be emphasised that the results of these preliminary tests have been obtained with an impure

substance and such slight toxic effects as have been noted may possibly be due, in part at least, to these impurities.

EFFECTS ON BACTERIA IN VITRO

In view of this slight evidence of tissue toxicity it is all the more striking that the substance in a dilution of one in several hundred thousand inhibits in vitro the growth of many micro-organisms, including anaerobes. Of those so far tested in this laboratory the following are sensitive to the inhibiting action of our preparation: *Clostridium welchii* (2 strains); *Cl. septique* (1 strain, Nat. coll. type-cultures No. 458); *Cl. œdematiens* (1 strain, N.C.T.C. No. 277); *C. diphtheriæ* (1 strain, mitis type); *Streptococcus pyogenes* (Lancefield group A); *Str. viridans* (1 strain from tooth); *Str. pneumoniæ* (type 8); staphylococci (3 strains). Penicillin is not immediately bactericidal but seems to interfere with multiplication.

THERAPEUTIC EFFECTS

From all the above tests it was clear that this substance possessed qualities which made it suitable for trial as a chemotherapeutic agent. Therapeutic tests were therefore done on mice infected with streptococci, staphylococci and *Cl. septique*; the results are summarised in the accompanying table, the preliminary trials on small numbers of mice being omitted.

Bacteriological methods: streptococcus and staphylococcus.—The staphylococcus was kept in culture in meat extract broth and the streptococcus in the same with serum. Both organisms were repeatedly passed through mice and were used for experiment 2–4 days after the last passage. The experimental infection was induced with 20–24 hour broth cultures injected intraperitoneally. According to opacity measurements with Brown's tubes, doses of 450 and 350 million cocci, living and dead, were given respectively in the two streptococcal experiments, and doses of 760 and 200 million in the staphylococcal experiments.

Pathogenic anaerobes.—The therapeutic effects were tried in mice infected with spores of *Cl. septique* in the manner described by Henderson and Gorer (1940). Attempts to carry out similar tests with *Cl. welchii* were temporarily abandoned owing to the difficulty of establishing in mice a type of infection which is both certainly fatal and allows adequate time before death for treatment to take effect. A spore suspension of *Cl. septique* was made by anaerobic growth at 37° C. for 48 hours on Dorset's egg slopes, suspension of the growth of each slope (surface about 15 sq. cm.) in about 1 c.cm. of sterile distilled water, centrifugalisation, once washing with distilled water, recentrifugalisation and resuspension in the original volume of distilled water. The suspension was then heated to 75° C. for one hour. The virulence of the suspension, when injected with an equal volume of 5% calcium chloride was roughly titrated by injection into the thigh muscle of mice, and a dose was chosen for the therapeutic experiment such as would kill for certain without being unnecessarily severe, viz., 0·05 c.cm. of the suspension diluted one in three, mixed with 0·05 c.cm. of the calcium chloride solution. This was injected into the thigh muscles of the 75 mice whose subsequent fate is recorded in the table.

Treatment.—The general principle has been to keep up an inhibitory concentration of the substance in the tissues of the body throughout the period of treatment by repeated subcutaneous injections. No extended tests have been made to determine the minimum effective quantities or the longest intervals possible between injections. The doses employed have been effective and not toxic; they may have been excessive. The solution contained 10 mg. per c.cm. of substance.

In the first streptococcal experiment the treatment was continued for 12 hours only. That this was inadequate was shown by deaths occurring during the arbitrarily chosen 10-day period. In the second experiment the time of treatment was lengthened, with improved results. In this and the two staphylococcal experiments injections were given 3-hourly for the first 32–37 hours, then at longer intervals.

In preliminary experiments with *Cl. septique* it was found that the infection could be satisfactorily held in check so long as penicillin was being given (i.e., for 2 days) but when the administration was stopped the infection developed. In the experiment quoted, therefore, the injections were given for 10 days, 3-hourly for 41 hours, then at longer intervals, and twice daily for the last 2 days of the period. No deaths have subsequently occurred (22 days after beginning of experiment).

The behaviour of the mice infected with streptococci and staphylococci was interesting. For some hours after the start of treatment they looked sick—some even appeared to be dying—but as the experiment went on they progressively improved till at the end of 24 hours in the case of the streptococci and about 36 to 48 hours with the staphylococci it was difficult or impossible to distinguish them from normal mice. The survivors of the *Cl. septique* infection on the other hand remained well throughout, except for a few in which leg lesions appeared near the site of injection and cleared up in a few hours.

Summarising the data given in the table we see that in the final streptococcus experiment (no. 2) whereas 25/25 controls died, 24/25 treated animals survived. With *Staphylococcus aureus* the final experiment (no. 2) shows 24/24 deaths of the controls and 21/24 survivals among the treated. Lastly, with *Cl. septique* when the larger doses of penicillin were given (bottom line) the figures are 25/25 control deaths and 24/25 treatment survivals.

CONCLUSIONS

The results are clear cut, and show that penicillin is active in vivo against at least three of the organisms inhibited in vitro. It would seem a reasonable hope that all organisms inhibited in high dilution in vitro will be found to be dealt with in vivo. Penicillin does not appear to be related to any chemotherapeutic substance at present in use and is particularly remarkable for its activity against the anaerobic organisms associated with gas gangrene.

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REFERENCES

Clutterbuck, P. W., Lovell, R. and Raistrick, H. (1932) *Biochem. J.* **26**, 1907.
Dubos, R. J. and Cattaneo, C. J. (1939) *J. exp. Med.* **70**, 249.
Fleming, A. (1929) *Brit. J. exp. Path.* **10**, 226.
Henderson, D. W. and Gorer, P. A. (1940) *J. Hyg., Camb.* **40**, 345.
Hotchkiss, R. D. and Dubos, R. J. (1940) *J. Biol. Chem.* **132**, 791, 793.
McLeod, C. M., Mirick, G. S. and Curnen, E. I. (1940) *Proc. Soc. exp. Biol.* **43**, 461.
Reid, R. D. (1935) *J. Bact.* **29**, 215.

MENINGOCOCCAL MENINGITIS STARTING AS DIABETIC COMA

BY CECIL W. WARD, M.B. Leeds
AND
ALBERT A. DRIVER, M.B. Leeds, D.P.H.
ASSISTANT MEDICAL OFFICERS AT SEACROFT HOSPITAL, LEEDS

THE following unusual case of meningococcal meningitis presented a difficult problem in diagnosis.

A well-developed athletic man, aged 19, was apparently in perfect health on the morning of Feb. 26. He had little appetite for his midday meal and in the afternoon complained of a slight headache and drowsiness. During the evening this drowsiness became more pronounced, and when first seen by the doctor at 10.30 P.M. he was comatose and could only be roused with great difficulty.

The patient was admitted to Seacroft Hospital about midnight. He did not respond to any stimulation. Pupils half dilated and did not react to light. Swallowing reflex present; knee, ankle, and abdominal reflexes present but sluggish; plantar responses not obtained. Uniform flaccidity of all four limbs, with no perceptible difference between the two sides. No rigidity of neck. Breathing deep and regular; smell of acetone in breath and dryness of tongue led to immediate catheterisation. Sugar 3% and acetone bodies found in urine. Temperature 99·8° F., pulse-rate 84, respiration-rate 32.

Diabetic coma having been diagnosed with some hesitation, the patient was given saline containing 25 g. of glucose to the pint by the intravenous drip method and 25 units of insulin