drugs may in fact, by preventing the growth of secondary bacterial invaders, postpone the bacterial liquefaction of the tenacious secretion. Treatment should be directed primarily to the relief of anoxia, the liquefaction of mucus, and the mechanical removal of tenacious secretion.

Summary

A study was made of 35 children dying of acute laryngotracheobronchitis over a four-year period in Sheffield.

Twenty-nine children died of an exudative affection of the lower respiratory tree.

Laryngotracheobronchitis would appear to be a more common cause of death in children between the ages of 6 weeks and 2 years than the notifiable infectious diseases.

The aetiology of the condition is not known, and the infection is not necessarily specific.

Death seems to be due chiefly to mechanical suffocation by abnormal mucous secretion.

Treatment should be directed to maintaining an airway. Antibacterial drugs are unlikely to be effective in the early stages of the disease and may possibly postpone recovery.

It is a pleasure to acknowledge the interest and advice of Dr. E. H. Harding. The photographs are by A. K. Tunstall.

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The *Hospitals Year Book* for 1952 is a comprehensive guide to regional hospital boards, boards of governors of teaching hospitals, management committees, boards of management, etc. The bed and patient statistics section provides a complete analysis of the allocation of beds in all N.H.S. hospitals. In an introductory article the editor points out that public attention has been so concentrated on financial problems of the national hospital service that its achievements are in danger of being overlooked. In England 40,000 more beds are available than in 1948, and full-time nursing staff have increased by 25,000. Despite such progress the position in mental hospitals and mental-deficiency institutions is acute; 3,000 mental-hospital beds which were diverted to other uses during the war have not yet been brought back into service, and another 2,800 are closed for lack of staff. The *Year Book* is published by the Institute of Hospital Administrators, 75, Portland Place, London, W.1, and costs 45s.

EFFECT OF ANTIBIOTICS ON TISSUE CULTURES OF HUMAN SKIN

BY

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AND

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[WITH SPECIAL PLATE]

As several antibiotics are now available for the treatment of infection by a wide range of organisms, it is useful to consider their relative toxicity to the tissues. This is particularly important when antibiotics are used for local application, as in these circumstances high concentrations may be maintained in contact with the skin. In this unit the practical problem arises which of the antibiotics active against the flora of burns is likely to be least harmful when applied as a local dressing. Clearly the drugs should not inhibit the growth and migration of epithelium in the concentrations used.

A rapid method of assessing the relative toxicity of antibiotics to human skin is provided by the use of cultures of skin *in vitro*. This method does not involve exposing patients to the risk of harmful concentrations of antibiotics, and makes it possible to compare their effects under controlled conditions. In the experiments to be described, explants of human skin cultivated in a fluid medium were used as the test material. These were subjected to various concentrations of aureomycin, terramycin, chloramphenicol, polymyxin B and E, penicillin G, streptomycin, neomycin, and "A18" hydrochloride* in order to determine the highest concentration of each which had no adverse effect upon the growth and migration of epithelium.

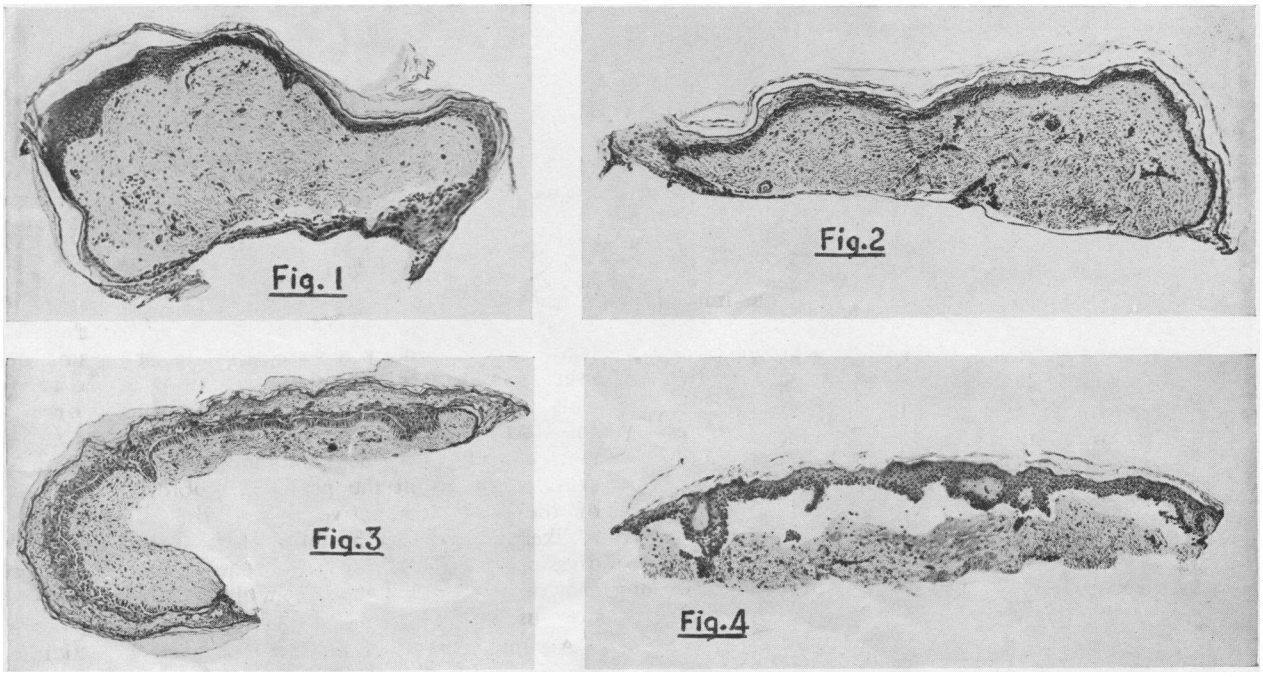
Methods

Cultivation of Human Skin in Vitro.—Adult human skin was obtained by using the excess of material which was removed for storage after skin-grafting operations, and was explanted either immediately or within 24 hours of removal. The skin was preferably of medium thickness—that is, containing about the same thickness of dermis and of epidermis. It was cut into small pieces about 1–2 mm.^{2} in size, and cultivated upon the surface of a fluid medium. The method used was a modification of that originally described by Medawar (1948) for the cultivation of adult rabbit skin, which has proved successful for guinea-pig skin (Cruickshank, 1951). Each explant was cultured in 0.5 ml. of medium in a 5-ml. rubber-stoppered bottle, in an atmosphere of 50% oxygen and 50% air at 37° C. The medium consisted of 5 parts fresh homologous serum, 3 parts Krebs–Ringer phosphate, 1 part 5% glucose in distilled water, and 1 part streptomycin (500 units/ml.) in Ringer's solution. Streptomycin in this concentration is free from toxic effects, and is included routinely to prevent infection of the cultures by organisms normally present on the skin surface. During cultivation the explants were rocked through 15 degrees each way from the horizontal about five times a minute on a specially devised platform.

In order to test the effects of the various antibiotics the appropriate solutions of these were substituted for the 1 part

*"A18" is an antibiotic in the process of development by Glaxo Laboratories Ltd.

C. N. D. CRUICKSHANK AND E. J. L. LOWBURY: EFFECT OF ANTIBIOTICS ON TISSUE CULTURES OF HUMAN SKIN



Human skin cultivated for three days. (Haematoxylin and eosin. ×75.) FIG. 1.—Normal control culture. FIG. 2.—Culture showing minor inhibition. FIG. 3.—Culture showing severe inhibition. FIG. 4.—Culture showing destruction of explant.

J. L. EMERY: ACUTE LARYNGOTRACHEOBRONCHITIS IN CHILDREN

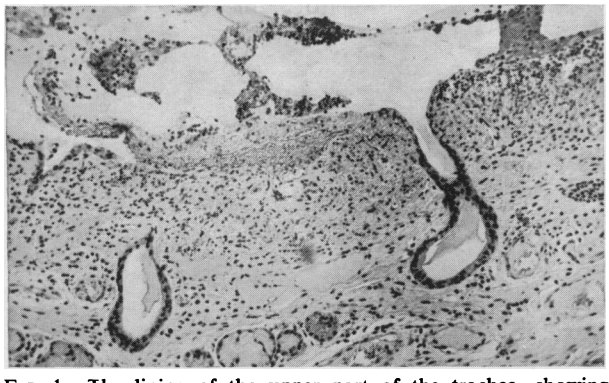


FIG. 1.—The lining of the upper part of the trachea, showing destruction of the epithelium and exudation from glands. (Haematoxylin and eosin. ×90.)

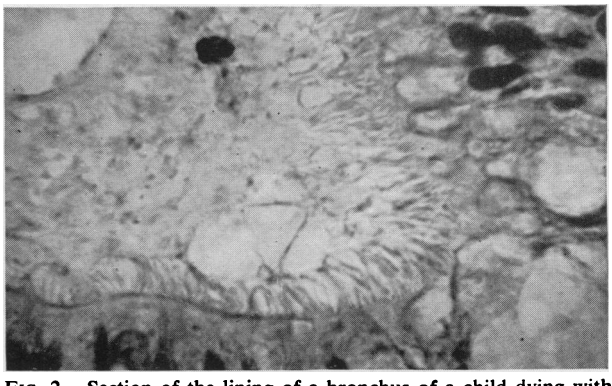


FIG. 2.—Section of the lining of a bronchus of a child dying with the exudative type of acute laryngotracheobronchitis, showing cilia and exudate. (Haematoxylin and eosin. ×700.)

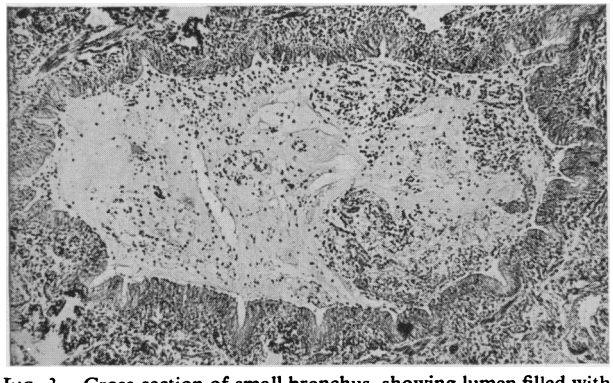


FIG. 3.—Cross-section of small bronchus, showing lumen filled with exudate and small pools of cells. (Haematoxylin and eosin. ×57.)

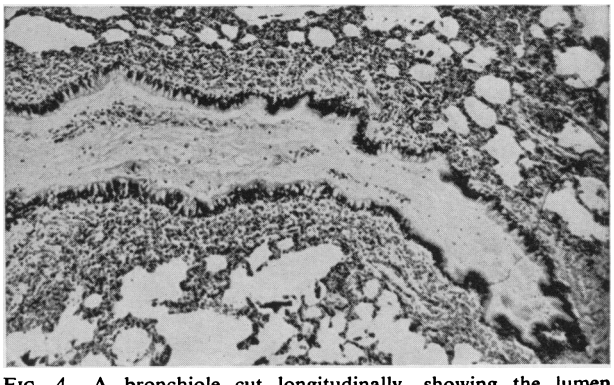


FIG. 4.—A bronchiole cut longitudinally, showing the lumen entirely filled with exudate. (Haematoxylin and eosin. ×60.)

of streptomycin in the medium. In each experiment the cultures were put up in groups of three—that is, there were three control cultures and three cultures of each of the antibiotics under test.

Standardization of Materials.—Antibiotics were dissolved and appropriately diluted in Ringer's solution, the concentration of the solution added to the medium being 10 times the desired final concentration. The concentrations of the different antibiotics which were tested are shown in the accompanying Table. Aureomycin and terramycin, which

Effects of Antibiotics upon Cultures of Human Skin

Antibiotic	Concentration (mg./ml.)	No. of Experiments	No. of Explants	Normal	Minor Inhibition	Severe Inhibition	Destruction
Control	—	5	14	14	0	0	0
Aureomycin hydrochloride	2·0	2	6	0	3	3	0
	1·0	1	3	1	2	0	0
	0·2	1	3	3	0	0	0
	0·02	1	3	3	0	0	0
Chloramphenicol ..	2·0	3	10	5	2	2	1
	1·0	2	6	1	3	1	1
	0·2	1	3	1	1	1	0
	0·1	2	5	3	1	1	0
	0·01	2	6	5	1	0	0
Terramycin hydrochloride	2·0	2	6	0	0	0	6
	1·0	1	3	0	0	0	3
	0·2	1	3	0	3	0	0
	0·02	1	3	3	0	0	0
Polymyxin E ..	10·0	1	3	0	0	0	3
	2·0	3	9	9	0	0	0
	1·0	1	3	3	0	0	0
Polymyxin B sulphate	2·0	3	9	0	1	5	3
	1·0	1	3	0	1	0	2
	0·2	2	6	6	0	0	0
Penicillin G ..	6·0*	1	3	0	0	1	2
	0·6	2	6	6	0	0	0
Dihydrostreptomycin sulphate	10·0*	1	3	0	0	0	3
	1·0	1	3	3	0	0	0
Neomycin sulphate	10·0	1	3	0	0	0	3
	1·0	1	3	3	0	0	0
	0·2	1	3	3	0	0	0
"A18" hydrochloride	2·0	1	3	0	0	0	3
	0·2	1	3	0	0	3	0
	0·02	1	3	3	0	0	0

* Equivalent to 10,000 units/ml.

were acid in solution, required neutralization by sodium hydroxide. Both of these antibiotics are less soluble at neutrality than in acid solution, and a moderate proportion of aureomycin was undissolved at the two highest concentrations (2 mg. and 1 mg.) in the medium. A small insoluble residue was also present in the highest concentration of terramycin and of chloramphenicol (2 mg./ml.). The solutions of these antibiotics were prepared from the capsules supplied for clinical use.

The *p*H of the medium in each culture bottle was roughly checked by B.D.H. indicator papers after three days' incubation and found in every case to be between 7 and 7.6. No bacterial growth was found on blood-agar plates inoculated from tissue-culture media at the same time.

Histological Methods.—After cultivation (usually for three days, but occasionally for shorter periods) the explants were fixed in formol-saline, dehydrated, cleared, and embedded in paraffin. Owing to the small size of the explants the periods required for dehydration and clearing could be considerably shortened. From each explant a median section was cut and stained with haematoxylin and eosin. When the interpretation of any result was doubtful, serial sections through the explant were cut and examined.

Results

Appearance of Normal Cultures.—Under normal conditions of culture, epithelium migrates from the edges of the explant over the cut surface of the dermis, which becomes completely encased in epithelium, so that the explant resembles a cyst. Epithelium migrating from the cut ends of the hair follicles may contribute to this layer, which is at first only one cell thick, but subsequently may increase by cell division until it may be three cells thick after three days. The dermis does not undergo any obvious changes, but appears to survive in a quiescent state (Special Plate, Fig. 1).

Assessment of Inhibition.—By this method of culture inhibition of growth cannot be measured quantitatively, but various degrees are clearly recognizable. For ease of comparison cultures which failed to grow normally have been grouped into three categories:

(*a*) *Minor Inhibition.*—Normal staining properties of epidermis and dermis are retained, but migration of epithelium is less than in the control cultures.

(*b*) *Severe Inhibition.*—The normal staining properties of the epidermis and dermis are retained, but no migration occurs.

(*c*) *Destruction.*—There is no evidence of migratory activity, and the staining properties of the cells of the explant are abnormal.

In some instances the explant becomes completely necrotic. Typical examples of these appearances are shown in Figs. 2, 3, and 4.

Effects of the Antibiotics

These effects are summarized in the Table. Triads of explants exposed to the same material showed close agreement in their appearance after incubation, and replication of results in different experiments was good. The following conclusions can be drawn on the action of the different antibiotics.

Aureomycin.—This did not exercise any harmful effects upon cultures in concentrations of 0.2 mg./ml. or less. Above this level, inhibition of the cultures was usually noticed. In most cases this was not severe—no culture being completely killed. The migration of epithelial cells, however, failed to reach completion. It will be noted that, at concentrations of 1 mg./ml. and above, a saturated solution with some turbidity was obtained.

Terramycin.—This antibiotic was more toxic than either aureomycin or chloramphenicol. At levels of 1 mg./ml. and above, the cultures were consistently killed. At 0.2 mg./ml. the cells survived and some migration took place; at 0.02 mg./ml. normal cultures were obtained.

Chloramphenicol.—The results obtained with this antibiotic were more irregular. Occasionally normal cultures were obtained with concentrations of 2 mg./ml., but again at 0.1 mg./ml. abnormal cultures were sometimes present. Variation in the source of the test material may partly account for the irregularity, but even within single experiments inconsistencies were noted. These inconsistencies make it difficult to assess the toxic level of this drug, but it can be concluded that concentrations higher than 0.01 mg./ml. are apt to prove toxic.

Polymyxins E and B.—Polymyxin E appeared to be slightly less toxic than polymyxin B. The former killed cultures at 10 mg./ml., but normal cultures were obtained at 2 mg./ml. and below. On the other hand, polymyxin B caused either death or severe inhibition at 1 mg./ml., but consistently normal cultures were obtained at 0.2 mg./ml. and below. Some of the cultures containing non-toxic amounts of the polymyxins showed forms of growth which differed from those of the normal culture. There seemed to be a stimulating effect on the epithelium, which grew out into a thick layer. Further investigations are being carried out upon this phenomenon.

Penicillin G.—6 mg./ml. (10,000 units/ml.) was toxic to skin in culture, but normal cultures were obtained at 0.6 mg./ml. (1,000 units/ml.).

Streptomycin.—10 mg./ml. proved toxic, but normal cultures were obtained at 1 mg./ml.

Neomycin.—10 mg./ml. caused either death or severe inhibition of the cultures; 1 mg./ml. allowed skin to grow normally.

"A18" Hydrochloride.—No normal cultures were obtained at 0.2 mg./ml. and above, but normal growth proceeded at 0.02 mg./ml.

Discussion

Caution is necessary in interpreting these results for clinical purposes. Although the proliferation of skin in the cultures described may be compared to that of skin over burns involving epidermis only, the effect of substances on tissue culture probably gives an overestimate of their toxicity *in vivo*. Any antibiotic applied to the surface of a wound is continuously diluted by the tissue fluids which are exuded, and is also removed from the site of application into the blood stream. Nevertheless, the relative toxicity of different antibiotics to the skin can probably be assessed by the method described. It is possible, however, that the toxic effects of aureomycin are somewhat underestimated in comparison with those of other agents, because of its instability at the *p*H and temperature of incubation used in the tests.

As penicillin has been used in this unit for several years as a routine for local application to burns, it is interesting to consider its toxicity to skin as shown by tissue culture. *In vitro*, 1,000 units (0.6 mg.)/ml. did not harm skin, while 10,000 units (6 mg.)/ml. caused severe inhibition or death of cells. When applied to burns, 1,000 units of penicillin per ml. has been found to have no harmful effects. For a short period a cream containing 10,000 units of penicillin per g. was applied to burns colonized by *Str. pyogenes* (Jackson *et al.*, 1951) and no ill effects were observed in individual cases. The tissue-culture tests would appear, in this instance, to overestimate the toxicity of penicillin G to epithelium. It is possible, however, that the higher concentration of penicillin might cause a minor degree of interference with healing, which could be detected only by comparing the healing times of a large number of treated cases with those of a comparable control series.

Another factor which must be considered in interpreting the results of these experiments for clinical purposes is the solubility of the antibiotic in tissue fluids. There is greater risk in using a high concentration of an antibiotic when it is very soluble than when it is relatively insoluble. For example, a saturated solution of aureomycin at an approximately neutral reaction does not cause any severe inhibition as judged by the present tests. On the other hand, polymyxin will easily dissolve in a concentration of 10 mg./ml., and at this level it is decidedly toxic to skin *in vitro*. Similarly, penicillin G will form solutions considerably stronger than that which will prevent the growth of epithelium in culture.

Lépine *et al.* (1950) tested the effect of aureomycin and of chloramphenicol on explants of chick embryo lung, and found that proliferating epithelial cells and fibroblasts were retarded in growth by 0.01 mg./ml. and completely inhibited by 1 mg./ml. of these substances. It is probable that the difference between these findings and those reported above can be attributed to the nature of the test materials. Adult human tissue which was used in the present experiment might reasonably be expected to show effects more comparable to those which occur in healing human burns.

Reports are available concerning the effects of penicillin and streptomycin on cells in culture. Both of these antibiotics are regarded for tissue-culture purposes as being non-toxic to normal cells at concentrations of 100 units/ml. (Cameron, 1950). It will be noted that in the present experiments the maximum non-toxic level is set higher at 1,000 units/ml. It must be understood, however, that the object of these experiments is to determine the maximum non-toxic level, with particular relation to clinical treatment, and that where there is a safe margin between toxicity and bactericidal properties it is clearly desirable for tissue-culture purposes not to exceed the bactericidal concentration more than is absolutely necessary.

Summary

The effects of varying concentrations of a range of antibiotics upon human skin cultivated *in vitro* are described.

By this method the highest concentrations of these substances free from toxic effects are assessed as follows: aureomycin hydrochloride, 0.2 mg./ml.; terramycin hydrochloride, 0.02 mg./ml.; chloramphenicol, 0.01 mg./ml.; polymyxin E, 2 mg./ml.; polymyxin B sulphate, 0.2 mg./ml.; penicillin G, 0.6 mg./ml. (1,000 units/ml.); dihydrostreptomycin sulphate, 1 mg./ml. (1,000 units/ml.); neomycin sulphate, 1 mg./ml.; "A18" hydrochloride, 0.02 mg./ml.

The relationship of these results to the clinical effects of local applications of antibiotics is discussed.

We wish to acknowledge the technical assistance of Mr. J. R. Cooper and Miss M. Hulse, and to thank Glaxo Laboratories Ltd., Burroughs Wellcome and Co., and the M.R.C. Antibiotics Clinical Trials (Non-Tuberculous Conditions) Committee for providing antibiotics.

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EXFOLIATIVE CYTOLOGICAL CONTROL IN OCCUPATIONAL CANCER OF THE BLADDER

BY

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[WITH SPECIAL PLATE]

The relative ease with which exfoliation of cells occurs from bladder tumours into the urine has been noted by Papanicolaou (1947, 1948) in the day-to-day cases of unknown aetiology, and by Goldblatt (1947) in cases of known aetiology in the dyestuffs industry. The utilization of this fact in the early diagnosis of bladder tumours in workers is the subject of this paper.

Whereas American authors have repeatedly emphasized (Papanicolaou, 1947; Fremont-Smith *et al.*, 1948) that exfoliative cytological diagnosis must be regarded as an adjunct to the more usual procedures (cystoscopy, biopsy, *x*-ray examination), the situation is somewhat different in the consideration of occupational bladder tumours in this country. Routine cystoscopy is stated to be practised in America, Switzerland, and Italy (Müller, 1951; di Maio, 1937, 1949; Barsotti and Vigliani, 1949) in factories where occupational bladder tumours have arisen among workers in certain departments that manufacture or use dyestuffs intermediates. Such a routine, at intervals of say six or twelve months, is the best if not an infallible way to detect tumours at a stage early enough to ensure hopeful or successful treatment.

In Britain, routine cystoscopy has never commended itself (Goldblatt, 1947) to workers. Only when, by the repeated and increasing appearance of red blood corpuscles in the urine, the presence of a tumour is rendered probable are workers amenable to persuasion and agree to undergo cystoscopy before and periodically after appropriate operation or treatment. This is quite understandable, inasmuch as the workman experiences