Brit. J. Pharmacol. (1959), **14**, 168.

THE COMPARATIVE TOXICITY OF ANTIBIOTICS TO SKIN

BY

J. C. LAWRENCE

From the Medical Research Council Industrial Injuries and Burns Research Unit, Birmingham

(RECEIVED NOVEMBER 13, 1958)

The toxicity of sixteen antibiotics to skin *in vitro* was investigated by a microrespirometer technique and by the histological appearance of 3-day cultures. There was close agreement between the results obtained by the two methods and between tests with human and with guinea-pig skin. Antibiotic concentrations which caused more than 30% respiratory damage after one day invariably produced abnormal cultures, and more than 60% respiratory damage often resulted in necrotic cultures. Dose/response curves were obtained for the effect of antibiotics upon skin respiration. Mathematical evaluation had little advantage over visually fitted dose/response curves. The ED50 provided a reproducible measure of the relative toxicity of different antibiotics to skin, but the ED30 probably provided a better comparison because this was the highest concentration of antibiotic which permitted migration of epithelium. Many antibiotics at therapeutic concentrations show little toxic effect upon skin *in vitro*. It is suggested that the healing of surface lesions might well be retarded if the antibiotic concentration found toxic *in vitro* were maintained at a similar concentration *in vivo*.

As high concentrations of antibiotics are frequently applied on surface wounds such as burns, it is desirable to know the local toxicity of the antibiotics, since impairment of wound healing could possibly offset advantages gained by elimination of infecting micro-organisms.

Tissue culture offers a convenient approach to this problem and was used by Cruickshank and Lowbury (1952) to assess the effect of some antibiotics on the migration of epithelium around small explants of human skin. Hu, Livingood, and Hildebrand (1956) used a roller tube technique to study the effect of some antibiotics upon human skin cells. Other workers have used other culture materials; Lépine, Barski, and Maurin (1950) used chick-embryo cultures for tests on the toxicity of chlortetracycline and chloramphenicol, and Schrek (1953) tested oxytetracycline on rat thymus cells.

Though clinical conditions are doubtless different from those of *in vitro* experiments, cultures of adult mammalian skin are obviously more appropriate to this problem than chick-embryo cultures and the technique of skin culture used by Cruickshank and Lowbury (1952) is somewhat analogous to conditions found in a healing surface wound. However, the use of histological criteria in this method has the disadvantage that many tests are necessary in order to determine the maximum tolerable antibiotic concentration. In the present investigation a microrespirometer technique (Cruickshank, 1954) has been applied to this problem and the results have been compared with those obtained by the histological method (Cruickshank and Lowbury, 1952). This gives a rapid and reproducible method for determining the immediate toxicity of antibiotics to skin.

METHODS

Guinea-pig and human skin slices were used in this investigation; guinea-pig skin was cut free-hand from the dorsum of the ear (Cruickshank, 1954), human skin was that left over after the grafting of burns. Since human skin obtained in this manner has been previously found to be less reliable for metabolic studies than guinea-pig skin owing to variations in thickness and the amount of keratin present (Lawrence and Ricketts, 1957), preliminary experiments with guinea-pig skin were followed by experiments with human skin.

The following standard skin culture medium was used for all the experiments: homologous serum 5 parts; Krebs-Ringer phosphate 3 parts; 2% glucose 1 part; dihydrostreptomycin sulphate 500 μg./ml. 1 part (Cruickshank and Lowbury, 1952).

For toxicity tests antibiotics were dissolved in physiological saline at 10 times the required strength. Where necessary, the *p*H was adjusted to 7.0. These solutions were incorporated into tissue cultures by making 1:10 dilutions with skin culture medium. Sparingly soluble materials were employed as

suspensions ; these were agitated violently immediately before use so that the suspension was as uniform as possible.

The differential microrespirometer described by Cruickshank (1954) was used for skin respiration studies. With this device aerobic respiration of small skin slices could be measured accurately over long periods of time. Test solutions were added after respiration measurements had been made for 2 hr. Readings were then taken for the next 4 to 5 hr., discontinued at night (about 15½ hr.), recommenced the following day and continued until 24 hr. after addition of the test solution. The mean respiratory rate of the 22 to 24 hr. period was then expressed as % of the 2 hr. control period before addition of the test material. It was found convenient to terminate experiments at 24 hr., because control cultures respired steadily over this period ; some decrease in respiration occurred over longer time periods. Respiratory damage caused by toxic agents after 48 hr. was proportional to that occurring after 24 hr.

A preliminary experiment on each antibiotic discovered the approximate concentrations producing slight and severe inhibition of respiration after 24 hr. Having established this range, a series of intermediate concentrations were prepared and tested. A control culture in which saline was added to the respirometer was always included with every experiment.

Three-day cultures of skin were prepared in the manner described by Cruickshank (1951). Antibiotic concentrations causing about 5%, 25%, 50% and 95% respiratory damage were chosen. Histological appearance of these cultures was classified into the four groups defined by Cruickshank and Lowbury (1952): (*a*) Normal cultures: epithelium migrates over the cut surface of the dermis so that the explant resembles a "cyst" of dermis within epidermis. (*b*) Minor inhibition: these stain normally but epithelial migration is less than in normal cultures, usually only around the "shoulders" of the explant. (*c*) Severe inhibition: these cultures also stain normally but no migration of epithelium occurs. (*d*) Necrotic cultures: these stain normally and, in some instances, the explant is destroyed.

Results

Respiration Studies.—Sixteen antibiotics were tested by the same experimental technique. To illustrate the procedure, results obtained with vancomycin (McCormick, Stark, Pittenger, Pittenger, and McGuire, 1955–56) will be quoted in full.

Fig. 1 shows the respiratory changes of guinea-pig skin caused by various concentrations of vancomycin. Table I shows the corresponding % respiratory damage of guinea-pig and human skin. No change in respiration could be detected until the second day. This "delayed" toxic action was common to all antibiotics tested except novobiocin ; toxic concentrations of this substance caused an appreciable decrease in respiration 2 hr. after addition. With the other antibiotics some toxic effects could be discerned 8 to 10 hr. after addition and by 16 hr. toxic

TABLE I

THE RESPIRATORY DAMAGE TO SKIN CAUSED BY VANCOMYCIN

Drug Conc. (mg./ml.)	Guinea-pig				Human		
	O_2 Uptake (µl./mg./hr.)		% Respiratory Damage	Curves in Fig. 1	O_2 Uptake (µl./mg./hr.)		% Respiratory Damage
	0 to 2 hr. Before	22 to 24 hr. After			0 to 2 hr. Before	22 to 24 hr. After	
0	0·96	0·97	0	Not shown	0·36	0·38	0
1·0	1·00	0·95	5	A	0·42	0·39	7
2·5	0·99	0·89	10	B	0·40	0·34	15
5·0	0·89	0·74	17	C	0·32	0·25	22
7·5	1·01	0·77	24	D	0·35	0·24	31
10·0	1·00	0·47	53	E	0·44	0·18	59
20·0	1·05	0·37	65	F	0·41	0·11	75

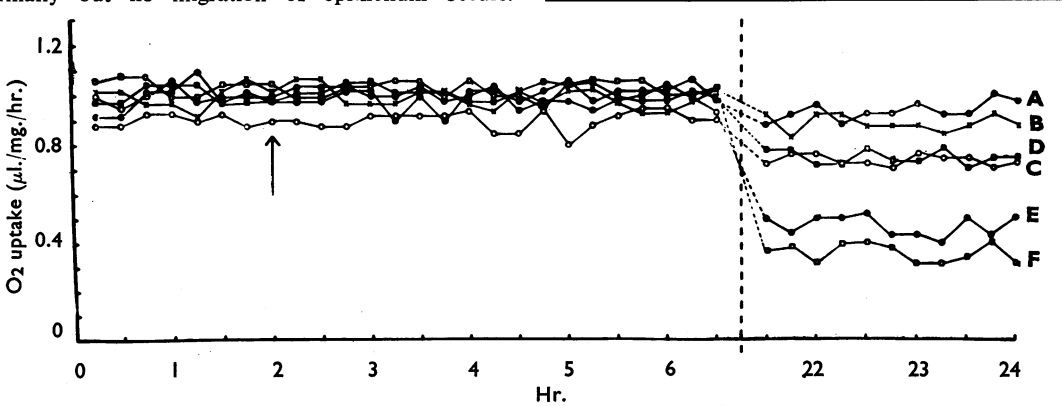

FIG. 1.—The effect of vancomycin on the respiration of guinea-pig skin. Vancomycin was added at the time indicated by the arrow in the following concentrations: A, 1.0 mg./ml.; B, 2.5 mg./ml.; C, 5.0 mg./ml.; D, 7.5 mg./ml.; E, 10.0 mg./ml.; F, 20.0 mg./ml. The broken vertical line denotes the overnight period during which observations were discontinued.

effects were obvious. These respiratory changes reached a maximum 18 to 20 hr. after the antibiotic had been added to the culture. None of the antibiotics caused any initial stimulation of respiration, in contrast to observations made with certain other bacterial products (Lawrence, 1958, 1959).

Log drug concentration plotted against % respiratory damage gave a sigmoid curve and a linear dose/response curve was obtained by transferring the observed % to a probability scale. Fig. 2 shows the dose/response curve for vancomycin.

The relative toxicities of antibiotics to skin were assessed by comparing the median effective concentration (ED50) of each antibiotic with the ED50 of penicillin. Penicillin was chosen as the standard with a toxicity of 1.0 because it is one of the least toxic substances to skin so far discovered. Table II shows the relative toxicity indices of the antibiotics computed on weight/unit volume and molar bases. It is important to note that these indices are valid only for the 50% effect; it is only when the slopes of the dose/response curves are the same that the relative toxicity index is constant for all percentage effects. The slope values of these curves were calculated from the expression (Gaddum, 1948), slope $\simeq 1/\log.ED69 - \log.ED31$, and have been included in Table II for comparison since it is generally accepted that substances producing

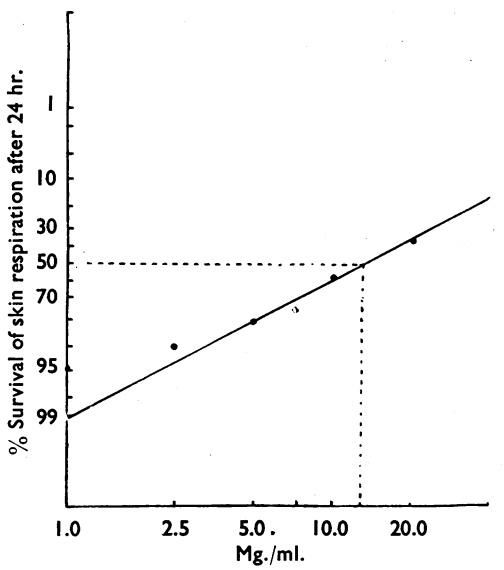

FIG. 2.—Dose/response curve for the effect of vancomycin on skin respiration. M.W. of vancomycin is 3,350 (approx.). ED50 13 mg./ml. (38.8×10^{-5} M). Ordinate, probability scale.

TABLE II

CONCENTRATIONS OF ANTIBIOTICS REQUIRED TO REDUCE THE RESPIRATION OF SKIN BY 50% AFTER 24 HR.

The dose/response curves of antibiotics marked with an asterisk were extrapolated because of their low solubility.

Antibiotic	M.W.	ED50 (mg./ml.)	Toxicity Relative to Penicillin	ED50 (M × 10^{-5})	Molar Toxicity Relative to Penicillin	Slope of Dose/Response Curve
Penicillin	356	7·4	1·0	208	1·0	2·78
Cephalosporin N	359	23·4	0·32	652	0·32	0·75
Vancomycin	3,350	13	0·6	38·8	5·4	2·63
Oleandomycin*	828	36·3	0·16	438	0·47	2·08
Erythromycin*	725	4·6	1·6	63·5	3·3	2·33
Carbomycin	953	0·26	28·5	27	77	5·88
Streptomycin	964	7·8	0·95	80·9	2·6	1·54
Neomycin	?1,000	4·4	1·7	4·4	4·7	1·23
Chloramphenicol	323	8·7	0·85	269	0·77	0·69
Chlortetracycline	514	0·25	29·6	4·9	42·4	2·63
Oxytetracycline	481	0·3	24·7	6·2	33·6	2·70
Tetracycline	465	0·26	28·5	6·0	34·7	2·58
Polymyxin B	1,280±70	0·4	18·5	2·9	71·7	1·49
Bacitracin	>1,500	10 mg./ml. non-toxic				
Neopumilin	>2,000	Saturated solution non-toxic				
Novobiocin	612	0·3	24·7	4·9	42·4	2·94

dose/response curves with similar slopes probably have comparable modes of action (Finney, 1947; Gaddum, 1948).

Dose/response relations for the tetracycline antibiotics were also evaluated by the mathematical method of Finney (1947) and compared with the results obtained by the method of Gaddum (1948). This comparison is shown in Table III.

TABLE III

COMPARISON OF RELATIONSHIPS BETWEEN DOSE AND RESPONSE FOR THE TETRACYCLINE ANTIBIOTICS OBTAINED BY DIFFERENT METHODS OF CALCULATION

F: method of Finney (1947). G: method of Gaddum (1948).

Antibiotic	F ED50 (mg./ml.)	Slope	G ED50 (mg./ml.)	Slope
Chlortetracycline ..	0·22±0·07	2·3	0·25	2·6
Oxytetracycline ..	0·32±0·08	2·4	0·30	2·7
Tetracycline	0·25±0·12	2·4	0·26	2·6

Both methods gave similar values for the ED50 and slope. Since the two methods were in such good agreement, visually fitted lines were used in this investigation.

Three-day Cultures.—The histological appearance of human and guinea-pig skin explants cultured in the presence of various antibiotics for 3 days is recorded in Table IV. The toxicity to skin of both species of all the antibiotics tested was found to be similar; these results have

TABLE IV

THE EFFECT OF ANTIBIOTICS ON 3-DAY SKIN CULTURES
Results marked with an asterisk should be read in conjunction with Table V.

Antibiotic	Conc. (mg./ml.)	Histological Appearance						% Resp. Damage after 24 hr.
		No. of Expts.	No. of Explants	Cultural Appearance				
				Normal	Minor Inhibition	Severe Inhibition	Necrosis	
Controls	—	27	54	49	5	0	0	0
Penicillin	1·0	2	4	4	0	0	0	2
	2·5	2	4	2	2	0	0	10
	5·0	3	6	1	2	2	1	32
	10·0	2	4	0	0	0	4	65
Vancomycin	1·0	2	4	4	0	0	0	5
	5·0	2	4	3	1	0	0	15
	10·0	2	4	0	2	2	0	38
	25·0	2	4	0	0	2	2	70
Oleandomycin	10·0	2	4	3	1	0	0	14
	20·0	4	8	5	2	1	0	30
Erythromycin	1·0 (sat. soln.)	4	8	6	2	0	0	10
Streptomycin	1·0	2	4	4	0	0	0	9
	2·5	2	4	2	2	0	0	23
	10·0	2	4	0	0	1	3	64
Neomycin*	2·5	2	4	0	3	1	0	38
Tetracycline	0·1	2	4	2	1	1	0	18
	0·25	2	4	1	1	2	0	48
	0·5	2	4	0	0	2	2	74
	1·0	2	4	0	0	1	3	90
Oxytetracycline*	0·3	2	4	0	0	3	1	50
Polymyxin B*	0·5	2	4	0	1	2	1	56
Bacitracin	10·0	5	10	9	1	0	0	0
Neopumilin	0·01 (sat. soln.)	4	8	6	2	0	0	0
Novobiocin	0·1	2	4	3	1	0	0	10
	0·2	2	4	1	3	0	0	30
	0·5	2	4	0	1	2	1	74
	1·0	2	4	0	0	0	4	93

therefore been grouped together. The % respiratory damage of skin cultured for 1 day in these antibiotic concentrations has also been included in Table IV for comparison.

Neopumilin, oleandomycin, and erythromycin were tested as saturated solutions because of their low solubility. It was not possible to investigate the effect of cephalosporin N or carbomycin upon 3-day cultures of skin.

DISCUSSION

The histological appearance of 3-day skin cultures made in the presence of antibiotics bore a close relationship to the amount of respiratory damage to skin after 1 day (Table IV). Concentrations of antibiotics causing more than 30% respiratory damage invariably produced abnormal 3-day cultures and 60% (or more) respiratory damage was usually associated with necrotic cultures. Antibiotic concentrations causing less than 30% respiratory damage did not generally interfere with the migration of epithelium around skin explants cultured for 3 days. Similarly, the earlier histological results of Cruickshank and Lowbury (1952) can be compared with the respiration results reported in this study (Table V).

TABLE V

THE EFFECT OF ANTIBIOTICS UPON THE CULTURAL APPEARANCE OF SKIN; THE CULTURAL APPEARANCE REPORTED BY CRUICKSHANK AND LOWBURY (1952) COMPARED WITH % RESPIRATORY DAMAGE

Antibiotic	Conc. (mg./ml.)	Histological Results						% Resp. Damage after 24 hr.
		No. of Expts.	No. of Explants	Cultural Appearance				
				Normal	Minor Inhibition	Severe Inhibition	Necrosis	
Neomycin	0·2	1	3	3	0	0	0	5
	1·0	1	3	3	0	0	0	20
	10·0	1	3	0	0	0	3	66
Chloramphenicol	0·1	2	5	3	1	1	0	13
	0·2	1	3	1	1	1	0	18
	1·0	2	6	1	3	1	1	30
	2·0	3	10	5	2	2	1	36
Chlortetracycline	0·2	1	3	3	0	0	0	40
	1·0	1	3	1	2	0	0	90
	2·0	2	6	0	3	3	0	99
Oxytetracycline	0·02	1	3	3	0	0	0	<1
	0·2	1	3	0	3	0	0	30
	1·0	1	3	0	0	0	3	90
Polymyxin B	0·2	2	6	6	0	0	0	33
	1·0	1	3	0	1	0	2	73
	2·0	3	9	0	1	5	3	85

Penicillin and chloramphenicol were exceptional in that concentrations which caused about 40% respiratory damage frequently resulted in severely inhibited or necrotic cultures. The reason for this anomaly is not yet clear; possibly some delayed secondary effect occurs. In contrast Cruickshank and Lowbury (1952) found that high concentrations of chlortetracycline led to inhibition but not necrosis of cultures; the present study showed that similar concentrations of this antibiotic caused extreme respiratory damage (>90%). This may have been due to the fact that chlortetracycline rapidly decomposes at 37°, or the antibiotic may perhaps act as a fixative, like formaldehyde.

These observations suggest that the antibiotic concentration which skin can tolerate without undue interference in the normal metabolic processes is the concentration which produces 30% respiratory damage after one day. Table VI

TABLE VI

THE TOXICITY OF ANTIBIOTICS TO SKIN. AT THESE CONCENTRATIONS, NAMELY THOSE PRODUCING 30% INHIBITION OF RESPIRATION AFTER 24 HR., THE MIGRATION OF EPITHELIUM IS JUST POSSIBLE

The dose/response curves of the antibiotics marked with an asterisk were extrapolated because of their low solubility.

Antibiotic	ED30 (mg./ml.)	Toxicity Relative to Penicillin	ED30 (Molar $\times 10^{-5}$)	Molar Toxicity Relative to Penicillin
Penicillin	5·0	1·0	140	1·0
Cephalosporin N	4·0	1·25	111	1·26
Vancomycin	8·1	0·62	24·2	5·8
Oleandomycin*	20·0	0·25	242	0·58
Erythromycin*	2·5	2·0	34	4·1
Carbomycin	0·19	26	2·0	70
Streptomycin	3·6	1·4	37	3·8
Neomycin	1·7	2·9	17	8·2
Chloramphenicol	4·0	1·25	124	1·13
Aureomycin	0·16	31	3·1	45
Terramycin	0·20	25	4·2	33
Tetracycline	0·14	36	3·0	47
Polymyxin B	0·17	29	1·3	108
Bacitracin	10 mg./ml. non-toxic			
Neopumilin	Saturated solution non-toxic			
Novobiocin	0·20	25	3·3	42

shows the relative toxicity of the antibiotics at this level (ED30). A comparison of this table with Table II shows that the relative toxicity of the antibiotics differs with the threshold selected (ED50 or ED30); for instance, cephalosporin N is less toxic than penicillin at the ED50 but more toxic at the ED30.

The dose/response curves of the related antibiotics of the tetracycline group (which share similar chemical structure and antibacterial action) had similar slopes. Erythromycin, oleandomycin, and carbomycin also have similar antibacterial activities (Garrod, 1957). Of these, erythromycin and oleandomycin appeared to have comparable effects on skin cultures (slopes 2.3 and 2.1 respectively); but carbomycin was very different (slope 5.9). On structural grounds cephalosporin N (aminoadipic acid penicillin) might have been expected to resemble penicillin in its action on skin, but the penicillin/dose response curve (slope 2.8) was very different from that of cephalosporin N (0.8). This is consistent with certain differences in the range of sensitivity of bacteria to these antibiotics (Newton and Abraham, 1954). It is also possible that the sample of cephalosporin N employed contained impurities which interfered with the test.

Though a similarity in the skin toxicity dose/response curves of two antibiotics may suggest a close relationship in the antibacterial mechanism or chemical structure, this is not necessarily so. For example, vancomycin has a similar slope to that of the tetracycline group (2.6), but bacterial cross-resistance between vancomycin and the tetracyclines has not been encountered (Ziegler, Wolfe, and McGuire, 1955–56). Similarly, polymyxin B and streptomycin share similar slopes (1.5), but their effect on isolated cells is known to be dissimilar. Streptomycin causes inhibition of oxalacetate-pyruvate condensation (Umbreit and Tonhazy, 1949) while polymyxin B is a surface active agent (Newton, 1953). The difference in chemical structure of these two antibiotics is well known. Confirmatory evidence from other sources would seem advisable before drawing conclusions on similarity of toxic action to skin.

The antibiotics may be classified into groups on the basis of their molar toxicities to skin (Table VI). Erythromycin, oleandomycin, bacitracin, and neopumilin are "safe" antibiotics as far as skin is concerned, saturated solutions having little toxic effect.

One large group, including cephalosporin N, vancomycin, chloramphenicol, streptomycin, and neomycin, has a toxicity index of the same order as penicillin (between 0.5 and 8.0). At therapeutic concentrations these materials have little appreciable toxicity towards skin.

The tetracyclines and novobiocin with relative toxicity indices between 35 and 45 form a third group which is somewhat more toxic than penicillin.

Polymyxin B and carbomycin have high indices, of 108 and 70 respectively. However, even these antibiotics have a low toxicity compared with a substance like mercuric chloride which is 424 times as toxic as penicillin (Lawrence, 1958).

The antibiotic concentrations permitting normal skin cultures (Table VI) are generally comparable with such similar values as are available from other workers. Hu *et al.* (1956) obtained the following minimum inhibitory concentrations for antibiotics on human skin *in vitro*: penicillin 1.8 mg./ml.; neomycin 1.0 mg./ml.; chlortetracycline 0.2 mg./ml.; oxytetracycline 0.1 mg./ml. These values, except that quoted for penicillin, agree well with those in Table VI; the present experiments suggest that skin can tolerate 5 mg./ml. penicillin without undue toxic effects.

Schrek (1953) demonstrated 20% mortality of rat thymus cells *in vitro* after 20 hr. in the presence of 0.125 mg./ml. oxytetracycline; the dose/response curve for oxytetracycline indicated that this concentration would be expected to reduce skin respiration by 15% after 1 day.

Lépine *et al.* (1950) using chick embryo fibroblasts found 0.1 mg./ml. chloramphenicol

"quite toxic" and 0.1 mg./ml. chlortetracycline "very toxic." The current investigation showed that 4.0 mg./ml. chloramphenicol and 0.16 mg./ml. chlortetracycline permitted normal skin cultures. The difference between the observations could be due to the diverse nature of the tissues used in the two tests.

Care is needed in applying these results for clinical purposes. Salle, McOmie, and Schechmeister (1937) consider that some form of therapeutic index is desirable in connexion with the use of antiseptics on surface wounds. Such an index is probably of greatest value when comparing substances lethal to bacteria and tissues at similar ranges of concentration. With antibiotics this method seems less appropriate; the enormous difference in susceptibility of bacteria and tissues to antibiotics leads to indices of quite a different order. A more important objection is based on the variations between strains and especially the existence of resistant bacteria which would render such an index invalid. Moreover, variation in susceptibility of bacterial strains to antibiotics makes the application of a single index for a single bacterial species impracticable. It seems, therefore, more useful to have a measure of the tissue toxicity of the antibiotics which can be used together with information on the sensitivity of the infecting bacteria to select an antibiotic for local application.

Cruickshank and Lowbury (1952) consider that tissue culture overestimates the toxicity of antibiotics. For example, Jackson, Lowbury, and Topley (1951) found that penicillin cream used at 10,000 i.u./g. had no apparent ill effect on wound healing although the same concentration often produced necrotic skin cultures and reduced skin respiration by 36% after one day. Clearly an antibiotic applied to a surface wound will be continually diluted by exuded fluid and also removed from the site by the blood stream. Skin respiration experiments suggest that most of the antibiotics take time to exert a toxic action. It may be that a concentration of antibiotic, found toxic *in vitro*, when applied *in vivo* would have this concentration reduced to a safe level before it had time to exert its full toxic effect. It is possible that, if a concentration of antibiotic found toxic *in vitro* could be maintained *in vivo*, wound healing might be impaired. This point requires investigation.

I should like to thank Dr. J. P. Bull and Dr. E. J. L. Lowbury for much invaluable advice and criticism.

REFERENCES

Cruickshank, C. N. D. (1951). *Nature (Lond.)*, **168**, 206.
—— (1954). *Exp. Cell Res.*, **7**, 374.
—— and Lowbury, E. J. L. (1952). *Brit. med. J.*, **2**, 1070.
Finney, D. J. (1947). In *Probit Analysis*, Cambridge University Press.
Gaddum, J. H. (1948). *Pharmacology*, 3rd ed. Oxford: University Press.
Garrod, L. P. (1957). *Brit. med. J.*, **2**, 57.
Hu, F., Livingood, C. S., and Hildebrand, J. F. (1956). *J. invest. Derm.*, **26**, 23.
Jackson, D. M., Lowbury, E. J. L., and Topley, E. (1951). *Lancet*, **2**, 705.
Lawrence, J. C. (1958). Ph.D. Thesis, University of Birmingham.
—— (1959). *Brit. J. exp. Path.*, **40**, 8.
—— and Ricketts, C. R. (1957). *Exp. Cell Res.*, **12**, 633.
Lépine, P., Barski, G., and Maurin, J. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, **73**, 252.
McCormick, M. H., Stark, W. M., Pittenger, G. E., Pittenger, R. C., and McGuire, J. M. (1955–56). *Antibiot. Ann.*, **3**, 606.
Newton, B. A. (1953). *J. gen. Microbiol.*, **9**, 54.
Newton, G. G. F., and Abraham, E. P. (1954). *Biochem. J.*, **58**, 103.
Salle, A. J., McOmie, W. A., and Schechmeister, I. L. (1937). *J. Bact.*, **34**, 267.
Schrek, R. (1953). *Antibiot. and Chemother.*, **3**, 783.
Umbreit, W. W., and Tonhazy, N. E. (1949). *J. Bact.*, **58**, 769.
Ziegler, D. W., Wolfe, R. N., and McGuire, J. M. (1955–56). *Antibiot. Ann.*, **3**, 612.