Journal of Medicinal and Pharmaceutical Chemistry

VOL. 2, No. 1 (1960)

The Effect of β-Diethylaminoethyl diphenylpropylacetate Hydrochloride (SKF 525–A) on the Therapeutic Action of a Variety of Antibacterial, Antiviral or Antiprotozoal Drugs

J. M. THORP, E. WESTON HURST and A. R. MARTIN, *Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire*

Introduction

The enhancing effect of β-diethylaminoethyl diphenylpropylacetate hydrochloride (referred to as SKF 525–A), which in the experimental animal prolongs the period of activity of a diversity of drugs, has been shown by Axelrod, Reichenthal and Brodie[1] to result from an inhibition of the metabolic transformation of these drugs. Using liver slices and homogenates, Cooper, Axelrod and Brodie[2] demonstrated that SKF 525–A inhibits the enzyme systems which bring about oxidation of alkyl side-chains, dealkylation of alkylamines, deamination of sympathomimetic amines, ether-cleavage and phenol-conjugation. Metabolic hydroxylation of aromatic nuclei is also blocked by SKF 525–A, though at a relatively high concentration of the compound. The whole subject has been reviewed by Brodie.[3] Diphenylpropylacetic acid (SKF–acid) is equally active at a cellular level, though only feebly so in the intact animal. A number of studies with the two compounds have confirmed the general nature of the phenomena, and it is apparent that SKF 525–A may sometimes be used to indicate whether the unchanged drug or a metabolite is responsible for a therapeutic or pharmacological response in treated animals.

We ourselves have used SKF 525–A in conjunction with a number of therapeutic agents, and report here our observations of its influence on the therapy of four bacterial, a viral and two protozoal infections.

1. A Streptococcal Infection in Mice

Methods

Groups of 12 or more mice received intraperitoneally 0·2 ml of a suitable dilution (10^{-4} to 10^{-6}) of *Streptococcus agalactiae* (Krüger strain), grown for 24 h in serum-broth or blood-broth. In earlier experiments the culture was derived from a stock conserved in Robertson's meat medium; later, owing to highly inconstant results, we passed the stock culture weekly in mice, re-isolated the organisms from the heart-blood, and used the culture so obtained to initiate that for infecting the experimental groups. Except where otherwise stated, treatments began 15 min after infection and continued twice daily for 3 days.

Sulphonamide therapy

Twice-daily oral dosing with 2·5 mg of sulphadiazine increased the mean period of survival of infected mice from approximately 1 day to over 4·5 days, and with 1 mg to nearly 4 days. Treated mice did not begin to die for several days after all the controls were dead. Doses of 5 mg of sulphanilamide more than trebled the period of survival, and of 2 mg increased it by around 50 per cent. None of these effects was modified by simultaneous intraperitoneal administration of 1 mg of SKF 525–A twice daily. These results accord with our knowledge of the metabolism of the sulphonamides and of the supposed action of SKF 525–A.

Aureomycin therapy

As seen in Table I, doses of 0·25 mg chlortetracycline* slightly diminished mortality and more than doubled the period of survival of those mice which died. Doses of 0·1 mg exerted a lesser effect. The conjunction of SKF 525–A did not influence mortality, but greatly increased the mean periods of survival. In a repeat experiment, in which a larger dose of chlortetracycline (0·4 mg) was included, the effect of SKF was similar, except that at the highest level of dosage there was in addition a significant reduction in the number of deaths among animals receiving the

* Aureomycin ®

combined treatment. Given by itself, SKF 525–A exerted no beneficial effect.

Table I. Effect of SKF 525–A on the activity of chlortetracycline in streptococcal infection of the mouse

Treatment	Deaths in 40 mice
None	40 (1·1)
SKF 525–A 1 mg i.p. b.i.d.	40 (1·1)
Chlortetracycline 0·25 mg orally b.i.d.	31 (2·4)
Chlortetracycline 0·1 mg orally b.i.d.	34 (1·5)
Chlortetracycline 0·25 mg+SKF 525–A	32 (4·4)
Chlortetracycline 0·1 mg+SKF 525–A	35 (3·9)

We gave streptococci intraperitoneally. Treatments began 15 min after infection and continued twice daily for 3 days. The figures in parentheses are the mean periods of survival (in days) of the cases proving fatal.

SKF 525–A, therefore, enhances the therapeutic effect of chlortetracycline in a streptococcal infection in mice, without itself exhibiting therapeutic properties.

Penicillin therapy

The effect of SKF 525–A on treatment with penicillin G was unexpected. In the experiment summarized in Table II, it was

Table II. Effect of SKF 525–A on the activity of penicillin G in streptococcal infection of the mouse

Treatment	Deaths in 40 mice
None	40 (1·2)
SKF 525–A 1 mg i.p. b.i.d.	40 (1·1)
Penicillin G 0·1 mg s.c. b.i.d.	8 (4·5)
Penicillin G 0·02 mg s.c. b.i.d.	13 (3·2)
Penicillin G 0·1 mg+SKF 525–A	32 (2·9)
Penicillin G 0·02 mg+SKF 525–A	38 (2·3)

Experimental procedure as in Table I.

responsible for a very sharp diminution of the therapeutic effect, both when judged by the numbers of mice surviving and by the periods of survival of those ultimately succumbing to the infection. In a further experiment in which the streptococci were rather more virulent and the doses of penicillin G were halved, the latter greatly increased the mean periods of survival without appreciably affecting mortality. On this occasion SKF 525–A almost completely abolished the effect of the penicillin. The phenomenon was confirmed in at least 20 experiments.

To explain the effect of SKF 525–A in diminishing the therapeutic action of penicillin, we considered the following possibilities.

(a) Increased or reduced metabolic transformation of the antibiotic by the host or by the bacterium to, respectively, less or more active derivatives.

(b) Reduced access of penicillin to the infecting organism by, (i) formation of an insoluble salt of penicillin in the host, (ii) enhanced excretion or modified distribution of penicillin in the host, or (iii) reduced permeability of the bacterial cell to penicillin.

(c) Direct antagonism of the antibacterial action of penicillin within the bacterial cell.

(d) A modified response of the host to infection, brought about in one of several possible ways.

The results of experiments to test some of these hypotheses follow.

Metabolism of penicillin. An extensive search of the literature failed to reveal any precise analytical study of the products of penicillin metabolism, with the single exception of the work of Ullberg,[4] who demonstrated that ^{35}S-labelled benzyl penicillin was degraded (to the extent of about 10 per cent in 3 h) to ^{35}S-penicilloic acid by the cat and guinea-pig. Penicilloic acid is devoid of antibacterial activity. No suggestion that biologically active metabolites of penicillin may be formed *in vivo* has been made. That one such metabolite of penicillin G might be *p*-hydroxybenzyl penicillin (penicillin X) seemed possible, since SKF 525–A is known to block aromatic hydroxylation, and since penicillin X has been reported to be two to three times as active as penicillin G (on a weight basis) against certain bacterial

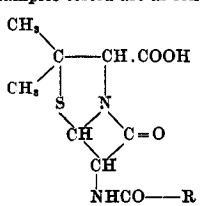
infections (*Str. pyogenes* and *Str. pneumoniae*) in mice (Eagle,[5] Hobby, Lenert and Hyman[6]). If penicillin X were the active metabolite formed from penicillin G, it would be expected from the experimental findings detailed above that its therapeutic effects would not be blocked by SKF 525–A. As shown by the results presented in Table III, neither penicillin X nor any

Table III. Effect of SKF 525–A on the antistreptococcal activity of a variety of penicillins

Treatment	Deaths in 20 mice	
	Exp. 1	Exp. 2
None	20 (1·0)	20 (1·0)
SKF 525–A 1 mg i.p. b.i.d.	20 (1·0)	20 (1·2)
Penicillin G 0·05 mg s.c. b.i.d.	0	1 (7·0)
Penicillin G+SKF 525–A	16 (3·9)	16 (3·9)
Penicillin X 0·025 mg s.c. b.i.d.	1 (6·0)	1 (7·0)
Penicillin X+SKF 525–A	6 (4·8)	13 (3·6)
Penicillin K 0·25 mg s.c. b.i.d.	—	2 (4·0)
Penicillin K 0·1 mg s.c. b.i.d.	15 (3·9)	—
Penicillin K 0·25 mg+SKF 525–A	—	11 (4·4)
Penicillin K 0·1 mg+SKF 525–A	17 (3·2)	—
Penicillin D 0·04 mg s.c. b.i.d.	0	—
Penicillin D+SKF 525–A	14 (4·5)	—
Penicillin V 0·05 mg s.c. b.i.d.	0	0
Penicillin V+SKF 525–A	16 (3·4)	15 (3·5)
Penicillin F 0·05 mg s.c. b.i.d.	—	1 (4·0)
Penicillin F+SKF 525–A	—	12 (4·0)

Experimental procedure as in Table I.

The chemical constitution of the above penicillins and the potency in u/mg (assayed against *Staph. aureus*—Oxford 6571) of the samples tested are as follows:



G; R=benzyl (1660), X; R=*p*-hydroxybenzyl (700), K; R=*n*-heptyl (2661), D; R=*n*-amyl (1713), V; R=phenoxymethyl (1580) and F; R=Δ^2-pentenyl (1784).

of the other penicillins available to us escaped the dystherapeutic effect of SKF 525–A. Since the therapeutic activity of all the penicillins tested was affected by SKF 525–A, it appeared that the variable side-chain R— was unlikely to be the *sole* point of SKF 525–A-blocked metabolism, if this were the explanation of the phenomena observed.

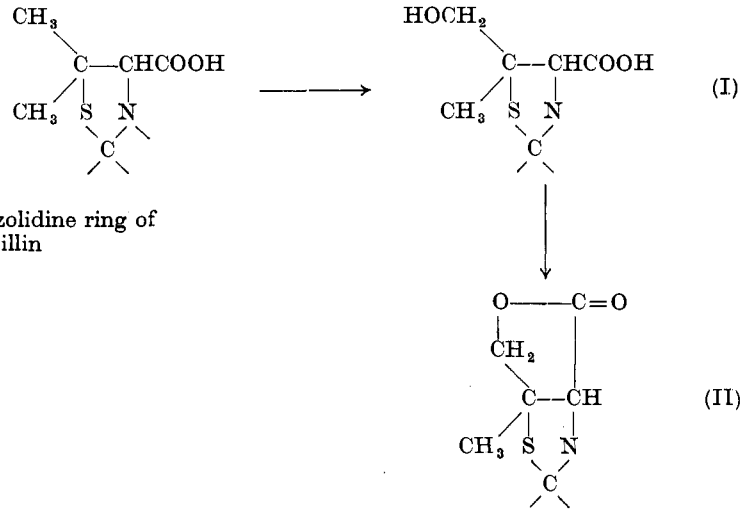
Other sites of the penicillin molecule might of course be subject to metabolic attack. Oxidation of the sulphur atom of the thiazolidine ring gives rise to the sulphone or sulphoxide, but once again the antistreptococcal activity of methyl penicillinate sulphone and methyl penicillinate sulphoxide was blocked by SKF 525–A.

Conjugation with the carboxylic acid group of penicillin has been suggested as a stage in the renal tubular excretion of penicillin, involving conjugation with glycine. We have found, however, that no detectable glycine conjugate of penicillin occurs in the urine of penicillin-dosed animals (Thorp and Pickstock, unpublished). Nor has it been shown that penicillin occurs in the urine in a form other than the free acid, or that SKF 525–A blocks conjugation. Thus this would seem an unlikely metabolic explanation.

Oxidation of a methyl group. We have shown (Francis *et al.*)[7] that 2,3-dimethylquinoxaline-di-*N*-oxide is rapidly and extensively metabolized to the corresponding 2-hydroxymethyl derivative. Such oxidation of penicillin would give rise to (I), from which the lactone (II) could be formed. Bachhawat *et al.*[8] have recently demonstrated the enzymatic carboxylation of β-hydroxyisovaleric acid (III) to β-hydroxy-β-methyl glutaric acid (IV). A similar carboxylation of penicillin would give rise to the dicarboxylic acid (V).

Since none of the metabolites postulated were available for direct test, evidence for their occurrence was sought by other means. Paper chromatographic analysis, by our colleague Dr. R. R. Goodall, of the urine of mice treated with penicillin G disclosed the presence of a bacteriostatic substance, not present in the original penicillin or in normal urine; this took up an intermediate position between penicillin G and penicillin X in the system of Goodall and Levi.[9] The amount of the metabolite was decreased in mice given SKF 525–A, and its excretion was blocked similarly to that of penicillin by a renal blocking agent related to

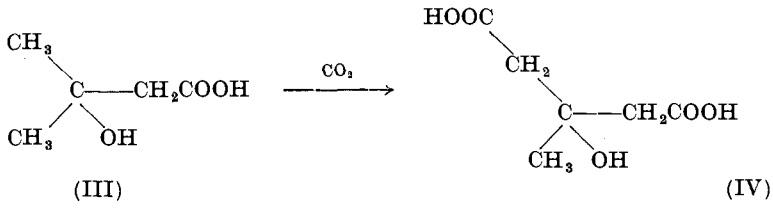
probenecid. Penicillin V gave two derivatives, the appearances of which were completely abolished by simultaneous administration



Thiazolidine ring of penicillin

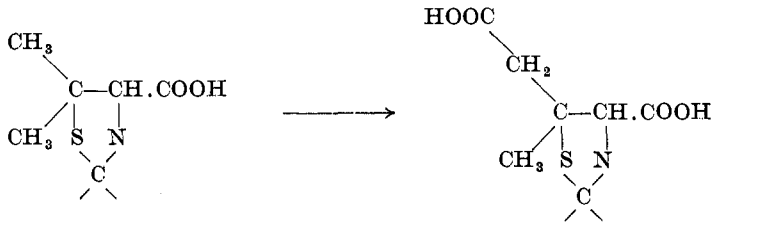
(I)

(II)



(III)

(IV)



(V)

of SKF 525–A. However, no clear evidence has yet been obtained that these substances are concerned in the abnormal response to penicillin of mice dosed with SKF 525–A.

Insoluble salt formation. The salt formed between SKF 525–A and penicillin G is only sparingly soluble. To exclude the unlikely possibility that the latter might be inactivated *in vivo* due to this, we administered penicillin G with other substances capable of forming sparingly soluble salts, namely procaine hydrochloride and dibenzyl-ethylenediamine diacetate. In a single experiment using groups of 10 mice, penicillin G and penicillin G combined with SKF 525–A gave results almost identical with those in Table II; neither of the other bases diminished the effect of penicillin G and if anything they tended to enhance it.

The Action of SKF 525–A on bacteria in vitro. Meanwhile collateral biological work suggested that SKF 525–A possesses pharmacological properties other than those currently attributed to it. We therefore considered whether it might act by antagonising the antibacterial action of penicillin within the bacterial cell or by reducing the permeability of the bacterium to the antibiotic. *In vitro* bacteriostatic tests against the same streptococcus demonstrated no antagonism; mixtures of penicillin G and SKF 525–A in the proportions of 1 : 50, 1 : 20 and 1 : 5 showed the same end-points as did penicillin itself. In the initial experiments the two substances were presented simultaneously to the bacteria, which had not yet entered the log-phase of growth during which the bactericidal action of penicillin is exerted. To exclude the possibility that different rates of penetration of the substances may have favoured penicillin, and that SKF 525–A already present in the bacteria might antagonise the action or might block the entry of antibiotic arriving later, in subsequent experiments the SKF 525–A was added at the time of subculture and the addition of the antibiotic deferred until various stages during the phase of rapid growth. These experiments equally demonstrated no *in vitro* inhibition of the action of penicillin. Thus, SKF added immediately before incubation of the culture in a concentration 20 times that of penicillin ($1 : 10^6$–$1 : 128 \times 10^6$) did not influence the action of penicillin added after 0, 1, 2 or 4 h of incubation.

Similar mixtures of penicillin and SKF-acid (which, as stated above, inhibits *in vitro* but not *in vivo* the same enzyme systems as SKF 525–A, and to which the latter would be expected to be converted in the body) were *more* bacteriostatic than penicillin G

alone by a factor of about 25. By itself the acid was bacteriostatic only at a concentration of 1 : 250, while SKF 525–A alone inhibited growth to about 1 : 12,000.

Action of SKF 525–A on the host. Finally, there remained the possible effect of SKF 525–A on the response of the host to infection.

The experiments to date had shown that SKF 525–A itself has no beneficial influence on streptococcal infections in mice. Working, however, with doses of the organism which kill virtually all control mice within 24 h, it is difficult, with certainty, to exclude a noxious effect on the host; such an effect could conceivably explain the results with penicillin G. Accordingly we studied the effect of twice-daily dosing in mice infected with successive dilutions of a culture, from one of 10^{-4} to one of 10^{-10}. Two experiments on groups of 20 mice gave dissimilar results. In the first it seemed that SKF 525–A had slightly worsened the infection, in that mortality among animals receiving the 10^{-8}, 10^{-9} and 10^{-10} dilutions was higher in the treated groups. In the second experiment there was no such difference, except that with one dilution of the culture (10^{-8}) the mean period of survival of treated mice was about half-a-day shorter than that of the controls (1·5 and 2·2 days respectively). Two further experiments yielded similarly equivocal results. We may, however, conclude that if SKF 525–A exercises a deleterious effect upon the infection, the effect is only slight; considering also the results of combining SKF 525–A with other therapeutic agents, it seems certain that this effect is insufficient to account for the marked reduction in the therapeutic action of penicillin.

As a further means of detecting a deleterious effect on the host, we examined the effect of pre-treatment with SKF 525–A on the therapeutic effect of both penicillin G and chlortetracycline. The results of one of three experiments appear in Table IV; the others showed trends in the same direction. Pre-treatment with SKF 525–A may have had a slightly favourable effect on the outcome of infection, as shown by the rather longer mean period of survival; this effect was constant in all three experiments. Pre-treatment definitely increased the effect of penicillin G in two experiments—penicillin was not included in the third—while as before combined treatment with SKF 525–A diminished the

Table IV. Effect of time of treatment with SKF 525–A on the activity of penicillin and chlortetracycline

Treatment	Deaths in 20 mice
None	20 (1·1)
SKF 525–A *before* infection	19 (1·8)
SKF 525–A *after* infection	20 (1·0)
Penicillin G 0·1 mg s.c. b.i.d.	6 (4·7)
Penicillin G 0·1 mg+SKF 525–A *before* infection	0
Penicillin G 0·1 mg+SKF 525–A *after* infection	14 (3·4)
Chlortetracycline 0·4 mg orally b.i.d.	17 (4·5)
Chlortetracycline 0·4 mg+SKF 525–A *before* infection	19 (4·0)
Chlortetracycline 0·4 mg+SKF 525–A *after* infection	10 (4·3)

Treatment with SKF 525–A was (a) 1 mg intraperitoneally twice daily for 3 days ending 18 h *before* infection, or (b) a similar dose twice daily for 3 days beginning 15 min *after* infection. Other treatments began after infection.

effect of penicillin G. With chlortetracycline, on the other hand, pre-treatment acted unfavourably; in the two other experiments the effect was rather more marked than in the one tabulated, while, as previously, combined treatment enhanced the effect of chlortetracycline.

The findings that pre-treatment with SKF 525–A reverses the effects observed when it is given simultaneously with penicillin and chlortetracycline, and that the effects with penicillin and with chlortetracycline are in opposite directions while SKF 525–A has no effect on sulphonamide therapy, all combine to render unlikely a non-specific influence on the peritoneal cavity into which SKF 525–A and streptococci were both injected within a short period of time. Nevertheless, the reversal of its effect on the therapeutic action of the antibiotics according to whether it preceded or accompanied these suggests a mechanism which primarily is concerned with the host rather than with the infecting organism. The phenomenon calls to mind adaptive and 'rebound' responses which are often associated with endocrine imbalance and which may be accompanied by marked changes in levels of enzyme-activity.

Such a reversal of initial effect occurs with SKF 525–A and the reticulo–endothelial system. As will be reported in detail

elsewhere (Thorp, unpublished), SKF 525–A given orally or intraperitoneally to mice causes an initial increase in reticulo-endothelial function as measured by the rate of clearance of intravenously administered carbon by the method of Biozzi, Benacerraf and Halpern.[10] The increase is followed by a marked and sustained depression of phagocytic activity, occurring in spite of an increase in the relative weight of the liver, the main site of clearance. This finding suggests another factor influencing the effect of SKF 525–A on the therapeutic activities of chlortetracycline and penicillin, activities which, as we have seen, differ not only between themselves but also according to whether the SKF 525–A accompanies or precedes treatment with the antibiotic. Since the action of penicillin is exerted in the extracellular and intravascular environment in which it occurs predominantly, the initial phase of enhanced phagocytic activity due to SKF 525–A would tend to remove bacteria from the environment of the penicillin and thus act dystherapeutically; prolonged or preliminary treatment with the compound would have the opposite effect. Conversely, with a compound such as chlortetracycline which is said to be concentrated in the liver and excreted in the bile, reduced reticulo–endothelial function in this site might possibly assist in separating the antibiotic from the bacteria.

However this may be, the data established serve to emphasise the multiple actions of SKF 525–A and to suggest complex inter-relationships where its effect on the therapeutic activity of other substances is concerned. It should not be assumed immediately that all of these effects can be explained in terms of the undoubted ability of the compound to block certain enzyme-systems, and probably in instances like the present much further work is needed to explain fully the experimental observations.

Chloramphenicol and streptomycin therapy

SKF 525–A enhanced the therapeutic activity of chloramphenicol, as might be expected from Fouts and Brodie's demonstration[11] that the compound inhibits enzymatic reduction of the antibiotic. With streptomycin the therapeutic effect was diminished by SKF 525–A (Table V).

Table V. Effect of SKF 525–A on the activity of chloramphenicol and of streptomycin in streptococcal infection in the mouse

Treatment	Deaths in 12 mice
None	12 (1·2)
SKF 525–A 1 mg i.p. b.i.d.	12 (1·0)
Chloramphenicol 5 mg orally b.i.d.	2 (4·5)
Chloramphenicol 1 mg orally b.i.d.	11 (4·2)
Chloramphenicol 5 mg+SKF 525–A	3 (4·5)
Chloramphenicol 1 mg+SKF 525–A	3 (3·8)
Streptomycin 2 mg s.c. b.i.d.	0
Streptomycin 1 mg s.c. b.i.d.	0
Streptomycin 2 mg+SKF 525–A	1 (8·0)
Streptomycin 1 mg+SKF 525–A	4 (4·5)

Experimental procedure as in Table I.

2. Other Bacterial Infections in the Mouse and Rabbit

The foregoing experiments concerned one infective agent and one host. To confirm the general nature of the phenomena observed with penicillin, we studied three other bacterial infections in the mouse and one of these in the rabbit. SKF 525–A greatly diminished the effect of suboptimal doses of penicillin G on infections in mice evoked by a haemolytic streptococcus and a staphylococcus (*Staphylococcus pyogenes* 'Smith' MP 224), and in both mice and rabbits by a Type I pneumococcus (Pneumococcus 7465 of the National Collection of Type Cultures). All these organisms were sufficiently virulent to kill every control in average periods of 1·0 to 1·8 days after intraperitoneal injection, and all could be countered by suitable doses of penicillin G when this was unaccompanied by SKF 525–A.

3. Equine Encephalomyelitis in the Mouse

It seemed desirable also to move outside the field of antibacterial therapy, and to this end we determined the effect of SKF 525–A on several other infections—one of these viral, the others protozoal.

We have described elsewhere the marked prophylactic action

of mepacrine against equine encephalomyelitis in the mouse (Hurst, Melvin and Peters,[12] and Hurst, Snow and Roberts[13]). At near-minimally effective levels of dosage, the simultaneous exhibition of SKF 525–A largely abolished the prophylactic effect. Table VI presents the results of one experiment; a repeat

Table VI. Effect of SKF 525–A on the activity of mepacrine against Eastern equine encephalomyelitis of the mouse

Treatment	Deaths in 30 mice
None	27 (5·7)
SKF 525–A 1 mg i.p. b.i.d.	25 (5·3)
Mepacrine 4 mg orally once	5 (9·2)
Mepacrine 4 mg+SKF 525–A	19 (7·6)

We gave mepacrine 24 h before virus. Dosing with SKF 525–A began 3 h before virus and continued twice daily for 3 days.

gave nearly identical results. The mice received a single dose of mepacrine 24 h before intramuscular infection with virus, and twice daily doses of SKF 525–A beginning 3 h before virus and continuing for 5 days.

Malarial Infections in the Mouse and the Chicken

The adverse influence of SKF 525–A on the treatment with mepacrine of equine encephalomyelitis in mice suggested enquiry as to its action on antimalarial therapy with the drug. Dr. D. G. Davey kindly undertook the necessary experiments and also included proguanil in the investigation. The results with *P. berghei* in mice appear in Table VII, and a second smaller experiment yielded confirmatory results. The SKF 525–A was given intraperitoneally twice daily in the unit doses stated, beginning immediately after infection of the mice. Mepacrine and proguanil were given subcutaneously once daily, beginning about 6 h after infection. Treatments continued on each of four successive days. Dr. Davey concluded that SKF 525–A possesses slight but definite antimalarial activity in its own right. It enhances considerably the antimalarial action of mepacrine. For example, in doses of 0·05 mg, mepacrine by itself is devoid of activity;

Table VII. Effect of SKF 525–A on the antimalarial activity of mepacrine and of proguanil in mice

(*a*) Activity of drugs given singly

Drug	SKF 525–A			Mepacrine				Proguanil	
Unit dose (mg/20 g)	0·25(*a*)	0·5(*b*)	1·0(*c*)	0·025(*d*)	0·05(*e*)	0·075(*f*)	0·1(*g*)	0·1(*h*)	0·25(*i*)
Result	VSL	VSL	SL	NA	NA	VSL	SL	NA	SL

(*b*) Activity of drugs given together

Mixture	*a*+*d*	*a*+*e*	*b*+*d*	*b*+*e*	*b*+*f*	*b*+*g*	*c*+*e*	*c*+*f*	*c*+*g*	*b*+*i*	*c*+*h*	*c*+*i*
Result	VSL	A	VSL	A	A	A	A	A	A	SL	A	A

The infection was with *P. berghei*. Subcutaneous dosing with mepacrine and proguanil began 6 h after infection and continued daily for 4 doses. SKF 525–A was given twice daily intraperitoneally beginning immediately after infection.

NA: no action, parasites 10–20 per field
VSL: very slight action, parasites slightly reduced in most animals
SL: slight action, parasites obviously reduced but found easily, e.g. one per field or one in two fields
A: marked action, parasites found only by searching

combined with SKF 525–A it possesses marked antimalarial action. SKF 525–A also enhances the effect of proguanil, though to a lesser degree. In young chickens infected with *P. gallinaceum* the effect of SKF 525–A is less marked with both mepacrine and proguanil.

Comment

SKF 525–A has been described as prolonging the period of activity of a variety of drugs by impeding their metabolic degradation within the body. On some other drugs it has no action. In this report we describe a third contingency, namely reduction of therapeutic activity through administration of the compound.

In conjunction with a number of well-known antibacterial, antiviral or antiprotozoal drugs SKF 525–A appeared, (i) to have no influence on the therapy of a streptococcal infection of mice with sulphanilamide or sulphadiazine, (ii) to enhance the effect of chlortetracycline, and possibly of suboptimal doses of chloramphenicol, on a streptococcal infection of mice, (iii) to enhance the effects of mepacrine and of proguanil in malarial infections of mice and chickens; it also appeared to possess some antimalarial activity of its own right, (iv) to reduce the therapeutic effect of streptomycin on a streptococcal infection of mice, and of penicillin on two streptococcal infections of mice, a staphylococcal infection of mice, and a pneumococcal infection of mice and rabbits, and (v) to reduce the prophylactic action of mepacrine against equine encephalomyelitis in mice.

Administered prior to therapy with penicillin or chlortetracycline, SKF 525–A produced an opposite effect to that resulting from simultaneous exhibition with the antibiotic.

In standard bacteriostatic tests SKF 525–A did not diminish the effect of penicillin, and SKF-acid tended to enhance it. SKF 525–A itself possessed some inhibitory properties against bacterial growth.

The *in vivo* effect of SKF 525–A against penicillin did not appear to result from direct inactivation of the antibiotic through formation of an insoluble salt.

Although SKF 525–A affects the metabolism of penicillin, no certain evidence has yet accrued that this fact explains its action on penicillin therapy.

SKF 525–A first stimulates and then depresses reticulo–endothelial function; it seems that this observation may well explain, at least in part, its 'biphasic' effects on therapy with chlortetracycline and with penicillin.

Summary. SKF 525–A modifies the therapeutic effect of an antibiotic (chlortetracycline, penicillin G, X, K, D, V, and F, or streptomycin) in a bacterial infection (streptococcal, staphylococcal, or pneumococcal) in the mouse or rabbit. The therapeutic effect of penicillin is increased or decreased by SKF 525–A, in the opposite direction to that of chlortetracycline, depending upon the timing of administration of the SKF 525–A relative to the antibiotic. These results are interpreted in relation to the alterations in metabolism and reticulo–endothelial function caused by SKF 525–A, and to its *in vitro* activity.

SKF 525–A has no influence on sulphonamide therapy of a streptococcal infection in mice. Whilst SKF 525–A reduces the antiviral activity of mepacrine, it enhances the antimalarial action of this drug, as also that of proguanil.

(*Received 13 October, 1959*)

References

[1] Axelrod, J., Reichenthal, J. and Brodie, B. B. *J. Pharmacol.* **112,** 49 (1954)
[2] Cooper, J. R., Axelrod, J. and Brodie, B. B. *J. Pharmacol.* **112,** 55 (1954)
[3] Brodie, B. B. *J. Pharm. Lond.* **8,** 1 (1956)
[4] Ullberg, S. *Acta Radiol. Stockh.*, Suppl. 118 (1954)
[5] Eagle, H. *J. exp. Med.* **85,** 175 (1947)
[6] Hobby, G. L., Lenert, T. F. and Hyman, B. *J. Bact.* **54,** 305 (1947)
[7] Francis, J., Landquist, J. K., Levi, A. A., Silk, J. A. and Thorp, J. M. *Biochem. J.* **63,** 455 (1956)
[8] Bachhawat, B. K., Robinson, W. G. and Coon, M. J. *J. biol. Chem.* **219,** 539 (1956)
[9] Goodall, R. R. and Levi, A. A. *Analyst* **72,** 277 (1947)
[10] Biozzi, G., Benacerraf, B. and Halpern, B. N. *Brit. J. exp. Path.* **34,** 441 (1953)
[11] Fouts, J. R., and Brodie, B. B. *J. Pharmacol.* **116,** 20 (1956)
[12] Hurst, E. W., Melvin, P. and Peters, J. M. *Brit. J. Pharmacol.* **7,** 455 (1952)
[13] Hurst, E. W., Snow, G. A. and Roberts, D. C. *Brit. J. exp. Path.* **36,** 215 (1955)