REVIEW ARTICLE

ANTIFOLIC ACIDS AND ANTIPURINES IN CHEMOTHERAPY

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In this review an attempt will be made to illustrate the uses and potential uses of antifolic acids and antipurines in chemotherapy. These two types of agent can probably be best defined, in brief, by referring to their ability to inhibit the growth of various organisms; this inhibition can be prevented or reversed by the addition respectively of folic acid (I) or one of the natural purines, usually adenine (II; $R' = NH_2$, $R'' = H$) or guanine (II; $R' = OH$, $R'' = NH_2$). Antagonism experiments in small animals have proved difficult to assess accurately, and the most commonly employed organisms are bacteria, e.g., *Lactobacillus casei*, *Streptococcus faecalis* and *Escherichia coli*. By their use we can ascertain whether the ratio of the potential antagonist to the amount of folic acid, or the purine, required to relieve inhibition remains roughly the same whatever the actual concentrations of the antagonistic substances. In this instance a true competitive antagonism would exist and, when present, constitutes usually the best available evidence for a true folic acid or purine antagonist, since when the antagonism is irreversible or not competitive, we do not know whether the metabolite is essentially involved or not, although the bacteria concerned may normally require folic acid or a purine for growth. It must be emphasised that these simple and ideal conditions seldom, if ever, apply wholly in practice, and they are here used mainly as an illustration of the principle of competitive antagonism and the logical criterion for its presence. For an example of the detailed treatment of this subject the reader may be referred to reference 1. In this review, except where stated, only reversible antagonisms will be considered.

Folic Acid Antagonists

Antifolic acids and antipurines are logically related, since recent work on the biosynthesis of nucleic acids[2–4] reveals the essential role of folinic acid (Citrovorum factor) in the synthesis of their purine constituents. This derivative of folic acid is necessary for the synthesis of glycinamide ribotide, and at a later stage the ring closure of 5-amino-4-iminazole-carbonamide ribotide. Folinic acid (5-formyl-5 : 6 : 7 : 8-tetrahydropteroyl-glutamic acid, III) is formed from folic acid, and it has been shown that, for example, the two powerful antifolic acids, aminopterin (IV; $R'=NH_2$, $R'' = H$) and amethopterin (Methotrexate IV; $R' = NH_2$, $R'' = Me$) inhibit growth by preventing the conversion of folic to folinic acid in various organisms. In these cases also there is probably a direct interference with folinic acid[5–9].

The first chemotherapeutic developments in this field were the successful use of aminopterin and Methotrexate in inducing in some proportion,

remissions in children with acute leukaemia[10]. Rather less useful are the folic acid analogues which contain a hydroxy group in the 4-position, e.g. (IV; R′ = OH, R″ = Me) and they have now fallen out of general clinical use. In general, the clinical effect of these antifolic acids is reflected in experiments with leukaemia in mice[11–15]. The fact that the clinical use of antifolic acids does sooner or later induce a drug resistance is a serious disadvantage. This effect has been alleviated in experimental leukaemias by the combination of two drugs which do not show cross-resistance, e.g., by the use of 6-mercaptopurine (*vide infra*) with Methotrexate[16]. Similar clinical attempts have so far been promising.

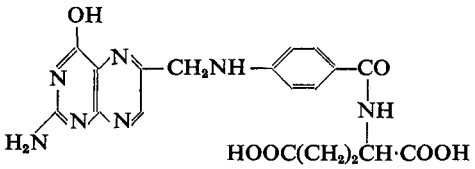
The field of the antifolic acids may still, nevertheless, be worth cultivating further, since Farber[17] has found that drugs of the Methotrexate type have produced impressive improvement in rhabdomyosarcoma, Hodgkin's disease, lymphosarcoma, neuroblastoma and chronic lymphoid leukaemia. Also, Colsky[18] reported improvement in carcinoma of the breast treated with Methotrexate.

Amongst simpler types of folic acid analogues which have yielded, at the least, significant antifolic activity, are the 6:7-substituted diaminopteridines (V). In this type R′ and R″ have been phenyl or substituted phenyl[19,20], R′ phenyl or substituted phenyl and R″ amino[21] or R′ and R″ have both been alkyl or aralkyl, or R′ aryl and R″ alkyl[22–24]. Antifolic acid activity has also been found in more complex types, for example, the naphthopteridine (VI)[25,26] and the indolopteridines (VII; R=alkyl)[22–24]. None of these types has yet yielded compounds of value in leukaemia, but high antimalarial activity has been found in some of the 2:4-diamino-6:7-dialkylpteridines and 2:4-diamino-6-aryl-7-alkylpteridines; e.g., V (R′ = anisyl, R″ = *iso*propyl) which equals proguanil (Paludrine) in activity. But for the emergence of the pyrimidine derivative pyrimethamine (Daraprim, *vide infra*), a drug of wide usefulness clinically would probably have arisen from this series.

Another potentially interesting type of activity was found both in the 6:7-dialkyl-2:4-diaminopteridines and in the indolopteridines, (e.g., VII; R = Me) where substances with high activity *in vitro* against *Vibrio cholerae* were found.

High antifolic and antimalarial activity has also been found in structures simpler than pteridines, viz., the 2:4-diamino-5-chlorophenyl pyrimidines[27] (e.g., VIII), and the 4:6-diamino-1-chlorophenyl-1:2-dihydrotriazines (e.g., IX)[28–32]. 2:4-Diamino-5-3′:4′-dichlorophenyl-6-methylpyrimidine (VIII; R = Me) resembles aminopterin and Methotrexate in its antifolic acid properties, but it has had only slight success in the treatment of acute leukaemia in children[33] and seems to be inferior to Methotrexate owing to its toxicity. Slight inhibition of growth of the mouse sarcoma 180 has been caused by this type of compound where the methyl group is replaced by hydrogen, ethyl or *n*-amyl groups[34]. Of much greater clinical interest in this group is pyrimethamine[35] (Daraprim, VIII; R = C_2H_5), a widely used antimalarial drug.

The series of dihydrotriazines (e.g., IX), studied by Modest and Foley and their collaborators, are non-competitive inhibitors of folic acid and



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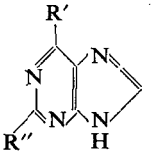

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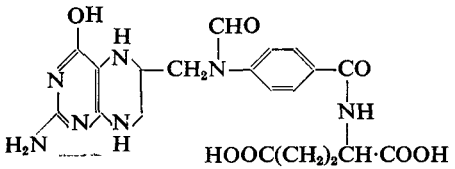

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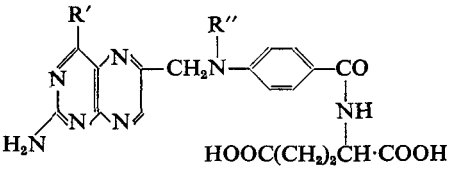

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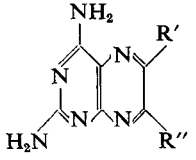

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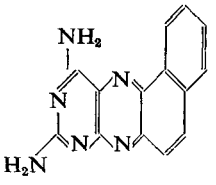

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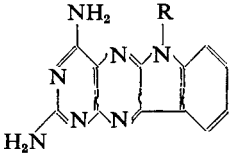

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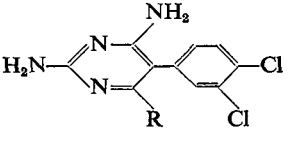

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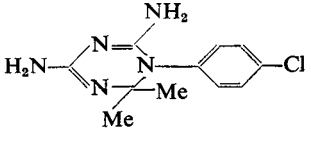

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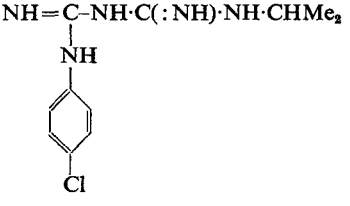

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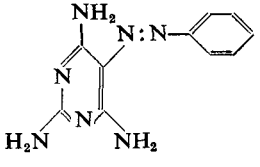

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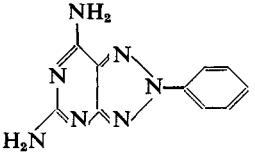


(XII)

folinic acid. Although active against experimental leukaemias, clinical experiments with these compounds have been found unpromising possibly because an adequate dose cannot be tolerated[36]. The series has provided many compounds active against experimental malaria[37,38] and experimental tumours[39], but although compound IX is the active metabolite[40] of the widely used antimalarial proguanil (X), and has been found to be more active in experimental animals, its clinical use is at least uncommon, and the same applies to the other members of the series.

Recently discovered antifolic acids are the amino-5-arylazopyrimidines (XI) and the amino-8-aryl-8-azapurines (XII)[1,41]. In neither series is activity high. Of the arylazopyrimidines the 2′:4′-dibromophenyl- and the β-naphthyl-analogues were the most active, having about 1/300th the activity of Methotrexate. The most active of the 8-azapurines was the 2′:4′-dibromophenyl-analogue which had 1/60th of the activity of Methotrexate, tested with *Str. faecalis*. However, antifolic acid activity associated with the 8-azapurine structure is apparently a new observation. The possibility that folic acid fulfils an essential role in the multiplication of viruses has not been neglected. Psittacosis virus (6BC) in chick embryonic tissue was inhibited by aminopterin, Methotrexate and amino-an-fol, which is an analogue of aminopterin (IV; R′ = NH_2, R″ = H) differing only by the substitution of an aspartic acid for the glutamic acid moiety. Since the inhibition caused by the last two compounds was annulled by Citrovorum factor, there is evidence that the effect arises from a direct interference with the folic acid metabolism of the virus[41a].

Antipurines

In the field of antipurines the number of compounds and types of structure discovered with appreciable activity has been much smaller than amongst the antifolic acids. The first example to show any marked activity as an antagonist of adenine in *L. casei* was 2:6-diaminopurine (XIII; R′ = R″ = NH_2)[41b]. The clinical trials[42] in leukaemia were disappointing, although a significant effect had been observed on a transplanted mouse leukaemia.[43]

At about this time the antiguanine activity of 8-azaguanine (XIV; R′ = OH, R″ = NH_2) was discovered with *Tetrahymena* as the organism,[44] but clinically its effect has been disappointing in trials spread over some six years, and its attempted use seems to have been abandoned.

Of the simple purines, 6-mercaptopurine[45] (XIII; R′ = SH, R″ = H) is by far the most interesting since it is the drug of choice in the treatment of acute leukaemia in adults[46]. Also it is effective in children and in those instances in which resistance has been induced to the antifolic acids and to cortisone. It is also of some value in chronic myeloid leukaemia. 6-Chloropurine (XIII; R′ = Cl, R″ = H) has been tried clinically in chronic and acute leukaemia without marked success[47]. Similarly, trials of 2-amino-6-mercaptopurine (XIII; R′=SH, R″=NH_2)[47] and purine itself (XIII, R′ = R″ = H), which is an adenine antagonist, have revealed, at most, no advantage over 6-mercaptopurine[48].

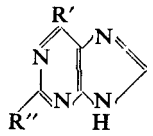
The most complex antipurine of any so far produced is puromycin, 6-dimethylamino-9-(3′-*p*-methoxy-L-phenylalanyl-amino-3′-deoxy-D-ribosyl)purine (XV). This evinces an antiguanine action in *E. coli*[55] and it has been shown that its trypanocidal action in *T. equiperdum* was due to interference with the purine metabolism of the parasite[49]. This trypanocidal action was reversed by adenine and other purines[50]. After showing activity against experimental tumours and leukaemias, all clinical trials in various malignant diseases were disappointing; but effectiveness as an amoebicide was uncovered first in guinea pigs[52] and then in man[53]. When the structure of puromycin was simplified by removal of the *p*-methoxyphenylalanyl residue (at the dotted line shown in XV) the amine obtained, 6-dimethylamino-9-(3′-amino-3′-deoxy-D-ribosyl) purine was similar to puromycin in its activity against experimental tumours and trypanosomiasis. Like puromycin, this compound has not so far shown promise in clinical trials with malignant disease.

New types of purines and azapurines have recently been synthesised as potential antipurines[54] on the hypothesis that cyclic substituents in the 9 position might be of value since they bear a spacial relation to the furanose ring (cf. XVI) which appears, in the form of the phosphate ester, as a stage in the normal biosynthesis of the hypoxanthine and presumably other purine moieties in ribonucleic acid, and possibly as the deoxy form in deoxyribonucleic acid.

The formulae XVII to XIX illustrate four active compounds, all of them showing essentially an antiguanine type of activity when tested with *E. coli*[55]. The azapurine (XVII) is about three times as active in this respect as is 6-mercaptopurine. The other compounds are less active; XVIII (R = Me or H) also show a less competitive type of action than does XVII, and XIX has a less competitive kind of inhibition than XVIII.

Of purine analogues, a little more removed from purine in structure, a series of thiazolopyrimidines (3:4:6-triaza-1-thiaindenes) (e.g., XX) and a series of pyrazolopyrimidines (1:2:5:7-tetrazaindenes) (XXI) have yielded active compounds. In the 1:2:5:7-tetrazaindene series[57] activity against those experimental tumours usually susceptible to the action of antipurines has been found[58]. In *E. coli*, XXI (R = Me) is about three times weaker than 6-mercaptopurine as an antiguanine, and had very little adenine activity[59]. Very recently, partially competitive antiguanine activity has been found in the 8-thiapurine derivative XXII[60]. Also, in the 2-azapurine series, 2-azaadenine (XXIII; R = NH_2) and 2-azahypoxanthine (XXIII; R = OH) are antipurines[62].

Benziminazole (XXIV) and its derivatives and benzthiazole (XXV) and benztriazole (XXVI) are the antipurines furthest removed from a structural analogy with purine. Benziminazole was first found to have anti-adenine activity in various organisms[63], and an antiguanine activity in *E. coli* was found later[55]. Benzthiazole and benztriazole were similarly found to be weak antiguanines[55]. Comparison of benztriazole with 8-azapurine (XXVII)[1] showed that the latter had a similar weak, mainly antiguanine, type of activity. Thus, in this instance, substitution of the



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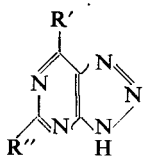

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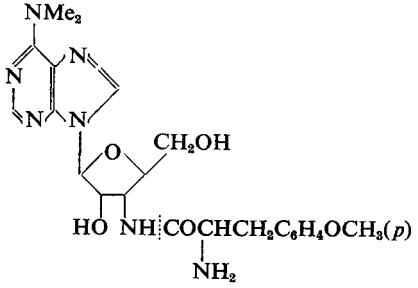

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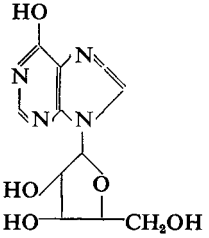

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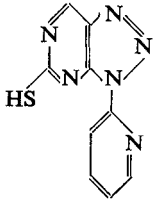

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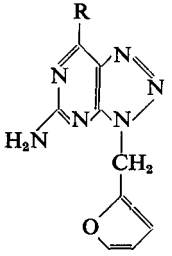

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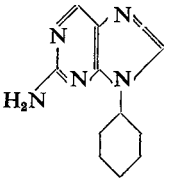

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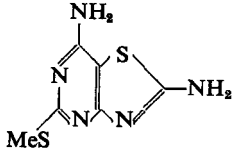

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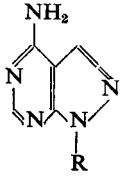

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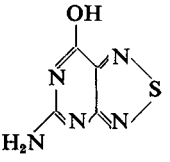

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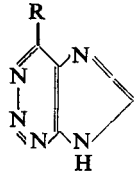

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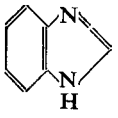

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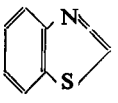

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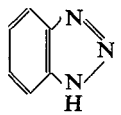
benzene ring by the pyrimidine ring, which is chemically very different and resembles much more a 2:4-dinitrobenzene ring, made little difference.

Antipurines and Viruses

It is convenient at this point to consider the effect of antipurines on viruses. 8-Azaguanine (XIV; R′ = OH, R″ = NH_2) has been extensively investigated and causes a marked inhibition of systemic development of lucerne mosaic virus in *Nicotiana glutinosa*, and tobacco and cucumber mosaic virus in cucumbers and *Nicotiana sp.*[64]. 8-Azaadenine (XIV; R′ = NH_2, R″ = H) has also some inhibitory effect on lucerne mosaic, cucumber mosaic and tobacco mosaic viruses. The virus inhibition by 8-azaguanine can be reversed by spraying the plants with adenine, guanine or hypoxanthine indicating an antipurine mechanism for the inhibition. Furthermore, 8-azaguanine is incorporated into the virus nucleic acid[65] and the infectivity of the virus was markedly reduced[65].

Vaccinia virus in tissue culture was also inhibited by 2:6-dichloro-8-hydroxypurine (XXVIII; R = R′ = Cl, R″ = H, R‴ = OH), 2:6:8-trichloropurine (XXVIII; R = R′ = R‴ = Cl, R″ = H) and 2:6-dichloro-7-methylpurine (XXVIII; R = R′ = Cl, R″ = Me, R‴ = H), but the inhibition was not reversed by normal purines[66].

2:6-Diaminopurine (XXVIII; R = R′ = NH_2, R″ = R‴ = H) was more active and its action was reversed by adenine and hypoxanthine[66]. Benziminazole[67–70] and derivatives of benziminazole have, however, led to more interesting results than purine derivatives or close analogues, and a detailed and systematic exploration of structure-activity relations in this field has been carried out[71,72]. By the attachment of a ribose moiety to the most active of the methyl and chlorine substituted benziminazoles, even more active compounds were obtained of which 4:5:6-trichloro-1-β-D-ribofuranosylbenziminazole (XXIX) was the best, being 760 times more active than benziminazole in the inhibition of Lee



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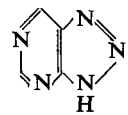

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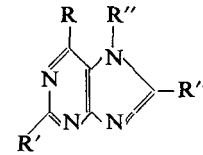

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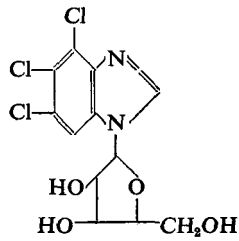

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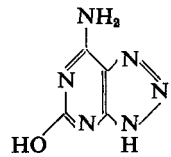

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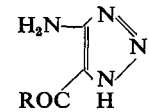
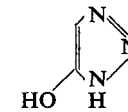


(XXXI)



(XXXII)

influenza virus in chorio-allantoic membrane tissue culture[73,74]. Although no unequivocal evidence was obtained that the mechanism of action involved an antipurine or antinucleoside effect, it was thought, after elimination of other possible mechanisms, that the action is intracellular and involves disturbance of the metabolism of ribosides. It must be added that no purine analogue or benziminazole derivative has so far been successfully used clinically in a virus disease.

Mechanism of Action of Antipurines

A great deal of work has been directed towards elucidating the essential mechanism of action by which the antipurines inhibit growth. This may be conveniently divided into the conception of the antipurine being incorporated into the nucleic acid molecule, thus producing dysfunction, and the concept that the agent interferes initially with some enzyme or co-enzyme concerned in some way with growth or interferes with the synthesis of nucleic acid from preformed purines. The evidence suggests that any of these mechanisms can operate according to the particular antipurine and also to the organism involved.

8-Azaguanine, and also 8-azaxanthine, are incorporated principally into the ribonucleic acid (RNA) of *B. cereus*, the azaxanthine being converted into azaguanine[75]. Incorporation into the deoxynucleic acid (DNA) was only slight. In both instances marked inhibition of growth occurred. Furthermore, comparison of the effects of 8-azaguanine, 8-azaadenine, 8-azahypoxanthine, 8-aza*iso*guanine (XXX), 4-amino-triazole-5-carbonamide (XXXI; R = NH_2), 4-aminotriazole-5-carboxylic acid (XXXI; R = OH) and 5-hydroxytriazole (XXXII) on tobacco mosaic virus and *E. coli* showed that where growth inhibition occurred, so did incorporation into RNA and where there was no inhibition RNA was not incorporated[76]. Since the evidence is strong that RNA plays, at least, a much bigger part in protein synthesis than does DNA, the comparison of the actions of these closely related compounds provides good evidence for associating incorporation with growth inhibition. Again with 8-azaguanine in *E. coli*, inhibition of growth does not begin until one to two generation times after the inhibitor has been added. The time lag in the onset of growth inhibition, which does not occur with other bacterial growth inhibitors, suggested that time was necessary for the accumulation of damaged and ineffective RNA before an effect on growth was evident. On the other hand, a comparison with the inhibition of growth of *B. cereus* by azaguanine shows that for a roughly equivalent amount of inhibition much less azaguanine is incorporated into the RNA[77]. This result suggests that another mechanism may be playing a larger part in inhibiting *B. cereus* than is seen with *E. coli*. This idea has been confirmed by experiments with 8-azaguanine (labelled with ^{14}C at the carbon in the 4-position) and *B. cereus*, from which it was concluded that inhibition was produced either by the incorporation of the inhibitor into a particularly sensitive portion of the nucleic acid or by interference with some co-factor[78]. Study of the effect of 6-mercapto-purine on the growth of *E. coli* showed that at low concentrations of the

inhibitor which produced inhibition, no effect on nucleic acid biosynthesis could be detected. Mechanisms other than an effect on nucleic acid must therefore be sought[79] and the inhibitors may well interfere in some way with co-enzyme A[80,81], pantothenic acid[81] or with diphosphopyridine nucleotide (DPN)[83]. In this connection it should be noted that co-enzyme A contains an adenine moiety linked via ribose and phosphoric acid to the pantothenic acid amide of β-mercaptoethylamine, whilst DPN also contains an adenine residue linked through ribose and phosphate to nicotinamide.

Hitherto there has been a tendency, when attempting to design new drugs intended to improve on the results so far obtained with antipurines, to consider only the structures of the established links in the chain of nucleic acid synthesis, or smaller moieties, e.g., purines, derivable therefrom, and then to think of analogous structures which might interfere with their function. A probably important consequence of the recent evidence on the mechanism of the inhibitory action of 8-azaguanine and 6-mercaptopurine will be to focus more attention on the possibility of interfering with the action of the two co-factors mentioned above and also with others.

The relation of the action of the sex hormones to the metabolism of folic acid and purines has received very little attention except from the work of Hertz and Tullner[84]. They found that the growth of chick oviduct, a tissue dependent on oestrogens, is much stimulated by administration of stilboestrol and folic acid and that this effect is competitively reversed by aminopterin, Methotrexate and 4-aminopteroylaspartic acid (amino-an-fol.). Similar results were obtained with the uterus of the sexually immature rat. Similarly an antagonism between an antipurine (2:6-diaminopurine) and adenine was demonstrated. Analogous antagonisms were observed between certain steroid hormones[84,85], and the results of this and further work might well help to explain, for example, why both antifolic acids and cortisone are beneficial in acute leukaemia.

Summary

A review of the field of antifolic acids and antipurines made with the intention of illustrating their use, or potential use, in chemotherapy, reveals drugs useful, but by no means ideal, for the palliative treatment of leukaemia, and the discovery of one valuable antimalarial drug, which is an antifolic acid. There is also evidence that an antipurine mechanism can be involved in trypanocidal and also in antiviral action, but successful clinical applications of these leads have not yet been achieved. Again, there is a preliminary indication that antifolic acids might perhaps be of some value as antiviral agents and here, as with the antipurines, further work will doubtless be stimulated by the enormous importance of a successful chemotherapy of virus diseases, which is yet to be achieved.

References

1. Timmis, Felton, Collier and Huskinson, *J. Pharm. Pharmacol.*, 1957, **9**, 46.
2. Schulman and Buchanan, *J. biol. Chem.*, 1952, **196**, 513.
3. Greenberg, *Fed. Proc.*, 1953, **12**, 651.

4. Hartman, Levenberg and Buchanan, *J. Amer. chem. Soc.*, 1955, **77**, 501.
5. Nichol and Welch, *Proc. Soc. exp. Biol., N.Y.*, 1950, **74**, 52, 403.
6. Sauberlich, *J. biol. Chem.*, 1949, **181**, 467.
7. Hendlin, Koditschek and Soars, *J. Bact.*, 1953, **65**, 466.
8. Broquist, Kohler, Hutchison and Burchenal, *J. biol. Chem.*, 1953, **202**, 59.
9. Nichol, Zakrzewski and Welch, *Proc. Soc. exp. Biol., N.Y.*, 1953, **83**, 272.
10. Farber, *Blood*, 1949, **4**, 160.
11. Weir, Welch and Heinle, *Proc. Soc. exp. Biol., N.Y.*, 1949, **71**, 107.
12. Kirschbaum, Geisse, Judd and Meyer, *Cancer Res.*, 1950, **10**, 762.
13. Law, Dunn, Boyle and Miller, *J. Nat. Cancer Inst.*, 1949, **10**, 179.
14. Burchenal, Johnston, Burchenal, Kushida, Robinson and Stock, *Proc. Soc. exp. Biol., N.Y.*, 1949, **71**, 381.
15. Burchenal, Burchenal, Kushida, Johnston and Williams, *Cancer*, 1949, **2**, 113.
16. Skipper, Thomson and Bell, *Cancer Res.*, 1954, **14**, 503.
17. Farber, Toch, Sears and Pinkel, *Advances in Cancer Research*, Academic Press, New York, 1956, **4**, p. 39.
18. Colsky, *Chemistry and Biology of Pteridines*, J. and A. Churchill, London, 1954, p. 413.
19. Daniel, Norris, Scott and Heuser, *J. biol. Chem.*, 1947, **169**, 689.
20. Daniel and Norris, *ibid.*, 1947, **170**, 747.
21. Spickett and Timmis, *J. chem. Soc.*, 1954, 2887.
22. Campbell, Dunsmuir and Fitzgerald, *ibid.*, 1950, 2743.
23. Campbell, Collier, Dunsmuir and Fitzgerald, British Patent No. 666,788/1952.
24. Dunsmuir, British Patent Application No. 6514/1954.
25. Felton and Timmis, *J. chem. Soc.*, 1954, 2881.
26. Collier, 1954, unpublished.
27. Hitchings, Falco, Vanderwerff, Russell and Elion, *J. biol. Chem.*, 1952, **199**, 43.
28. Modest, Foley, Pechet and Farber, *J. Amer. chem. Soc.*, 1952, **74**, 855.
29. Modest, Farber and Foley, *Proc. Amer. Assoc. Cancer Res.*, 1954, **1**, 33.
30. Modest, *J. org. Chem.*, 1956, **21**, 1.
31. Foley, *Proc. Soc. exp. Biol., N.Y.*, 1953, **83**, 733, 740.
32. Carrington, Crowther and Stacey, *J. chem. Soc.*, 1954, 1017.
33. Murphy, Ellison, Karnofsky and Burchenal, *J. clin. Invest.*, 1954, **33**, 1388.
34. Clarke, Buckley, Sternberg, Stock, Rhoads and Hitchings, *Cancer Res.*, 1952, **12**, 255.
35. Falco, Goodwin, Hitchings, Rollo and Russell, *Brit. J. Pharmacol.*, 1951, **6**, 185.
36. Farber, Toch, Sears and Pinkel, *Advances in Cancer Research*, Academic Press, New York, 1956, **4**, p. 40.
37. Hewitt, Wallace, Gumble, White and Williams, *Amer. J. trop. Med. and Hyg.*, 1954, **3**, 225.
38. Schmidt, Loo, Fradkin and Hughes, *Proc. Soc. exp. Biol., N.Y.*, 1952, **80**, 367.
39. Farber, Appleton, Downing, Heald, King and Toch, *Cancer*, 1953, **6**, 135.
40. Carrington, Crowther, Davey, Levi and Rose, *Nature, Lond.*, 1951, **168**, 1080.
41. Modest, Schlein and Foley, *J. Pharm. Pharmacol.*, 1957, **9**, 68.
41*a*. Morgan, *J. exp. Med.*, 1952, **95**, 269.
41*b*. Hitchings, Elion, Vanderwerff and Falco, *J. biol. Chem.*, 1948, **174**, 765.
42. Burchenal, Karnofsky, Kingsley-Pillers, Southam, Myers, Escher, Craven, Dargeon and Rhoads, *Cancer*, 1951, **4**, 459.
43. Burchenal, Bendick, Brown, Elion, Hitchings, Rhoads and Stock, *ibid.*, 1949, **2**, 119.
44. Kidder and Dewey, *J. biol. Chem.*, 1949, **179**, 181.
45. Elion, Burgi and Hitchings, *J. Amer. chem. Soc.*, 1952, **74**, 411.
46. Hall, Richards, Willett and Feichtmeir, *Ann. N.Y. Acad. Sci.*, 1954, **60**, 374.
47. Murphy, Tan, Ellison, Karnofsky and Burchenal, *Proc. Amer. Assoc. Cancer Res.*, 1955, **2**, 36.
48. Farber, *Advances in Cancer Research*, Academic Press, New York, 1956, **4**, p. 43.
49. Agosin and Brand, *Antibiotics and Chemotherapy*, 1954, **4**, 624.
50. Hewitt, Gumble, Wallace and Williams, *ibid.*, 1954, **4**, 1222.
51. Wright, Dolgolpol, Logan, Prigot and Wright, *Proc. Amer. Assoc. Cancer Res.*, 1955, **2**, 55.
52. Taylor, Sherman and Bond, *J. Amer. chem. Soc.*, 1954, **76**, 4497.
53. Faiguenbaum and Alba, *Boletin chileno de parisitologia*, 1954, **9**, 94.
54. Timmis, Cooke and Spickett, *The Chemistry and Biology of Purines*, J. and A. Churchill, London, 1957, p. 148.

55. Collier and Huskinson, *The Chemistry and Biology of Purines*, J. and A. Churchill, London, 1957, p. 134.
56. Hitchings, Elion and Sherwood, *Fed. Proc.*, 1950, **9**, 185.
57. Robins, *J. Amer. chem. Soc.*, 1956, **78**, 784.
58. Skipper, Robins and Thomson, *Proc. Soc. exp. Biol., N.Y.*, 1955, **89**, 594.
59. Collier and Huskinson, 1956, unpublished.
60. Timmis, *J. chem. Soc.*, 1957, in the press.
61. Woolley and Shaw, *J. biol. Chem.*, 1951, **189**, 401.
62. Shaw and Woolley, *ibid.*, 1952, **194**, 640.
63. Woolley, *ibid.*, 1944, **152**, 225.
64. Matthews, *J. gen. Microbiol.*, 1953, **8**, 277.
65. Matthews, *ibid.*, 1954, **10**, 521.
66. Thompson, Price, Minton, Elion and Hitchings, *J. Immunol.*, 1950, **65**, 529.
67. Brown, *ibid.*, 1952, **69**, 441.
68. Thompson, *J. Bact.*, 1946, **51**, 629.
69. Thompson, *J. Immunol.*, 1947, **55**, 345.
70. Morgan, *J. exp. Med.*, 1952, **95**, 277.
71. Tamm, Folkers, Shunk, Heyl and Horsfall, *ibid.*, 1953, **98**, 245.
72. Tamm, Folkers, Shunk and Horsfall, *ibid.*, 1954, **99**, 227.
73. Tamm, *Science*, 1954, **120**, 847.
74. Tamm, Folkers and Shunk, *J. Immunol.*, 1956, **72**, 54.
75. Mathews and Smith, *Nature, Lond.*, 1956, **177**, 271.
76. Mathews and Smith, *Biochem. J.*, 1954, **57**, vi.
77. Mathews, *Chemistry and Biology of Purines*, J. and A. Churchill, London, 1957, p. 270.
78. Mandel, *J. biol. Chem.*, 1957, in the press.
79. Bolton and Mandel, *J. Pharmacol.*, 1957, in the press.
80. Biesele, *Ann. N.Y. Acad. Sci.*, 1954, **60**, 228.
81. Garattini, Mopurgo and Passerini, *Giorn. ital. Chemterap.*, 1955, **2**, 29.
82. Caliari and Garattini, *ibid.*, 1955, **2**, 23.
83. Kaplan, Goldin, Humphreys, Ciotti and Stolzenbach, *J. biol. Chem.*, 1956, **219**, 287.
84. Hertz and Tullner, *Ann. N.Y. Acad. Sci.*, 1950, **52**, Art. 8, 1260.
85. Hertz, *Antimetabolites and Cancer*, American Association for the Advancement of Science, Washington, 1955, p. 253.