# *A Chemotherapeutic Search in Retrospect.*

THE TILDEN LECTURE, DELIVERED AT THE ROYAL INSTITUTION, LONDON, ON JUNE 7TH, 1951, AND IN BANGOR ON NOVEMBER 23RD, 1950.

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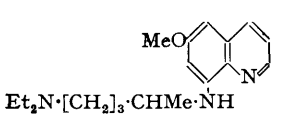
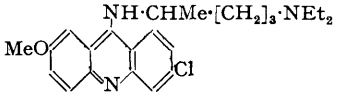
A CHEMOTHERAPEUTIC search is a quest for a chemical substance having a specific use in medicine, and as such is an exercise in applied science, although its execution may call for a high degree of scientific ingenuity from both the chemist and the biologist. Nevertheless, the objective has to be kept constantly in view and the temptation to wander from the path that leads to it resisted. When the search was conducted under the stress of war, as was the case with the early stages of the attempts to discover new antimalarial agents here described, this outlook had to be adhered to ruthlessly, so that many interesting side-observations had to be disregarded at least temporarily. Only later, when the emergency was past, was it possible to go back and examine the field of endeavour at greater leisure. Such a course is particularly apt in the present circumstance since the emergence of a highly active agent of novel type has tended to focus attention on the particular sequence of events and substances immediately concerned in its evolution, with a consequent neglect of numerous earlier types, many of considerable therapeutic interest at the time, but which for one reason or another could not be improved upon, and had in consequence to make way for more fruitful leads. The purpose of this lecture is to rectify these omissions by providing a fuller account of the research as a whole. By way of introduction, it is proposed to give a historical account of the events leading up to the present endeavours.

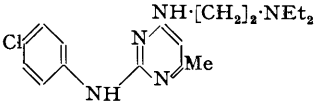
Although quinine has been recognised as a specific curative agent in malaria since its isolation from cinchona bark by Pelletier and Caventou in 1820, it is only within the last 25 years that its position has been challenged by the introduction of synthetic substitutes. The earlier misguided but in other ways significant attempts by Perkin to produce quinine by the oxidation of aniline being disregarded, the history of synthetic drugs goes back to the observation by Ehrlich and Guttman [1] in 1891 that methylene-blue possessed a certain amount of antimalarial action, and to the work of Skraup, Königs, Rabe, and others on the structure of the cinchona alkaloids. In Germany, Schulemann, Schönhöfer, and Wingler [2] utilised this knowledge, and by a series of steps arrived at Pamaquin (then called Plasmoquine) (I) first introduced clinically by Muhlens [3] without full disclosure of its constitution.




(I.)
Plasmoquine = pamaquin B.P.




(II.)
Atebrin = mepacrine B.P.




(III.)
" 2666."

In England, independently and more or less simultaneously, the main early attack on the preparation of synthetic antimalarials was carried out by Robinson, Kermack,[4] and their collaborators in co-operation with Keilin, by an arrangement with the Joint Chemotherapy Committee of the Medical Research Council and the Department of Scientific and Industrial Research. Here the important clues followed up were the constitution of quinine and the harmala alkaloids, and the later statement of the I.G. Farbenindustrie A.G. that Plasmoquine was the salt of an alkylamino-6-methoxyquinoline. The most active compounds were ultimately found, as with the latter, to be those carrying an alkylaminoalkylamino-grouping in position 8 of the quinoline nucleus. Before the War, related researches were also in progress in France under Fourneau, in Russia under Magidson, and in India under Brahmachari. The next important step forward was taken by the German workers in their exploitation of the pamaquin lead. Aminoalkylamino-groups had been introduced into many other heterocyclic systems including xanthens, azines, oxazines, thiazines, and acridines, but it was the last-

[1] Ehrlich and Guttman, *Berlin klin. Woch.*, 1891, **28**, 593.
[2] Schulemann, Schönhöfer, and Wingler, *Klin. Woch.*, 1932, **11**, 381.
[3] Muhlens, *Arch. Schiffs- u. Tropenhyg.*, 1926, **30**, Beiheft 3, 25.
[4] Robinson, Kermack, and co-workers, " Attempts to find New Antimalarials," Parts I—XXIX, *J.*, 1929 *et seq.*

named type only that provided drugs of special merit. Soon after the evaluation of pamaquin it was realised that this substance did not provide an adequate substitute for quinine, as it had no action on the asexual forms of malignant tertian malaria. In the course of an investigation of 10-aminoacridines, however, Mietzsch and Maus [5] finally succeeded in evolving a compound active against the schizonts of all three types of human malaria. The drug was first called Plasmoquine E, then Erion, and later Atebrin (now known in this country as mepacrine) (II). Then followed extensive researches on this class of compound, not only in Germany but again in Russia by Magidson and his collaborators who provided much useful information relating structure with activity, and in England by Clemo, Kermack, and workers in the D.S.I.R. laboratories. No significant improvement, however, was made.

Despite higher antimalarial potency, mepacrine had achieved little more before 1939 than having come to be regarded as a possible substitute for quinine, but with the imminence of war this aspect assumed more than ordinary importance, so much so that both pamaquin and mepacrine were included in a list of essential drugs proposed in 1938 for process study by the Association of British Chemical Manufacturers. In these laboratories the task of putting this proposal into effect was initially entrusted to the late Dr. F. H. S. Curd, and by September 1939 pilot-plant production of mepacrine had begun, soon afterwards to be transferred to full manufacture on a scale sufficient to make a considerable contribution to the needs of the armed forces operating in malarious areas. Meanwhile, the plans for novel research had been maturing and these became part of the national war-time scheme organised a little later by the Medical Research Council, and linked through that body with the efforts of the American National Research Council. Under the auspices of the former, prominent scientists in the universities were invited to take part in the researches, and there began a long period of happy and eventually fruitful collaboration. To illustrate this point it might be mentioned that of the 40 authors that have contributed to nearly 50 papers describing the chemical aspects of these labours, exactly one-half conducted their studies in academic laboratories. Further, some 1700 compounds were prepared and examined in the period up to 1947, of which nearly one-third showed highly significant effect in experimental infections. Clearly, a detailed review of an effort of such magnitude is beyond the scope of the present contribution. Therefore, items have been selected for discussion, not so much on the basis of the clinical utility of the substances concerned, but as illustrative of the apparent diversity of chemical types which provided activity in the experimental stages, and their relation to those which did not. In this, the Lecturer presumes to speak, not only for his own colleagues, but for all who contributed to the concerted effort.

At the outset it had been decided to attempt to break away from the quinoline and acridine types that had been so extensively exploited by earlier workers in this field, and in consequence a lengthy period of barren endeavour was expected. It was a more than pleasant surprise therefore to find activity in the third or fourth of the seemingly entirely novel structures that were prepared. In retrospect, it is clear that with the particular working hypothesis evolved as a guide to new synthesis, activity could hardly have been missed even at the earliest stages of the research. The principles for devising these particular drugs were compounded from known active substances, notably mepacrine and the sulphapyrimidines. The former was regarded as made up of methoxyl- and chlorine-substituted benzene nuclei associated with a potentially tautomeric amino-heterocyclic system and a " basic side chain " of the alkylaminoalkyl type. The latter, more especially a dimethyl derivative (" Sulphamezathine " : sulphadimidine B.P.C.), had just been shown to be active in human malaria, a property apparently to be associated with the pyrimidine ring, and moreover, the recent introduction of this " sulpha " drug into clinical practice as an antibacterial agent [6] and the variations made in its structure,[7, 8] had given a useful familiarity with the chemistry of pyrimidine. In consequence, and additionally because of the known biological importance of this ring system, it was decided to make it the central nucleus around which to array the " active " moieties deduced by consideration of the mepacrine molecule. The compound " 2666 " (III) was the first to show activity against chick malaria (*P. gallinaceum*) in the biological assays conducted throughout by Dr. D. G. Davey, and although clinical test proved abortive, it provided one of the important leads from which so much of the subsequent success was to follow.[9]

[5] Mietzsch and Maus, *Klin. Woch.*, 1933, **12**, 1276.
[6] Rose, Martin, and Bevan, *J. Pharmacol.*, 1943, **77**, 127.
[7] Rose and Swain, *J.*, 1945, 689.
[8] Rose and Tuey, *J.*, 1946, 81.
[9] Curd and Rose, *J.*, **1946**, **343**.

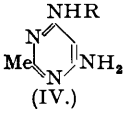
Parallel with this investigation, a cognate series of researches on pyrimidine derivatives was commenced by Todd and his group,[10] who elected to study compounds similar to those mentioned above, but without the aryl substituent. Here again, activity in the experimental infection was encountered at an early stage, providing an additional lead, although the greater relative toxicity of the non-aromatic preparations was ultimately to debar them from clinical consideration.

From this point and for some time to come, the scheme for development was almost automatic, and two broad lines were followed. In the first, the pyrimidine nucleus was retained and subjected to further embellishment, and in the second, the aniline and basic side-chain residues were substituted into yet other heterocyclic systems. As the researches progressed, the more promising leads were still further sub-divided, until some 40 distinct chemical classes were eventually examined, together with many more of which only a few examples each were made.

The sections which now follow outline the chemistry of some of the substances prepared, and provide comment on therapeutic effect. General discussion on structure in relation to activity appears later. To facilitate appreciation of the sequence of events, the various types have been grouped more or less on the basis of their conception, but not necessarily in exact chronological order since many were under investigation simultaneously.

In most of the structures formulated below the so-called basic side chain, usually the simplest dialkylaminoalkylamino-groups, such as 2-diethylaminoethylamino- or 3-diethylaminopropylamino-, figure prominently, although much more complex types were also examined. No attempt has been made to formulate all the variations made, although specific points are dealt with in the final discussion. Instead, the occurrence of such a side chain is denoted by the symbol –NHR, with R defined more fully if occasion demands.

*Pyrimidines.*—(a) *Non-aryl pyrimidine derivatives.*[10, 11] As a matter of deliberate policy, the first preparations of this type contained the pyrimidine nucleus as lightly substituted as possible,[10] and included the simple derivatives (IV)—(IX). These substances were prepared for the most part from the appropriate chloropyrimidines by direct reaction with the side-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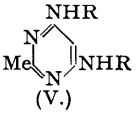
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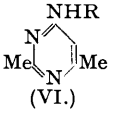
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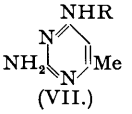
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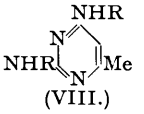
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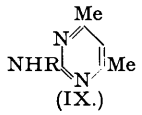
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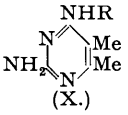
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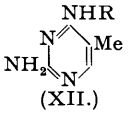
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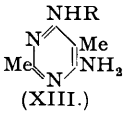
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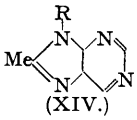

(XIV.)

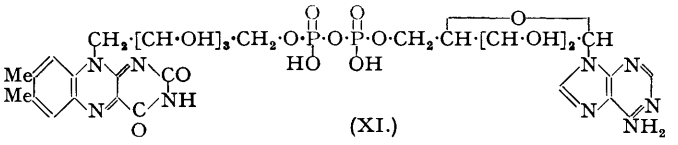
chain amine. Not one compound in this series was active. Antimalarial activity was first encountered, however, when a 5-methyl group was introduced into type (VII) giving (X), although most of the compounds prepared were also highly toxic. Omission of the 2-amino-group, or its replacement by methyl, *i.e.*, to give the 5-methyl compound corresponding to (VI), reduced activity very markedly without materially affecting toxicity, while replacement of the 2-amino-group by a second dialkylaminoalkylamino-group completely destroyed therapeutic effect.

These findings naturally led to a thorough study [11] of the effect of varying the nature of the basic side chain and the substituents at positions 5 and 6. At this stage consideration was given to the possible mode of action of the 5-substituted compounds so that this further development could be as rational as possible. Since an antagonism for riboflavin had already been proposed and demonstrated experimentally by use of *Lactobacillus casei* to explain the therapeutic action of the arylamino-pyrimidines described below, it seemed likely that the simpler compounds might in their turn act through interference with the structurally related purine component of a riboflavin–adenine dinucleotide (XI), either with synthesis or function.

It was further postulated on formal grounds that pyrimidine derivatives substituted in the 4 : 5-position might present optimum conditions for such an antagonism, in particular those bearing a side chain of moderate size in position 4 (=6) corresponding to that of the carbo-

[10] Hull, Lovell, Openshaw, Payman, and Todd, *J.*, 1946, 357.
[11] Hull, Lovell, Openshaw, and Todd, *J.*, 1947, 41.

hydrate residue in adenosine, and it was in fact subsequently observed that only those substances fulfilling these conditions showed marked antimalarial activity. For instance, marked

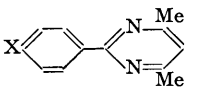


(XI.)

therapeutic effect was found in (XII) (compare inactive VII) while the highest activity in this series occurred with (XIII; R = $[CH_2]_3 \cdot NEt_2$) (compare inactive IV). Substituents other than methyl in position 5 also promoted activity, including ethyl, benzyl, phenoxy-, and ring structures such as that provided by 5 : 6-*cyclo*hexeno-. Curiously enough, the related purines themselves, for example (XIV), and others prepared later, have been entirely devoid of positive effect.

(b) *Aryl-substituted pyrimidines.* This type included the earliest compounds made in this research, and because of this, and a capacity for almost unlimited variation, it has received by far the fullest exploitation of any chemical class. For convenience in discussion, the preparations have been sub-divided into pyrimidines carrying a direct aryl or arylamino-substituent, and others in which the aryl residue is variously connected to the heterocyclic system, for example, through guanidino-, amidino-, ureido-, thioureido-, ether, and thio-ether linkages.

(i) Aryl- and arylamino-pyrimidines.[9, 12–18] Compound (XV; X = H) perhaps deserves special note as the first preparation of the campaign.[9] Its relation to sulphadimethylpyrimidine



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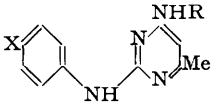
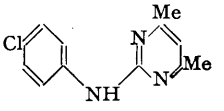


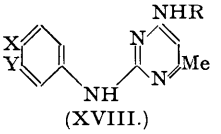
(XVI.)



(XVII.)

(sulphadimidine B.P.) is obvious. It was, however, inactive like the second substance (XV; X = OMe) and a slightly later preparation (XV; X = Cl). The introduction of the concept of tautomeric possibility and the need for marked basicity led to (XVI; X = Cl or OMe) and the immediate observation of antimalarial activity in the chick. The influence of the basic side chain was stressed by the inactivity of the parent amine (XVI; R = H), the corresponding hydroxy-derivative, and (XVII).

Early developmental work based on the novel (XVI) was necessarily somewhat empirical, but even so, it was influenced to no small extent by speculations regarding mode of action. The notion of riboflavin antagonism was postulated almost immediately, and indeed some two years before it was demonstrated experimentally with respect to the growth of *Lactobacillus*



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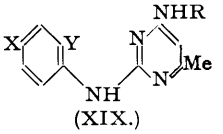

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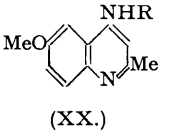


(XX.)

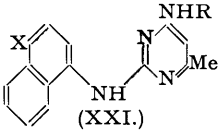
*casei*. This hypothesis [12] led, for example, to the preparation of compounds of type (XVIII) substituted in the 3 : 4-positions in the benzene ring (compare XI).

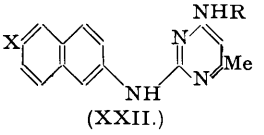
A wide variety of *p*-substituents introduced into (XVI) gave positive activity including, in addition to those already mentioned, bromo, iodo, fluoro, nitro, cyano, phenyl, dimethylamino, and carbomethoxy. The parent anilino-derivative (XVI; X = H) was inactive. In general, *m*- or *o*-substitution gave activity of a reduced order. 3 : 4-Substitution did not lead to enhanced effect, nor did 2 : 4-substitution (XIX; Y = Me or Cl) where it was expected

[12] Curd, Davis, and Rose, *J.*, 1946, 351.
[13] Curd, Raison, and Rose, *J.*, 1946, 366.
[14] Curd, Richardson, and Rose, *J.*, *Russia*, 1946, 378.
[14a] Magidson and Rubtsov, *J. Gen. Chem. Russia*, 1937, **7**, 1896.
[15] Curd, Davis, Owen, Rose, and Tuey, *J.*, 1946, 370.
[16] *Idem*, *ibid.*, p. 720.
[17] Basford, Curd, and Rose, *J.*, 1946, 713.
[18] Basford, Curd, Hoggarth, and Rose, *J.*, 1947, 1354.

that the *o*-substituent would provide a structure more nearly equivalent to the *iso*alloxazine system of riboflavin. The 2 : 5-dichloro-analogue was completely inactive.

An additional form of disubstitution, equivalent to 2 : 3- and 3 : 4-respectively, was provided by preparation and examination of a number of naphthylamine derivatives of types (XXI) and (XXII).[13] The latter included one of the most active compounds in the arylaminopyrimidine class (XXII; X = Br), and (XXII; X = OMe) was almost as effective. The α-naphthylamine derivatives (XXI) were very much less active.


(XXI.)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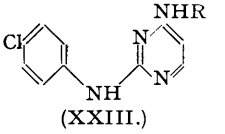
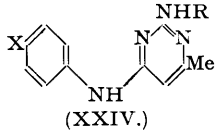
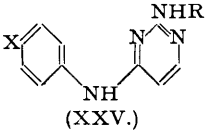

(XXIII.)

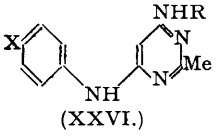
The need for the 6-methyl group in the pyrimidine nucleus was next investigated.[14] Its occurrence in many of the early structures was associated with the greater accessibility of the compounds so substituted, but clearly its intrinsic importance had to be assessed, particularly since amongst the quinoline antimalarial agents is was known that the introduction of methyl adjacent to the heterocyclic nitrogen atom, as in (XX), had a dystherapeutic influence.[14a] In this series, however, its omission, giving (XXIII), was attended by lowered activity. Activity persisted with phenyl in position 6 but much reduced, while chlorine and methoxyl derivatives were inactive.

With regard to substitution in position 5, we had before us knowledge of the profound effect produced in the non-aryl type.[14] It appeared possible that enhancement of therapeutic effect might again result through the consequent hypothetical widening of the range of antagonism to include enzyme systems based on purine nucleosides. This result was not achieved, however, since groups such as methyl, ethyl, propyl, butyl, benzyl, phenyl, phenoxy-, and bromine, and ring closure to 5 : 6-*cyclo*penteno- or -*cyclo*hexeno-, all reduced activity, the reduction being roughly proportional to the mass of the substituent.

All the above substances carried the aryl group in position 2 of the pyrimidine ring, and the basic group at 4. The next major modification was the transposition of these substituents to give the isomeric (XXIV).[15] Activity again resulted and the variations already applied to (XVI) were made.[16] In general, very similar effects were observed. For example, chlorine

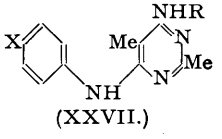

(XXIV.)

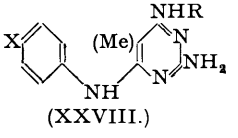

(XXV.)

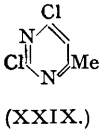

(XXVI.)

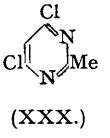
in the phenyl residue could be replaced by nitro- or cyano- to give equally active, if not more active, agents. The *p*-anisidino-derivatives were inactive, however, as were also the 3′ : 4′-dichloroanilino-compounds. The naphthylamines were very much less active in this series. Substitution in position 5 of the pyrimidine ring reduced activity somewhat, as did replacement of 6-methyl by hydrogen (XXV).

Finally, the third isomeric type (XXVI) was examined. The usual variations of *p*-substituent and basic side chain were made [17] but without revealing more than a trace of antimalarial action among the first dozen compounds, and that at doses which killed a proportion


(XXVII.)

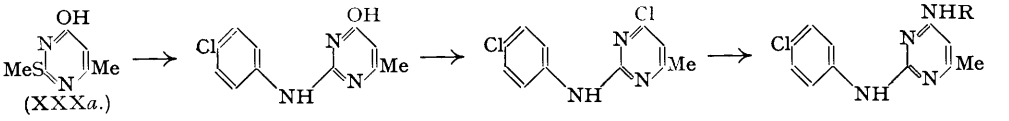

(XXVIII.)


(XXIX.)


(XXX.)

of the test birds. The influence of a substituent in position 5 of pyrimidine in the earlier type (X) (compare VII) being borne in mind, similar substitution was made in (XXVI) to give, not only (XXVII), but the analogous 5-ethyl, -amino-, and -phenyl derivatives.[18] Likewise the methyl group in position 2 of (XXVI) and (XXVII) was replaced by higher alkyl, by phenyl, and by amino- to give, in the last instance, compounds (XXVIII) derived from 2 : 4 : 6-triaminopyrimidine. Of a total of 40 such preparations, however, only a few showed activity, and that marginal.

With regard to the synthesis of the three types of isomeric arylaminoalkylaminopyrimidines discussed above, the first step was to introduce one or other of the main amine substituents into the pyrimidine ring, by replacement for example of chloro- or methylthio-groups, leaving a second grouping, usually hydroxyl, to be then converted into chloro- by the action of phosphoryl chloride, and subsequently causing this to react with the second amine component, *e.g.*:

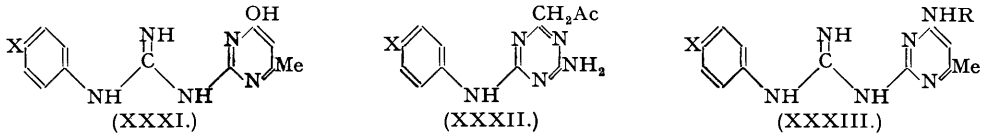


(XXX*a*.)

This stepwise production of, and reaction with, labile groupings, was found preferable to attempted reaction of the amine components with only one of the chlorine atoms in, *e.g.*, (XXIX) or (XXX), when mixtures either of isomers or of mono- and di-substitution products were frequently obtained, which were often extremely difficult to fractionate.

(ii) Aryl-guanidino-, -ureido-, -thioureido-, -oxy-, and -thio-pyrimidines.[19–23] The aryl derivatives of pyrimidine described above all had the aryl group linked to the heterocyclic system either directly or through amino-. Other methods of linkage required investigation and those based on ·NH·C(:NH)·NH·, ·NH·CO·NH·, ·NH·CS·NH·, ·O·, and ·S· were first examined. Of these, only the first three provided scope for the operation of tautomerism then being developed as a potentially significant feature governing antimalarial effect, and it seemed that the outcome of this section of the research would provide useful evidence for or against the hypothesis.

Access to the phenylguanidinopyrimidines was provided by a convenient reaction between aryldiguanides and β-keto-esters to give almost exclusively compounds, *e.g.*, of type (XXXI), accompanied in some instances by small amounts of the guanamine (XXXII).[19] Conversion



(XXXI.) (XXXII.) (XXXIII.)

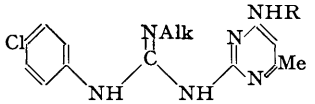
of the hydroxyl substituent into chloro-, followed by reaction with the second amine component, gave type (XXXIII). It was almost immediately apparent that this class provided a higher order of antimalarial activity at a lower level of general toxicity in experimental assays than the arylaminopyrimidines previously described, and it was one such preparation (XXXIII; X = Cl, R = $CH_2$·$CH_2$·$NEt_2$) that first showed useful clinical effect in man. A detailed investigation followed to determine optimal substitution in the molecule.[20] About 70 compounds were made of which 60 showed a significant degree of therapeutic effect. Since the parent (XXXIII; X = H) was quite active, it was possible to define approximately the relative influence of different substituents in the benzene ring. Potentiation was given by chlorine, fluorine, bromine, iodine, nitro-, cyano-, methylthio-, and phenyl in descending order of magnitude, optimum activity being usually associated with the *p*-position. *p*-Methyl or -methylsulphonyl was about as effective as hydrogen, but sulphonamido-, methoxyl, and dimethylamino- deactivated. Substituents in position 5 of the pyrimidine ring also had a dystherapeutic effect.

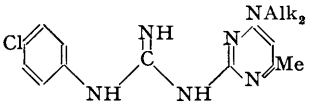
A further variation was the introduction of alkyl (*e.g.*, Me, Et, Bu) at the central nitrogen atom of the guanidine linkage to give type (XXXIV).[21] Activity still remained, though of a lower order than in the parent (XXXIII).

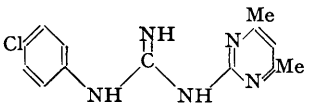
Because of the greater base strength associated with the arylguanidino-residue than with arylamino-, it was argued that it might be possible to omit or simplify the basic side chain and still retain activity. The related types (XXXV) and (XXXVI) were therefore made,[20] in the former the dialkylamino-group being diethylamino-, methyl*iso*propylamino-, or piperidino-. Both types did indeed provide quite a high degree of activity. It was also found that when

[19] Curd and Rose, *J.*, 1946, 362.
[20] Cliffe, Curd, Rose, and Scott, *J.*, 1948, 574.
[21] Crowther, Curd, and Rose, *J.*, 1948, 586.
[22] Ashworth, Crowther, Curd, and Rose, *J.*, 1948, 581.
[23] Curd, Davis, Hoggarth, and Rose, *J.*, 1947, 783.

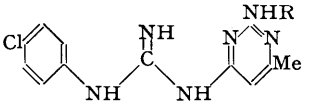
the alkyl groups in (XXXV) were both of the basic side chain type, an inactive compound resulted. The high therapeutic action of the 2-arylguanidinopyrimidines (XXXIII) clearly

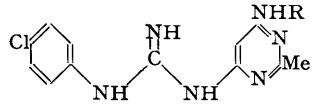

(XXXIV.)

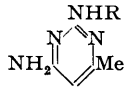

(XXXV.)

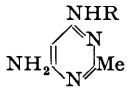

(XXXVI.)

called for an examination of the two other isomeric structures, namely, (XXXVII) and (XXXVIII).[21] The convenient method of preparation giving access to (XXXIII) was not, of course, applicable in these instances. Instead, an indirect route was used in which *p*-chlorophenyl *iso*thiocyanate was condensed with (XXXIX) and (XL), severally, and the resultant

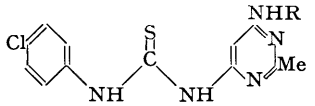

(XXXVII.)

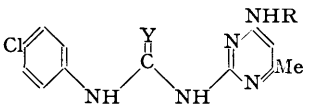

(XXXVIII.)


(XXXIX.)


(XL.)

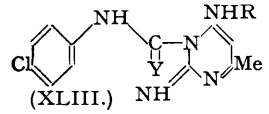
thioureas were converted into the guanidines by reaction with ammonia. In contrast to the corresponding arylaminopyrimidines, *both* of the new isomers provided active substances, and indeed, one compound of type (XXXVIII), which by analogy with (XXVI) should be quite inactive, was almost as effective as the best substance of type (XXXIII).

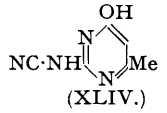

(XLI.)

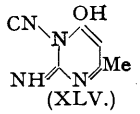

(XLII.)

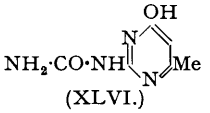
The thiourea (XLI; Y = S),[21] an intermediate in the preparation of (XXXVIII), together with (XLII; Y = S) [22] which resulted from the reaction of *p*-chlorophenyl *iso*thiocyanate with (VII), were themselves examined biologically and found to be entirely devoid of antimalarial effect. Likewise, the normal urea (XLII; Y = O) [22] from *p*-chlorophenyl *iso*cyanate and (VII) was inactive.

In the course of the investigation of these reactions, it was possible to settle constitutional doubts which existed regarding the precise structures of (XXXI), and hence of (XXXIII), and (XLII; Y = S or O). The several synthetic routes described above might equally have yielded isomeric substances such as (XLIII; Y = NH, S, or O). It was discovered, however, that the 2-methylthiopyrimidine (XXX*a*) when heated with *p*-chlorophenylguanidine [21] gave a product identical with (XXXI; X = Cl) which along with the derivative (XXXIII) must

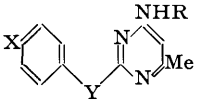

(XLIII.)

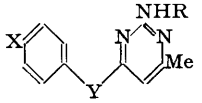

(XLIV.)


(XLV.)


(XLVI.)

therefore have the orientation indicated; (XXXI; X = Cl) was also obtainable from *p*-chloroaniline and (XLIV), the reaction product from dicyandiamide and ethyl acetoacetate, which cannot then have the alternative structure (XLV); (XLIV) on hydrolysis gives (XLVI), and when this is heated with *p*-chloroaniline and its OH group is converted into NHR, it yields, and establishes the constitution of (XLII; Y = O). Finally, since (XXXIII; X = Cl) resulted from the reaction of ammonia and mercuric oxide with (XLI), the latter had the structure shown.

(XLVII.) 

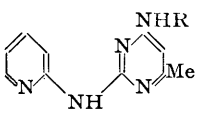

(XLVIII.)

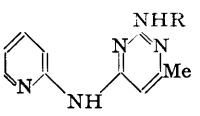
The investigation of the ether and thioether analogues of (XVI) and (XXIV) was called for,[23] if only because of the formal resemblance that these compounds (XLVII; Y = O or S) and (XLVIII; Y = O or S) still retained to riboflavin. Access to these substances was

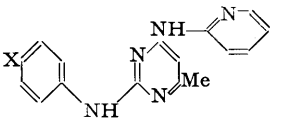
through condensation of the appropriate 2(or 4)-chloro-4(or 2)-basic side chain pyrimidine with a nuclear-substituted phenol or thiophenol. Of the 20 substances prepared, antimalarial activity was detected only in type (XLVIII; X = MeO, Y = O or S), with the thio-compounds markedly more effective than the oxygen ether type. Indeed, (XLVIII; X = MeO, Y = O, R = $CH_2 \cdot CH_2 \cdot NEt_2$) was almost as active as (III). The *p*-chlorophenyl ethers were entirely devoid of therapeutic action.

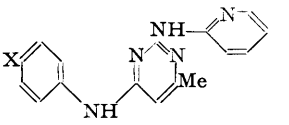
(c) *Various heterocyclic systems linked to pyrimidine.* Up to this stage of the research it seemed that the most useful therapeutic agents resulted from the association of pyrimidine with a benzenoid system. A further variation was now made in which the latter was replaced by a second heterocyclic structure, notably pyridine, quinoline, pyrimidine, or benziminazole.

(i) Pyridylaminopyrimidines.[24] The first compounds made were (XLIX) and (L), analogous to (III) and (XXIV), respectively, and prepared by similar methods. In addition, because


(XLIX.)

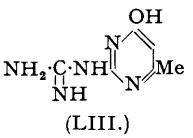

(L.)

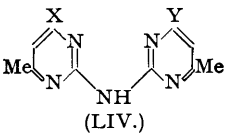

(LI.)

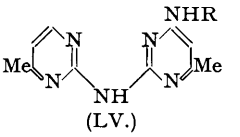

(LII.)

of the intrinsic basic properties of aminopyridine, it was thought that this residue might replace the basic side chain in the arylaminopyrimidine class. Compounds type (LI) and the isomeric (LII) were accordingly made. Not one of the several preparations, however, showed positive activity.

(ii) Dipyrimidylamines.[24] After a few abortive attempts to prepare substances of this class by causing chloropyrimidines to react with aminopyrimidines, a method involving total synthesis of the second pyrimidine ring was selected. Thus (LIII), from (XLIV) and ammonia,


(LIII.)

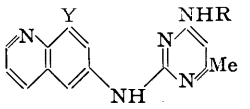

(LIV.)

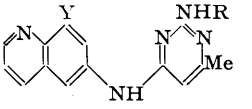

(LV.)

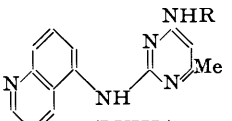
or diguanide and ethyl acetoacetate, condensed either with acetylacetone or more ethyl acetoacetate to give (LIV; X = Me, Y = OH, or X = Y = OH). Conversion of hydroxyl into chloro-, followed by reaction with alkylamines, gave, *e.g.*, (LV) and the related (LIV; X = Y = $NMe_2$ and $NHPr^i$). Here again, not one single substance exhibited antimalarial activity.

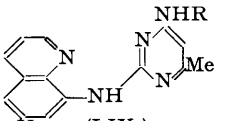
(iii) Quinolylaminopyrimidines.[25] Two possibilities were evident in this class, since the amino-linkage to the quinoline could be in either the heterocyclic or the benzene ring. The

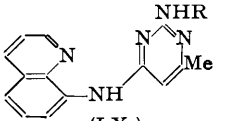
(LVI.)




(LVII.)


(LVIII.)

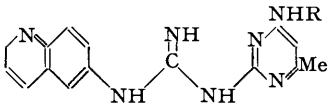

(LIX.)

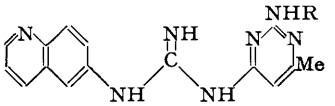

(LX.)

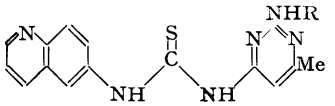
former were, of course, related to pyridylaminopyrimidines (XLIX), while the latter could be regarded as di-*o*-substituted arylaminopyrimidines. Examples of the latter only were made [25] (LVI—LX; Y = H or OMe), essentially the same synthetic route being utilised as that in which (XXX*a*) was the starting material, the linkage to the quinoline residue being at positions 5, 6, and 8. Of these preparations, only those based on (LVI; Y = H) were active. The corresponding (LVI; Y = OMe) was, surprisingly, quite inactive, as was also (LIX; Y = OMe) which was closely related to pamaquin (I).

[24] Curd, Graham, and Rose, *J.*, 1948, 594.
[25] Curd, Graham, Richardson, and Rose, *J.*, 1947, 1613.

At this point, it is convenient to include the quinolylguanidinopyrimidines [26] of which two types were made, (LXI) and (LXII), corresponding to the highly active (XXXIII) and (XXXVII) in the phenylguanidinopyrimidine series. The preparative methods were analogous to those used for the latter, namely, beginning with the condensation of 6-diguanidoquinoline and ethyl

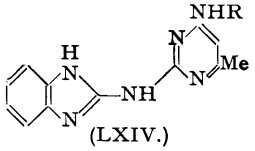

(LXI.)

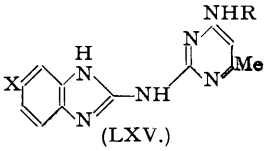

(LXII.)

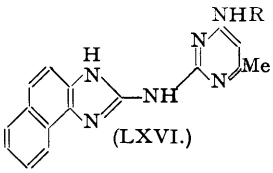

(LXIII.)

acetoacetate for (LXI), and amidation of the thiourea (LXIII), obtained by the interaction of 6-*iso*thiocyanatoquinoline and (XXXIX), for (LXII); (LXI) alone exhibited antimalarial activity, and that but slight at high dosage.

(iv) Benziminazolylaminopyrimidines.[27] Only derivatives of 2-aminobenziminazole were made, which could therefore be regarded as derived from (XXXIII). The 15 preparations included the parent benziminazole (LXIV), those with substituents at position 5 (=6) (LXV), and the naphthiminazole (LXVI). The simplest synthesis of these compounds involved the


(LXIV.)

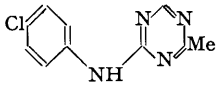

(LXV.)

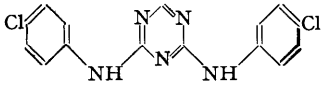

(LXVI.)

initial condensation of the appropriate *o*-phenylenediamine with (XLIV), followed by halogenation and reaction with the basic side-chain amine. Type (LXVI) was inactive, but (LXIV) had a marked therapeutic effect, greater than that of the related (XXXIII; X = H). Methyl and chloro-substituents (LXV; X = Me or Cl) both activated, but methoxyl deactivated.

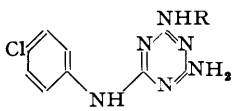
1 : 3 : 5-*Triazines*.[28]—The investigation of derivatives of 1 : 3 : 5-triazines was made after the discovery of activity in the diguanide class, but many of the compounds prepared so closely resemble the early arylaminopyrimidines that they are more conveniently described at this point. Two synthetic routes were used: the first, based on ring closure of diguanides with acetic anhydride, gave guanamine derivatives (LXVII), while the second was based on step-

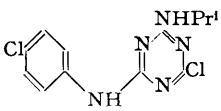
(LXVII.)




(LXVIII.)

(LXIX.)



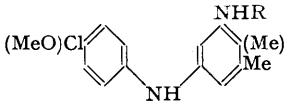

(LXX.)

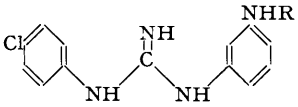
wise replacement of the three chlorine atoms of cyanuric chloride. In the latter type, one chlorine atom was replaced by the aniline residue (*p*-chloroaniline or *p*-anisidine), the second by another amine residue which was either a further aniline or an alkylamine, sometimes of the basic side-chain class, while the third chlorine, now much less reactive, was either left intact, or by heating the compound with the appropriate substance converted into hydroxy-, amino-, ethoxy-, or dialkylaminoalkylamino-. The compounds (LXVIII—LXX) were typical of the 20 or more of the members of this class that were made, not one of which, together with (LXVII), the exact analogue of (III), showed even the slightest trace of antimalarial activity.

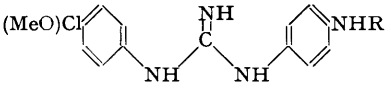
*Diphenylamines and Diphenylguanidines*.[29, 30]—The discovery of therapeutic activity in the arylamino- (XVI) and arylguanidino-pyrimidines (XXXII) seemingly lent support to the working hypothesis by means of which they had been evolved, while the experimental demon-

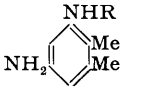
[26] Gulland and Macey, *J.*, 1949, 1257.
[27] Basford, Curd, and Rose, unpublished work.
[28] Curd, Landquist, and Rose, *J.*, 1947, 154.
[29] Mann and Porter, *J.*, 1947, 910.
[30] Mann, Naylor, and Porter, *J.*, 1947, 914.

stration of anti-vitamin properties with respect to riboflavin and the growth of *Lactobacillus casei* suggested a mechanism for their plasmodicidal action. The two relevant factors, one associated with potential tautomerism, and the other a general structural resemblance to the growth factor might, or might not, have been related. An investigation was put in hand by

(LXXI.) 

 (LXXII.)

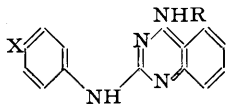
(LXXIII.) 

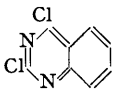
 (LXXIV.)

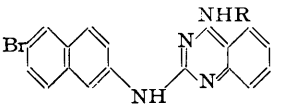
Mann and his co-workers which sought to throw some light on these matters by preparation of the compounds (LXXI) [29] and (LXXII) [30] formally analogous with (XVI) and (XXXIII), but having the pyrimidine nucleus replaced by benzene so that the tautomeric features characteristic of the former were abolished or at least greatly diminished. In addition, the *p*-isomer (LXXIII) of (LXXII) was made as a variant, and also the diaminoxylene (LXXIV) because of its formal resemblance to the active pyrimidine derivative (X). (LXXII) and (LXXIII) resulted from the condensation of the *p*-methoxy- or *p*-chloro-phenylcyanamide with the appropriate phenylenediamine. (LXXI) and (LXXIV) were prepared by standard procedures, except that the basic side chain was introduced by the device of reducing an anil group formed from primary amino- and 5-diethylamino-*n*-pentan-2-one diethyl ketal.

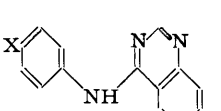
With regard to antimalarial activity, all the diphenylamines (LXXI), the simpler xylenes (LXXIV), and the diphenylguanidine (LXXIII) were entirely without action. On the other hand, (LXXII) showed quite marked activity, less than that of the pyrimidine analogue (XXXIII), but of the same order as (III).

*Quinazolines.*[32–35]—Since the association of a pyrimidine nucleus with a substituted benzene ring had been found to provide a basis for antimalarial activity, it was clearly of interest to examine derivatives of quinazoline in which these two cyclic systems were fused together. Additional support for this investigation also arose from the structural relationship between quinazoline and quinoline. Earlier work by Magidson and Golovchinskaya [31] had failed to disclose therapeutic effect in simple quinazolines carrying alkylaminoalkyl groups, but their preparations had not included arylamino-substituents. The latter were now introduced at position 2, yielding substances (LXXV) [32] analogous to the earliest arylaminopyrimidines (XVI), or more precisely the corresponding 5 : 6-disubstituted pyrimidines. The preparation

 (LXXV.)

 (LXXVI.)

 (LXXVII.)

 (LXXVIII.)

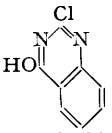
of these compounds was facilitated by the ease with which the basic side-chain amine could be made to react almost quantitatively with the 4-chloro-substituent of (LXXVI). A large series was made and activity was found in almost every member. A *p*-chloro-substituent again gave optimal effect, fully equal to that of type (XXXIII), but (LXXV; X = H, Me, MeO, MeS, and $NO_2$) were also quite highly active. In contrast to the arylaminopyrimidine series, the bromonaphthylamino-group in (LXXVII) was dystherapeutic.

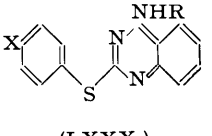
The influence of substitution in the benzene ring of the quinazoline system (LXXV; X = Cl) was examined.[34] Substituents such as chloro-, nitro-, amino-, methyl, methoxyl, and benzo- were variously introduced into positions 5, 6, 7, and 8. Activity was retained in every instance, but always less than that of the parent compound.

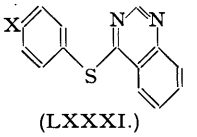
The isomeric type (LXXVIII) analogous to the active arylaminopyrimidines (XXIV) was also studied.[35] The preparative route from (LXXVI) and arylamines was first investigated,

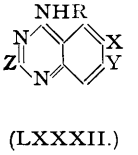
[31] Magidson and Golovchinskaya, *J. Gen. Chem. Russia*, 1938, **8**, 1797.
[32] Curd, Landquist, and Rose, *J.*, 1947, 775.
[33] Chapman, Gibson, and Mann, *J.*, 1947, 890.
[34] Curd, Landquist, and Rose, *J.*, 1948, 1759.
[35] Curd, Hoggarth, Landquist, and Rose, *J.*, 1948, 1766.

but although essentially pure 2-arylamino-4-chloroquinazolines were produced, these on further reaction with basic side-chain amines gave the 4 : 6-bisdialkylaminoalkylaminoquinazolines as the main products. A route starting with (LXXIX) [from hydrolysis of (LXXVI)],


(LXXIX.)


(LXXX.)

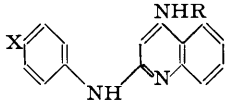

(LXXXI.)

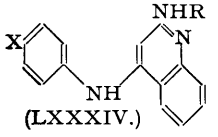

(LXXXII.)

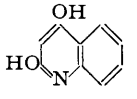
which reacted with basic side-chain amines, and then was converted into the 4-chloro-derivative, proved satisfactory, and in this way a number of compounds type (LXXVIII) was prepared, all of which, however, were devoid of antimalarial activity, as were also the related arylthioquinazolines (LXXX) and (LXXXI) [compare inactive (XLVII) and active (XLVII; Y = S)].

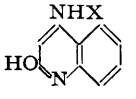
Finally, in the quinazoline series, the observation was made that the simple 4-basic side-chain derivatives were themselves active [33] (at variance with the findings of Magidson and Golovchinskaya [31] whose negative results were probably due to the insusceptibility of their test-strain), and this called for a systematic study of type (LXXXII; Z = H, X and/or Y = Cl, MeO, $NO_2$). Nearly 50 variants were made, nearly all of which were active. A 7-chloro-substituent provided highest activity, again of the same order as that of (XXXIII; X = Cl). Compound (LXXXII; Z = $NH_2$, X = Y = H) which was related to the active 5 : 6-dimethylpyrimidine (X), also showed considerable therapeutic effect.

*Arylaminoquinolines.*[36, 38]—Although, as recorded above, the quinoline nucleus had already been the subject of extensive chemotherapeutic researches, no derivatives had been prepared carrying arylamino-substituents analogous to the pyrimidines (XVI) and (XXIV). Compounds of type (LXXXIII) and (LXXXIV) were therefore prepared initially,[36] the starting material in both instances being 2 : 4-dihydroxyquinoline (LXXXV) which, when heated


(LXXXIII.)

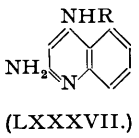

(LXXXIV.)

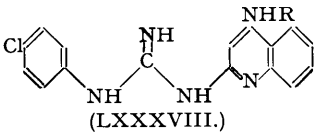

(LXXXV.)

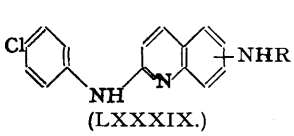

(LXXXVI.)

with excess of arylamine (in the presence of a salt such as hydrochloride), or excess of basic side-chain amine, gave the intermediate (LXXXVI; X = aryl or dialkylaminoalkyl). The latter, by the usual procedure, then led to (LXXXIV) and (LXXXIII), respectively. Of these two types, (LXXXIII) was much the more active, being comparable with the corresponding pyrimidines (XVI), and the highest activity was achieved, as before, with *p*-substituents such as chlorine, nitro-, and methoxyl. Additional substituents were introduced into the quinoline nucleus of type (LXXXIII), for example, 3-methyl and 7-chloro-, but without marked influence on the therapeutic effect. The 7 : 8-benzo-derivative was, however, inactive.

At this point, some attempt was made to determine whether the activity of these compounds was due to their structural relation to the earlier pyrimidines, or whether they were simply to be regarded as derivatives of 4-dialkylaminoquinolines which at that time had just become


(LXXXVII.)

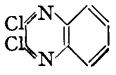

(LXXXVIII.)


(LXXXIX.)

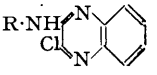
known as antimalarial agents.[37] Although closely allied compounds of type (LXXXVII) were made and found to be highly active, while the related (LXXXVIII), prepared from the latter *via* the intermediate thiourea (compare XLI), was completely inactive, no definite conclusions could be drawn. An additional aspect was provided by the preparation of isomers of (LXXXIII), but with the basic side chain in the benzene ring of the quinoline at positions 5, 6, and 8, respectively (LXXXIX).[38] These compounds were inactive.

[36] Curd, Raison, and Rose, *J.*, 1947, 899.
[37] Schönhöfer, *Z. physiol. Chem.*, 1942, **274**, 1.
[38] Bennett, Crofts, and Hey, *J.*, 1949, 227.

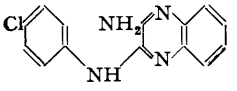
*Quinoxalines.*[39–41]—Apart from the obvious relation of quinoxaline to quinoline, its recognition as part of the riboflavin molecule provided an additional reason for the examination of this system as the basis for potential antimalarial agents. Syntheses in this series (45 compounds) were based initially on 2 : 3-dichloroquinoxaline (XC).[39] Stepwise replacement of



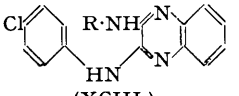
(XC.)



(XCI.)

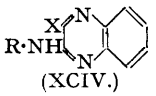


(XCII.)

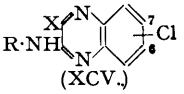


(XCIII.)

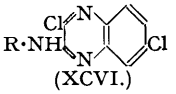
the chlorine atoms by amine residues gave compounds such as (XCI) (active), (XCII) (inactive), and (XCIII) (inactive). The importance of the chlorine substituent in (XCI) was demonstrated by the inactivity of (XCIV; X = H, Me, OH, or $NH_2$) obtained from the appropriate monochloroquinoxalines.[40] Substituents (Cl, Br, Me, OMe, $NO_2$) were introduced into the benzene



(XCIV.)



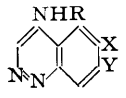
(XCV.)



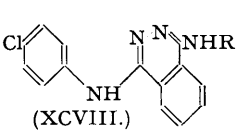
(XCVI.)

ring of the quinoxaline system.[41] These substituents in position 6 (some in 7), giving, *e.g.*, (XCV; X = NHPh, OH, S, OEt, or H), had no marked effect on the therapeutic properties of the parent structures, whether initially active or inactive. An exception was chlorine in position 6, which produced derivatives, notably type (XCVI), that were more active even than mepacrine (II) and (XXXIII).

*Cinnolines*[42] (*Phthalazines and* iso*Quinolines*).[39]—During the course of earlier work with cinnoline, Simpson and his co-workers had appreciated the potentialities of this nucleus for the production of chemotherapeutic agents. This section records those substances, of which some 20 examples were made, specifically designed as antimalarials.[42] They were all derivatives of 4-aminocinnoline (XCVII) with substituents (Cl, Me, OMe) in positions 6 and/or 7 and prepared by reaction of the appropriate amine with the 4-phenoxycinnoline. The parent



(XCVII.)



(XCVIII.)

(XCIX.)

compounds (XCVII; X = Y = H) showed feeble activity which was markedly strengthened, in particular, by the introduction of chlorine at position 7 (compare quinoxalines). A second chlorine at 6, giving (XCVII; X = Y = Cl), slightly reduced this activity. Therapeutic effect fell off rapidly with the heavier basic side-chains.

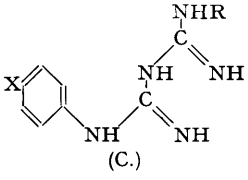
The preparation of single examples based on phthalazine (XCVIII) and *iso*quinoline (XCIX) may be mentioned here[39]—the former was weakly active at high doses, the latter quite inactive.

*Diguanides.*[43–50]—The sequence in which the foregoing chemical types have been recorded has been based largely on convenience of cross-reference, and only initially on approximate chronological order, so that, although it is most convenient to have left the diguanides until now, the first members of this series were actually synthesised within a year of the discovery by Dr. D. G. Davey of activity in the pyrimidines (XVI) against his experimental infections. By that time, exploitation of the lead presented by the latter compounds had produced so

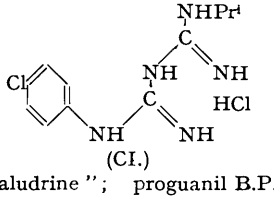
[39] Haworth and Robinson, *J.*, 1948, 777.
[40] Crowther, Curd, Davey, and Stacey, *J.*, 1949, 1260.
[41] Curd, Davey, and Stacey, *J.*, 1949, 1271.
[42] Keneford and Simpson, *J.*, 1947, 917.
[43] Curd and Rose, *J.*, 1946, 729.
[44] Curd, Hendry, Kenny, Murray, and Rose, *J.*, 1948, 1630.
[45] Crowther, Curd, Richardson, and Rose, *J.*, 1948, 1636.
[46] Birtwell, Curd, Hendry, and Rose, *J.*, 1948, 1645.
[47] Ainley, Curd, and Rose, *J.*, 1949, 98.
[48] Ashworth, Crowther, Curd, Hendry, Richardson, and Rose, *J.*, 1949, 475.
[49] Crowther, Curd, and Rose, *J.*, 1951, 1780.
[50] Crowther, Curd, Davey, Hendry, Hepworth, and Rose, *J.*, 1951, 1774.

many new highly active types that the emergence of yet another in the form of the diguanides created no novel situation, and it was not possible to appreciate at that stage its ultimate importance.

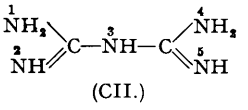
The selection of this particular system for study was based on earlier chemical experience gained from its incorporation in speculative drug molecules of the sulphanilamide class, and on the realisation that it provided an acyclic structure bearing a close formal resemblance to the early anilinopyrimidines (XVI), while retaining some of the features of potential tautomerism which at that time were considered necessary for therapeutic effect. The structure (C; X = Cl), carrying a basic side-chain radicle, was the first prepared.[43] It is here formulated in the manner in which a model of the molecule appears in the planar form, and which best shows its shape relationship to type (XVI). Surprisingly, it was entirely devoid of antimalarial activity.



(C.)



(CI.)

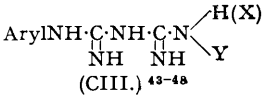


(CII.)

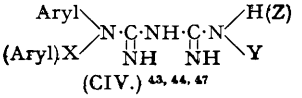
(" Paludrine "; proguanil B.P.C.)

Fortunately, experience of inactivity in earlier molecular types carrying two basic side chains immediately led to the suggestion that the dialkylaminoalkyl group of (C) was superfluous in association with the equally highly basic diguanide system. Its replacement, in the first instance by diethyl and piperidyl, led to the immediate re-emergence of antimalarial activity. Routine variation led in due course to " Paludrine " (= proguanil B.P.C.) (CI).[43] In addition to the normal therapeutic type of activity, compounds of the diguanide class were found by Dr. Davey to exhibit causal prophylactic action, in that they appeared to attack the parasite in the earlier pre- or exo-erythrocytic phase of its life cycle, and it was undoubtedly this unique observation that provided the stimulus for the very considerable effort then directed towards the type. In all, some 200 diguanides have been prepared and examined, together with probably an equal number of miscellaneous compounds whose molecules simulate in part, or in their entirety, the disposition of carbon and nitrogen atoms peculiar to the diguanide structure. A detailed record of these is clearly impossible here, and is indeed unnecessary since it is hoped shortly to publish elsewhere a full discussion of all the compounds made under this heading. Instead, it is proposed to record the main lines of investigation with some indication of the relative therapeutic effects.

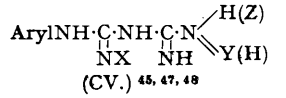
To assist appreciation of the structural chemical features, it is first necessary to point out the essential symmetry of the diguanide molecule (CII) and the actual equivalence of the nitrogen atoms $N^1$, $N^2$, $N^4$, and $N^5$, so that the conventional arrangement of the double bonds, and therefore of the hydrogen atoms, at any rate in diguanide itself, has no real significance. Substitution of hydrogen atoms by aryl and alkyl residues, especially poly-substitution, would



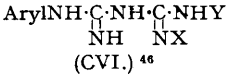
(CIII.) [43-48]



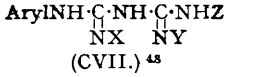
(CIV.) [43, 44, 47]



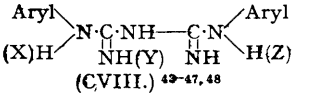
(CV.) [45, 47, 48]



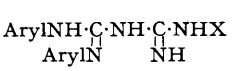
(CVI.) [46]



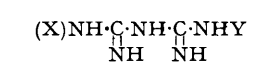
(CVII.) [48]



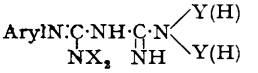
(CVIII.) [43-47, 48]



(CIX.) [48]



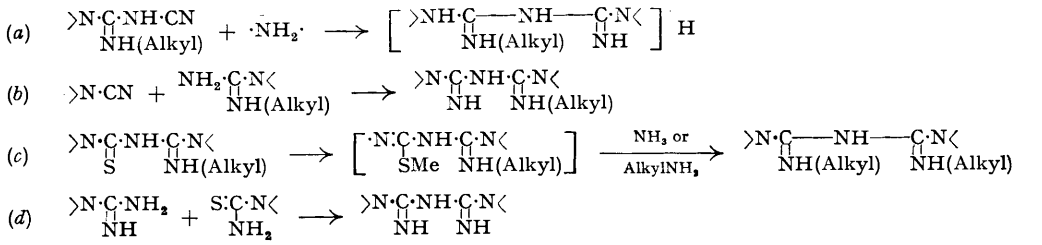
(CX.)



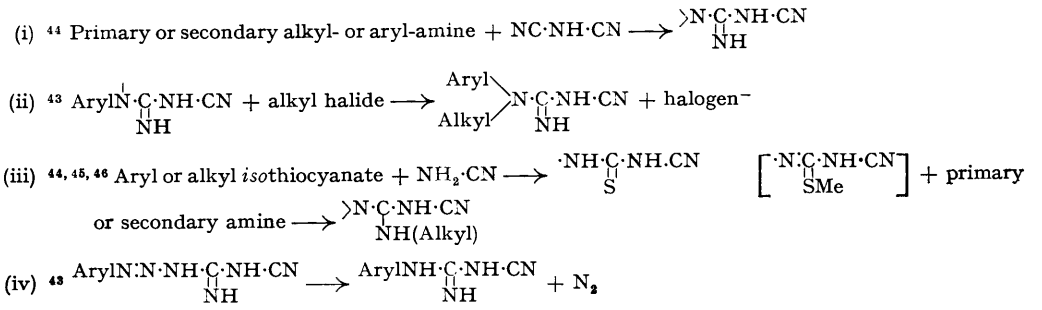
(CXI.) [48]

in certain instances fix the position of double bonds, but this aspect requires fuller discussion than is now convenient. Since all seven hydrogen atoms were potentially replaceable, a great number of variations was possible. Naturally, not all were made, but sufficient were investigated to enable working rules to be formulated. Some 14 main types were prepared, represented by the alternatives provided in (CIII—CX; X, Y, and Z = alkyl), together with many other individual variations, such as that seen in (CXI). The preparative routes were, in detail,

equally numerous, but it is possible to generalise. Most were based on the four fundamental reactions (*a*)—(*d*).



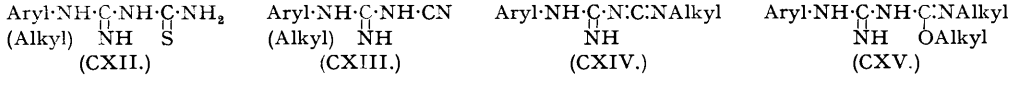
The dicyandiamide derivatives used in route (*a*) were themselves prepared by several reactions, *viz.*, (i)—(iv), and the guanylthioureas of route (*c*) resulted from interaction of



aryl or alkyl *iso*thiocyanates with guanidines, followed by methylation to give, where required, the guanyl *S*-methyl*iso*thioureas.[45]

The reaction of the dicyandiamides with amines (route *a*) was effected at temperatures between 50° and 150° with the amines present either as a salt,[44,45,46] or alternatively as the base in the presence of a copper salt,[43] in which case the diguanide was isolated as its copper complex. For route (*b*), the mono- or di-alkyl- or -aryl-cyanamides were brought into reaction with the mono- or di-substituted aryl- or alkyl-guanidines in boiling solvents such as butanol.[47]

Route (*c*) [45,46,48] was thought in some respects to follow a complex course. When the *N*-aryl- or *N*-alkyl-guanylthioureas (CXII) were converted into their *S*-alkyl *iso*-derivatives, reaction of these with amines yielded diguanides without the intervention of a desulphurising agent. When, however, they were treated with amines direct, a desulphurising agent such as mercuric oxide was required and the dicyandiamide (CXIII) was the main product, some of which then passed into the diguanide. Similar treatment of the *N*-arylguanyl-*N'*-alkylthioureas gave diguanides, but for a number of reasons it was considered likely that the reaction proceeded *via* the carbodi-imide (CXIV).[46] Similar products may also have intervened in the



conversion of the above-mentioned *S*-alkyl-*N'*-alkyl-*N*-arylguanyl*iso*thioureas into guanidines, since in the presence of alcoholic solvents good yields of *O*-alkylureas (CXV) were frequently obtained.

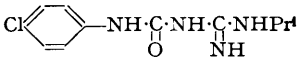
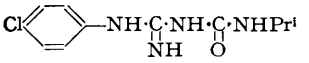
With regard to antimalarial activity, although so many different variants were examined, the results were capable of fairly simple analysis. The first important point was the parallelism between therapeutic and prophylactic effect which was complete throughout. The second was the unique position of the *iso*propyl group, since whatever variations were made elsewhere in the molecule, with regard to substitution either in the benzene ring or in the diguanide chain itself, this member of the homologous alkyl series, when present in a terminal nitrogen, always provided maximum activity, where activity was at all apparent. *n*-Propyl compounds were nearly as effective, but activity fell away rapidly on either side, the methyl homologues being inactive. The third important point was the need for one aryl group in the drug molecule on the nitrogen atom remote from that carrying the *iso*propyl group. More than one aryl residue

always deactivated completely. In brief then, the essential structural basis for high antimalarial activity was the diguanide molecule with a phenyl or substituted-phenyl group at $N^1$, and an *iso*propyl group at $N^5$. The introduction of a second alkyl group always deactivated, the order of diminishing therapeutic effect being with alkyl at $N^5$, $N^2$, $N^4$, and $N^1$, successively. The importance of the *iso*propyl group at $N^5$ in this series was well illustrated by the still high activity of the $N^2$-ethyl-$N^5$-*iso*propyl compounds (aryl = *p*-chlorophenyl) and the complete absence of activity in the isomeric $N^5$-ethyl-$N^2$-*iso*propyl compounds. (CI) with methyl groups at $N^2$ and $N^4$ retained slight antimalarial action, but heavier alkyl groups again deactivated completely.

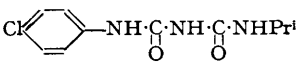
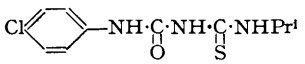
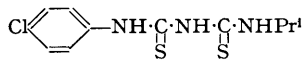
The influence of a wide range of substituents in the aryl (nearly always phenyl) nucleus was investigated. In general, the picture was similar to that seen in the arylamino- and arylguanidino-pyrimidine prototypes (XVI) and (XXXIII), respectively, but there were a few notable exceptions; for instance, while nitro-, methyl, and methylthio-substituents provided activity, cyano- did not. Moreover, the bromo- and iodo-analogues were at least as effective as the chloro-compounds.[49, 50] The mass of these heavier halogen substituents could be distributed in the form of two chlorine atoms at positions 3 and 4, giving a substance (CIII; Aryl = 3 : 4-$C_6H_3Cl_2$, Y = $Pr^i$) considerably more active, but also much more toxic, than (CI).[49, 50] Of the remaining dichloro-possibilities, only 3 : 5-disubstitution gave activity, but at a much lower level. The introduction of any substituent into one or both of the *o*-positions destroyed activity absolutely. Another contrast to the pyrimidine series was the inactivity of the 6-bromo-2-naphthyl analogue of (CI). Lastly, the $N^1$ : $N^5$-dialkyldiguanides (CX), even di*iso*propyldiguanide, were without antimalarial action.

*Guanylureas and Biurets.*[51, 52]—The preparation of guanylthioureas of types (CXVI) and (CXVII), for example, and of the derived guanyl-*S*-alkyl*iso*thioureas, as intermediates in the synthesis of diguanides was mentioned above. They were examined for plasmodicidal action,

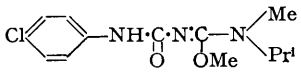
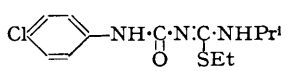
(CXVI.) Aryl·NH·C(=S)·NH·C(=NH)·NHPr$^i$ Aryl·NH·C(=NH)·NH·C(=S)·NHPr$^i$ (CXVII.)

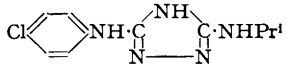
(CXVIII.)   (CXIX.)

with negative results. The guanylurea (CXVIII) analogous to (CXVI) was, however, quite highly active, but in the therapeutic assay only. The high activity of the homologous *N*-methyl derivative provided a further contrast to the related diguanide series. (CXVIII) resulted by direct synthesis from *p*-chlorophenyl *iso*cyanate and *iso*propylguanidine and indirectly from hydrolysis with cold dilute mineral acid of, or the action of nitrous acid on, the corresponding diguanide (CI; proguanil).[51, 52] The isomeric *N*-chlorophenylguanyl-$N^1$-*iso*propylurea (CXIX) was inactive.

(CXX.) (CXXI.) (CXXII.)

(CXXIII.)   (CXXIV.)

(CXXV.)   (CXXVI.)

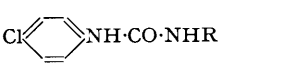
A series of related biurets,[51] monothiobiurets, and dithiobiurets [52] was also prepared and examined. These included (CXX), (CXXI), and (CXXII) which bore an obvious relationship to (CI), and also a number of *iso*-analogues, for example, (CXXIII), (CXXIV), and (CXXV). The latter were, for the most part, intermediate substances resulting from the action of *iso*cyanates and *iso*thiocyanates on ureas and *iso*ureas which were then hydrolysed or sulphurised to the corresponding normal biurets. (CXXV), obtained by further ethylation of the corresponding 1-*p*-chlorophenyl-4-ethyl-5-*iso*propyl-4-*iso*dithiobiuret (from *iso*propyl *iso*thiocyanate and *N*-*p*-chlorophenyl-*S*-methyl*iso*thiourea) was of particular importance since with hydrazine

[51] Curd, Davey, and Richardson, *J.*, 1949, 1732.
[52] Curd, Davey, Richardson, and Ashworth, *J.*, 1949, 1739.

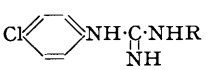
it gave the 1 : 2 : 4-triazole (CXXVI) [52] so closely related to (CI), but which, like all the other substances just described, was completely devoid of antimalarial activity.

*Miscellaneous.*—Many hundreds of compounds fall under this heading, resulting for the most part from single speculative variations on the main themes, and it is not possible to record them all here. There are a number of groups and individuals, however, the results from which provide some interest in relation to the main developments, and a selection is given below.

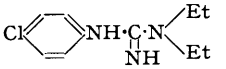
In most of the types discussed in the foregoing sections, the central nucleus could be regarded as providing a chemical link between an aromatic system and an alkylamino-group. At a comparatively early stage the possibility that therapeutic effect might result from a much less complex type of linkage was examined, and simple ureas and guanidines were made. These included compounds of type (CXXVII) (compare the active CXVIII), (CXXVIII), (CXXIX), CXXX), and (CXXXI). Of these, only (CXXVIII) showed activity, and that marginal.



(CXXVII.)

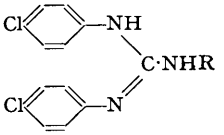


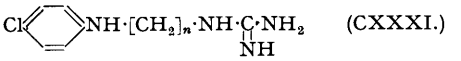
(CXXVIII.)



(CXXIX.)

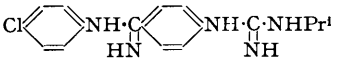
(CXXX.)



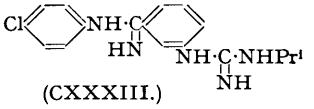


(CXXXI.)

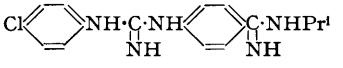
A further variation of the diguanide type was provided by a number of structures in which the diguanide chain was interrupted by a benzene ring, giving, *e.g.*, (CXXXII), (CXXXIII), and (CXXXIV), and the related simpler unalkylated types (CXXXV), (CXXXVI), and (CXXXVII). These substances were all derivatives either of diphenylamidine or diphenylguanidine [compare types (LXXI) and (LXXII)]. The former were made from the corresponding nitrobenzchloroanilides *via* the imino-chlorides and nitroamidines, and the latter from the diphenylguanidine-*p*-imino-ethers. (CXXXV) was slightly active, the remainder inactive.



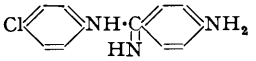
(CXXXII.)



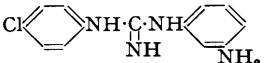
(CXXXIII.)



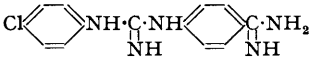
(CXXXIV.)



(CXXXV.)



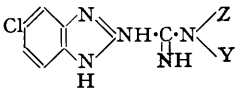
(CXXXVI.)

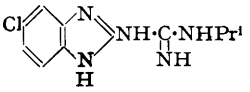


(CXXXVII.)

Benziminazolylaminopyrimidines (LXV), analogous to the original arylaminopyrimidines, have already been mentioned. Several benziminazoles similarly analogous to the diguanide agents were prepared, mainly by reaction of the appropriate alkyldicyandiamide with an *o*-phenylenediamine. Chief interest attaches to the series (CXXXVIII) and (CXXXIX). The

(CXXXVIII.)





(CXXXIX.)

latter, *i.e.*, the exact analogue of (CI; proguanil), was entirely devoid of activity. The former, especially (CXXXVIII; Z = Y = Me), was quite active at high and toxic doses.

## Discussion.

The beneficial effect of a chemotherapeutic agent may arise either from an indirect action involving stimulation of the host in some manner, or more commonly from a direct action on the pathogen. In the latter event, two factors have to be considered, access and intrinsic activity. The first is compounded of a number of variables such as solubility, absorption from the host alimentary tract or site of injection, rate and mode of excretion, metabolism, and so on, while the second, although at first glance simpler, might involve permeability considerations with respect to the cell, if there be such, of the disease incitant, diffusion to the ultimate site of action, and also consideration of the ability of the cell to detoxify or rid itself

of the foreign chemical substance. In a disease such as malaria, the situation is complicated by the existence of a life cycle for the causative parasite so that therapeutic effect might result from drug action at one or more different phases, some of which are intracellular in habitat.

The existence of this large number of variables, and many more, is frequently overlooked by those who would too easily rationalise the course of chemotherapeutic research, but equally it confounds those who attempt to explain observed facts in terms of simple and often entirely artificial systems. That is why the use of the " working hypothesis " concept based on observation and the intelligent development of leads, related where possible to a theoretical mode of action, must constitute the foundation for speculative research in this field for a long time to come.

For the same reasons, the mass of chemical and biological data that has arisen from the research under consideration is still insufficient for a critical analysis to be made, since modifications in chemical structure that bring about parallel changes in the overall chemotherapeutic effect of several drug types may do so by influencing different and separate pharmacological characteristics. For example, the basic alkyl side chain that appears in many active compounds may have a diversity of functions. It is known, for example, that its presence in a molecule leads to concentration in the erythrocytes and many other solid tissues of the animal body, clearly a desirable effect when dealing with an infection that is predominantly intracellular, but unpublished work, carried out in these laboratories by Dr. Madinaveitia on bacterial growth, would also suggest that the basic side chain exhibits specificity with regard to intrinsic antimicro-organismal action. For convenience, details of the basic side-chain groups in respect to the different drug types were not given in the preceding sections, but it was notable that in some instances optimum activity was provided by the lighter groups, *e.g.*, 2-dimethylaminoethylamino-, while in others the heavier (longer) and more complex groups were required (as in I). In still others, maximum activity resulted from the use of side chains of intermediate size. This would accord with the concept of multiplicity of function for the side chain, conditioned by the different physical and chemical properties inherent in the remainder of the drug molecule.

Study of many of the active types reveals, however, that strong basic properties can be provided by groups other than di- and tri-alkylamine systems. Thus, the phenylguanidinopyrimidine (XXXVI) was highly active, in contrast to the related inactive and much less basic (XVII). This result suggested that the phenylguanidine residue was operative. Physicochemical studies supported this view. For example, the dissociation constant of *p*-chlorophenylguanidine itself was found to be 10·6, while that for *p*-chloroaniline, the corresponding unit in (XVII), was 4·7.[53] The dissociation constant associated with the terminal nitrogen atom of typical basic side-chain amines was in the region 9·5—10·5.[51] On this basis, it is perhaps not surprising that the diphenylguanidine analogue (LXXII) of (XXXVI) exhibited some antimalarial activity, while the diphenylamine (LXXI) did not.

In the diguanide series, the diguanide chain was presumed to provide the necessary basic function. Thus, the higher of the two dissociation constants of *p*-chlorophenyldiguanide was found to be 10·4.[53] This was not varied more than a single unit by mono- or poly-alkylation of the nitrogen atoms, so that the various activations and deactivations produced by such modification could not be explained on this basis.

The presence of two basic groups each with a p$K$ value around 10 in the same molecule always deactivated in the numerous drug types examined. These could be two alkylamine systems, or two diguanide structures (of which several were made, but no examples are given above), or one of each type.

The need for one group in the molecule giving a high overall cationic charge thus seemed clear, but a more subtle requirement appeared in relation to a second basic function. The early working hypothesis on which these researches were launched took note of the presence within active molecules of potential tautomerism. Although initially concerned with a guanidine type of structure (acyclic or cyclic), further study showed that it could be associated with the simpler amidine configuration occurring either as such, or as a vinylogue, for example, in the 4-aminoquinolines and (II). As a consequence, it became much more attractive to abandon the principle of tautomerism, which in any event never had any foundation in fact, and reconsider the problem in the light of the properties of amidine groups, conditioned as they were by considerations of resonance energies. It then appeared that in all active molecules of the types being discussed an amidine group or a vinylogous structure was essential, associated

[53] Gage, *J.*, 1949, 221.

either with an aromatic or an alkyl group, or both, and so influenced by relevant substituents that at physiological hydrogen-ion concentrations it was present largely as the amidinium ion. This was an empirical observation that at the moment seemed quite incapable of explanation, but which accounted for a number of apparently anomalous therapeutic results.

For example, the second p$K$ constants associated with the active arylaminopyrimidines (XVI) and (XXV), which must be due to the heterocyclic system, were both around 6·6, while those of the analogous, but inactive arylaminopyrimidine (XXVI) and arylaminotriazine (LXVII) were 5·5 and 4·05, respectively.[55] The active 4-aminoquinazolines of type (LXXXII; Z = H) had a value of about 7·6.[54]

The diguanides apparently constituted a special case.[53, 54] These structures had a second dissociation constant, but too low (about 2) to have any biochemical significance. Instead, it must be assumed that the first dissociation constant not only provided the function normally associated in other types with the basic side chain, but also fulfilled the second criterion for activity in that it was of sufficient magnitude and arose from the amidino- or hetero-portions of the molecule, which must then be in the requisite protonised form under physiological conditions.

In considering antimalarial drugs as a whole, there are some notable omissions from the scheme, for example, quinine, the quinoline-carbinols, and pamaquin (I), except that all of these carry a side-chain alkylamine group of requisite base strength. At the moment, no explanation for these anomalies can be offered, except to comment that no other current theories of drug activity are universal in their application.

Before leaving the subject of basic function, more especially that associated with the side chains, there are further relevant points. The specificity of orientation and weight of the pendant dialkylaminoalkylamino-group for optimum activity in the various drug types under discussion has already been mentioned. In the diguanide class, however, no such specificity was seen, in that, whatever variation was made in the nature of the substituents either in the benzene ring or in the diguanide chain, optimum activity was always observed with a propylamino-, and in particular an *iso*propylamino-, as the terminal group (CI). Methylamino- or dimethylamino- at this position always gave inactivity. By contrast, these small groupings provided highest activity in the seemingly closely related guanylureas (CXVIII) and guanidinobenziminazoles (CXXXVIII). Indeed the *iso*propyl homologue of the latter type was inactive.

So far the profound influence of nuclear substitution of one kind or another into the many drug types described, has not been discussed. Changes in basic properties may sometimes be concerned, but usually these would be of insufficient magnitude to account for the all-or-none effects commonly observed. For example, activation of the simple pyrimidines (IV) and (VII) by the introduction of methyl groups into position 5 hardly influenced either p$K_1$ or p$K_2$, and moreover, similar substitution in other active types had the reverse effect on biological action. Likewise, the complete inactivity of the *o*-substituted phenyldiguanides could not be explained on this basis.

Undoubtedly, the most notable changes in activity were caused by variation of substituents in the aromatic groups of the many drugs described. The position of chlorine was outstanding, and it might usefully be recalled here that this substituent occurs time and again in chemotherapeutic researches where antagonism to a growth factor is being sought. In some of the antimalarial drugs, its effect was equalled by the other halogens, in others it was almost unique. Nitro- and cyano-groups could often replace chlorine, and still give high activity, but any hypothesis which attempted to sort out potentiating substituents on the basis of inductive effect, which at first sight seemed attractive, was invalidated by the host of anomalies that could be adduced. The effect of disubstitution in a single benzene ring also produced anomalies. For instance, the 3 : 4-dichloroanilino-residue, as in (XVIII; X = Y = Cl), had little influence on antimalarial activity by comparison with 4-chloroanilino-. In the isomeric (XXIV) it completely deactivated, while in the diguanide series (CI) it gave some of the most active antimalarials known. Indeed, the effects produced in the diguanide series with respect to halogeno-substitution in general were often the opposite of those seen in the arylamino- and arylguanidino-pyrimidine prototypes.

The influence of substituents in the fused-ring systems was equally unpredictable. For instance, in the arylaminoquinazoline (LXXV) and quinoline (LXXXIII) types, the introduction of chlorine into the benzene ring of the heterocyclic nucleus (positions 6 or 7) never increased but frequently decreased therapeutic activity.

[54] Gage, *J.*, 1949, 1458. [55] *Idem, ibid.*, p. 469.

In the simpler non-aryl-substituted dialkylaminoalkylamino-quinazolines (LXXXII), -quinolines (LXXXVII), -cinnolines (XCVII), and -quinoxalines (XCV), chlorine substituents at positions 6 and 7 gave marked potentiation of activity. In the last-named class, a chlorine substituent in position 2, that is α to a heterocyclic nitrogen and therefore labile, was sufficient to induce activity in an otherwise inactive type (compare XCI, and XCIV; X = H).

Finally, the possible relevance of the mass of facts to the mode or modes of action of the many compounds requires comment. The possible significance of a structural resemblance between some of the earlier drugs and prosthetic components of certain respiratory enzymes has already been mentioned. Apart from limited information gained by studying the effect of these drugs on bacterial growth, these notions still remain essentially speculative with respect to the substances examined, and even more so in consideration of some of the later types, in particular the diguanides, where some relation to the porphyrin enzymes has been proposed. Unfortunately, biochemical knowledge relating to the malaria parasite is extremely difficult to accumulate, and it might have been hoped therefore that some indirect evidence would have been forthcoming from a study of drug structure and antimalarial activity. The diversity of chemical types producing antimalarial action is, however, so great, that any possibility of this result's being achieved seems very remote at present. Indeed, one of the few conclusions to be drawn from this research is that a chlorophenyl residue, associated but not necessarily in conjugation with an amidine or extended amidine system, and in a structure that provides the necessary cationic functions, will more often than not lead to an active agent. What cannot be predicted on present knowledge is the intensity of action, or its relation to toxic side-effects. For the moment, then, the discovery of new antimalarial agents, and indeed of new chemotherapeutic agents of all types, still remains dependent upon the inventive and preparative ingenuity of the organic chemist.