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ANTIMALARIAL COMPOUNDS AS ANTAGONISTS OF ADENOSINE

BY

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The possible antagonism of the action of adenosine on some tissues by antimalarial drugs has been studied with the object of obtaining some information on the mode of action of this type of drug.

METHODS

Antagonism of adenosine

The following techniques were used in these experiments:—

(1) The isolated hen's caecum.—The action of adenosine on this tissue (Barsoum and Gaddum, 1935) was studied before and after the addition to the bath of known concentrations of antimalarials. Although it was possible to demonstrate an antagonism between these compounds and adenosine by this method, satisfactory results were not obtained because of the intense relaxation of the caecum produced by the antimalarial substances themselves.

(2) The measurement of the duration of the auriculo-ventricular (a.v.) block produced by intravenous administration of adenosine in the anaesthetized guinea-pig (Drury and Szent-Györgyi, 1929). The guinea-pig's heart is not so sensitive to adenosine as the hen's caecum, but this method has the advantage that the antimalarials do not alter the electrocardiogram (e.c.g.) in sub-toxic doses.

Three series of experiments were carried out with this technique.

(*a*) In the first series, hereafter called "acute," the compounds under test were administered by slow intravenous injection. Guinea-pigs of 250–400 g. body-weight were anaesthetized with pentobarbitone (nembutal), given intraperitoneally. A cannula was inserted into one of the jugular veins and the animals maintained under artificial respiration during the experiments by means of a Palmer's Starling Universal pump for small animals; $1:10^{-4}$ or $1:10^{-3}$ adenosine in normal saline was administered intravenously by the jugular vein cannula, never more than 1 c.c. of adenosine solution being injected. Electrocardiograms were taken, lead II, immediately after the administration of adenosine by means of a Cossor electrocardiograph modified for fast heart rates.

In each experiment a series of doses of adenosine from 0.1 to 0.3 mg./kg. was first administered in order to produce varying degrees of a.v. block. The cannula was then washed with the solution of antimalarial and the drug slowly infused by means of a motor-driven syringe which delivered the solution at the rate of 0.02 ml./min. This infusion was maintained for 30 min. after which the cannula was washed with adenosine solution and a second series of adenosine injections was administered in doses large enough to produce measurable heart blocks.

The dose of antimalarial was calculated in mg./kg./min. and adjusted by varying the concentration according to the body-weight of each animal. At least 3 guinea-pigs were used for each dose level of antimalarial.

This series was used mainly for the selection of chemical types able to antagonize the action of adenosine.

(*b*) In the second series the compounds were administered orally in a dosage regime similar to that used by Davey (1946) for the assessment of antimalarial activity against *P. gallinaceum* in chicks.

Batches, usually of 6 guinea-pigs each, were used for each dose level of every compound tested. The animals received two doses daily for $3\frac{1}{2}$ days, a total of 7 doses. The drugs were administered in solution in water, or in fine suspensions when insoluble, by means of a stomach tube. After the 7th dose, the guinea-pigs were anaesthetized with pentobarbitone and a series of adenosine injections was administered as described above.

These experiments will be referred to as "chronic" later on.

(*c*) Some experiments were carried out with heart-lung preparations of guinea-pigs. The method was essentially that described by Cruickshank (1945) for the rat; 25 ml. of heparinized guinea-pig's blood were used in the apparatus. The elastic peripheral resistance was adjusted to give a pressure of 80–100 mm. Hg in the aortic cannula; e.c.g. records were taken with leads placed in the superior vena cava and in the apex of the heart. Adenosine was injected into the venous cannula of the preparation.

Assessment of the adenosine action and of the antagonistic action of antimalarials

The action of adenosine in normal and treated guinea-pigs was assessed by the duration of the a.v. block as recorded in the electrocardiograms obtained during experiments. The duration of this action was measured as the time which elapsed between the first and last P wave not followed by a ventricular complex. No attempt was made to measure the duration of these effects by the inspection of the duration of the P–R intervals. From these results, the doses of adenosine which would have produced a.v. blocks of 5 seconds' duration were calculated by graphical methods.

An attempt to compare the intensity of the adenosine antagonism shown by the substances under test was made by calculating the doses of antagonists which would reduce the sensitivity of the guinea-pigs to adenosine to one-half of the normal (see Tables II and III).

The results so obtained were only semi-quantitative and can only be taken as a rough measurement of the intensity of the antagonism.

Antimalarial activity

The data on the antimalarial activity of the drugs used in this work was obtained in our laboratories by Dr. D. G. Davey using chicks infected with *Plasmodium gallinaceum* (Davey, 1940). Most of these results have already been published in the papers dealing with the synthesis of the compounds in question.

Materials

The substances used in this work can be classified in the groups indicated in Table I. In this Table the chemical constitution and the trivial name or code number of each compound is given. In the text these numbers and names will be used in preference to the full chemical names. In each group substances have been selected with as low and as high antimalarial action as possible.

Most of the substances used in our work were prepared in these laboratories (Curd and Rose, 1946a; Curd, Davies, and Rose, 1946; Curd, Davies, Owen, Rose, and Tuey, 1946; Curd, Richardson, and Rose, 1946; Curd and Rose, 1946b; Basford, Curd, and Rose, 1946; Curd and Rose, 1946c; Curd and Landquist, 1948; Curd, Landquist, and Rose, 1947). Others were obtained from Professor A. R. Todd (Hull, Lovell, Openshaw, Payman, and Todd, 1946; Hull, Lovell, Openshaw, and Todd, 1947). Quinidine and hydroquinidine were a gift of Messrs. Amsterdamshe Chininefabriek, Amsterdam. The adenosine used was purchased from Messrs. British Drug Houses, Ltd.

RESULTS

(1) *Antagonism in the hen's caecum*

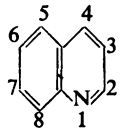
This antagonism was studied by measuring the relaxation of this tissue produced by known concentrations of adenosine in normal Tyrode's solution and in Tyrode's solution containing known quantities of antimalarial.

Mepacrine usually had practically no visible effect on the caecum in concentrations of $1 : 10^{-7}$ to $1 : 10^{-6}$, but it decreased the action of adenosine in this concentration range. Higher concentrations caused an intense relaxation of the caecum and at the same time reduced still further the action of

TABLE I

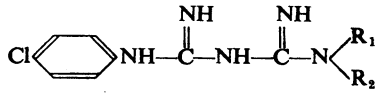
CHEMICAL COMPOSITION OF SOME OF THE ANTI-MALARIAL AND RELATED COMPOUNDS USED

Quinolines



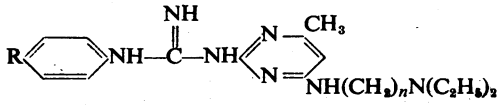
Name or code number		Substituents
Pamaquin ..	6 8	OCH_3 $NH.CH(CH_3)(CH_2)_3N(C_2H_5)_2$
Chloroquine	4 7	$NH.CH(CH_3)(CH_2)_3N(C_2H_5)_2$ Cl
5735 ..	2 and 3 4 7	CH_3 $NH.CH(CH_3)(CH_2)_3N(C_2H_5)_2$ Cl
3738 ..	2 4 7	OH $NH(CH_2)_3N(C_2H_5)_2$ Cl

Biguanides



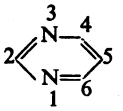
Name or code number	Paludrine	4430	5093
R_1	$CH(CH_3)_2$	$CH(CH_3)_2$	CH_3
R_2	H	CH_3	H

Arylguanidinopyrimidines



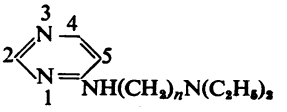
Code number	3349	3749	3742
R	Cl	Cl	CH_3O
n	2	3	2

Anilinopyrimidines



Code number	Substituents			
	2	4	5	6
2666	(*p*)Cl.C_6H_4NH	CH_3		$NH(CH_2)_2N(C_2H_5)_2$
3300	(*p*)Cl.C_6H_4NH	CH_3		$NH.CH(CH_3)(CH_2)_3N(C_2H_5)_2$
4070	CH_3	(*p*)Cl.C_6H_4NH		$NH(CH_2)_2N(C_2H_5)_2$
3756	(*p*)Cl.C_6H_4NH	—CH = CH — CH = CH—		$NH(CH_2)_2N(C_2H_5)_2$
4316	$(C_4H_9)_2N(CH_2)_3NH$	CH_3		(*p*)Cl.C_6H_4NH

Pyrimidines



Code number	Substituents			
	2	4	5	*n*
4747	CH_3	CH_3		2
4419	CH_3	NH_2	CH_3	2
3920	NH_2	CH_3		2
3448	NH_2	CH_3	CH_3	2
4746	NH_2	NH_2		3
4420	CH_3	NH_2		3
4450	CH_3	CH_3	$CH_2C_6H_5$	3
4184	NH_2	—$CH_2CH_2CH_2CH_2$—		3

adenosine. Fig. 1 summarizes the results of one of these experiments.

Similar results were obtained with quinine, 3349, and 2666, but the intense relaxation of the caecum produced by each of these antimalarials made the assessment of the antagonism somewhat difficult. For this reason this method was abandoned after a few trials with different antimalarial drugs.

(2) *Antagonism between adenosine and antimalarials on guinea-pig's heart*

The duration of the a.v. block produced by the intravenous administration of adenosine is proportional to the dose. The minimum effective dose of adenosine, in guinea-pigs anaesthetized with pentobarbitone, was 0.1 mg./kg., and this generally produced a block of short duration during which one or two independent P waves were recorded. The average duration of this block in a series of 40 guinea-pigs was found to be 0.67 sec. Blocks of 10–15 sec. duration were generally obtained with 0.3 mg./kg. of adenosine. The mean durations of the blocks produced in this control series with doses of adenosine from 0.1 to 0.3 mg./kg are given in Table III.

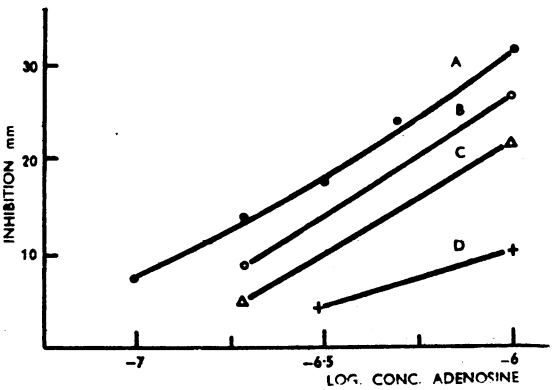


FIG. 1.—Influence of mepacrine HCl on the response of the hen's caecum to adenosine. Abscissae—log concentration of adenosine. Ordinates—relaxation of caecum in millimetres. A—normal Ringer. B—in mepacrine 10^{-7}. C—in mepacrine 10^{-6}. D—in mepacrine $10^{-5.5}$.

TABLE II

MINIMAL DOSES WHICH ANTAGONIZE THE ACTION OF ADENOSINE IN ACUTE EXPERIMENTS

Drug	Minimum antagonistic dose mg./kg./min.	Dose which halves sensitivity to adenosine mg./kg./min.
Quinine HCl ..	0.75	0.8
Mepacrine HCl	0.25	0.35
Pamaquin HCl	too toxic at 0.25	..
Chloroquine H_3PO_4 ..	0.25	0.45
Paludrine lactate	1.25	1.25
3349	3.0(?)	3.0(?)
2666	0.6	0.9
Procaine ..	Inactive at 2.0	..
Suramin ..	,, at 5.0	..
Methylene blue	,, at 2.0	..
Trypan blue ..	,, at 2.0	..
Trypan red ..	,, at 2.0	..
Sulphadimethyl-pyrimidine ..	,, at 8.5	..

A. "Acute" experiments

Table II gives the minimum antagonistic doses of the compounds tested in this series and the doses which reduce the action of adenosine to one-half.

Mepacrine

The duration of the blocks produced by adenosine after the continuous administration of 0.1 mg./kg./min. mepacrine for 30 min. did not differ significantly from that obtained before the antimalarial had been given. An appreciable reduction of the duration of the action of adenosine was observed after the administration of 0.25 mg./kg./min. mepacrine. The decrease in the effects of adenosine was more marked after 0.35 and 0.5 mg./kg./min. of antimalarial had been given. Fig. 2 shows these results.

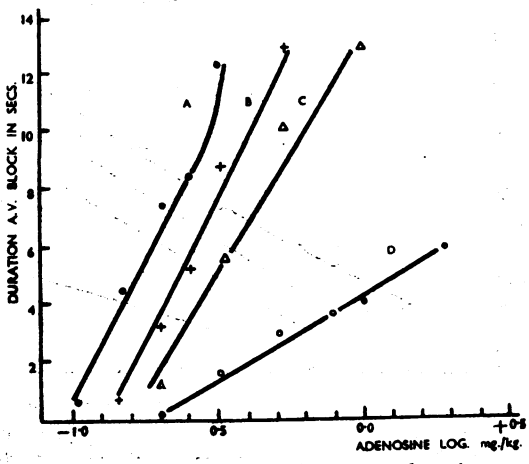


FIG. 2.—Acute antagonism between adenosine and mepacrine in the guinea-pig. Duration of the a.v. block produced by adenosine in guinea-pig after a continuous injection of mepacrine HCl for 30 min. Abscissae—log. dose of adenosine in mg./kg. Ordinates-duration of block in seconds. A—before administration of mepacrine. B—after 0.25 mg./kg./min. mepacrine. C—after 0.35 mg./kg./min. mepacrine. D—after 0.5 mg./kg./min. mepacrine.

This antagonism, however, is reversible. When the response to adenosine was examined at different times after the end of the infusion of mepacrine, it was found that the duration of the block became progressively longer, and in 90 min. the adenosine effects were about the same as those of the control series (Fig. 3).

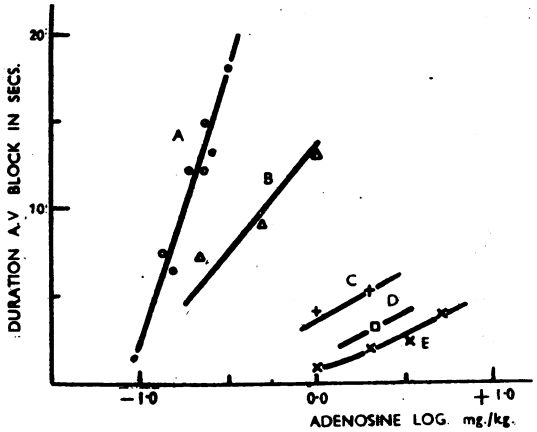


FIG. 3.—Duration of the a.v. block produced by adenosine before and at different times after the end of the continuous injection of mepacrine HCl of 0.5 mg./kg./min. for 30 min. Abscissae-dose of adenosine as log mg./kg. Ordinates-duration of block in seconds. A (dots)—before mepacrine. E—immediately after mepacrine. D—15 min. after mepacrine. C—30 min. after mepacrine. B—60 min. after mepacrine. A (circles)—90 min. after mepacrine.

Pamaquin

It was impossible to study the action of pamaquin by this method because of its high toxicity. Doses as small as 0.25 mg./kg./min. of pamaquin HCl were too toxic and caused bradycardia and right predominance in the e.c.g. Larger doses of pamaquin HCl killed the guinea-pigs before the end of the infusion period.

Chloroquine

Chloroquine is less toxic than pamaquin. Doses from 0.25 to 1 mg./kg./min. were administered without causing undue alterations of the e.c.g. The

minimum doses of chloroquine that antagonize the action of adenosine are of the order of 0.25 mg./kg./min.

Quinine

Quinine was inactive when administered in doses of 0.5 mg./kg./min. A good reduction in the duration of the a.v. block produced by adenosine was observed after doses of 0.75 mg./kg./min. had been infused.

Paludrine

Paludrine was found to be active in doses from 1.0 mg./kg./min. and the adenosine antagonism was very marked with 2 mg./kg./min. In some of these experiments the paludrine concentration of the blood was as high as 7 to 9 mg./l.

3349

3349 was found to be even less active than paludrine in antagonizing the effects of adenosine in this "acute" series. Doses of 2 mg./kg./min. were not antagonistic; doses of 3 mg./kg./min. showed a clear antagonism but were too toxic to the heart. The e.c.g. tracing showed cardiac irregularities and bradycardia, which made it impossible to assess the intensity of the antagonism.

2666

The antagonism to adenosine with this compound was more intense than with paludrine or 3349 but less than with quinine, mepacrine, or chloroquine. Doses of 1 mg./kg./min. of 2666 were strongly antagonistic, whereas 0.5 mg./kg. had no action on the adenosine effects.

Substances without adenosine antagonism

A selection of substances, some of them without antimalarial activity, were tested by this method. Procaine, suramin, methylene blue, trypan red, trypan blue, and sulphadimethylpyrimidine were tested; none of these antagonized the adenosine effects.

B. "Chronic" experiments

The adenosine antagonism shown by antimalarials of different types was studied in more detail in this series of experiments. Details of these experiments are given in Table III, which also gives the doses of antagonists which halve the sensitivity of the animals to adenosine.

Acridine derivatives: mepacrine

The heart blocks produced by adenosine in guinea-pigs that had received seven doses of mepacrine in 3½ days were shorter than those obtained in normal guinea-pigs.

The minimum dose of mepacrine which showed this effect was 2 × 5 mg./kg. daily. Larger reductions in the duration of the adenosine effects were observed when the dose of mepacrine was increased from 5 to 40 mg./kg. twice daily. With the last-mentioned dose, the minimum effective dose of adenosine was 0.3 mg./kg., which produced the same effect as 0.1 mg./kg. in normal guinea-pigs. Details of the results of these experiments are shown in Table III and Fig. 4.

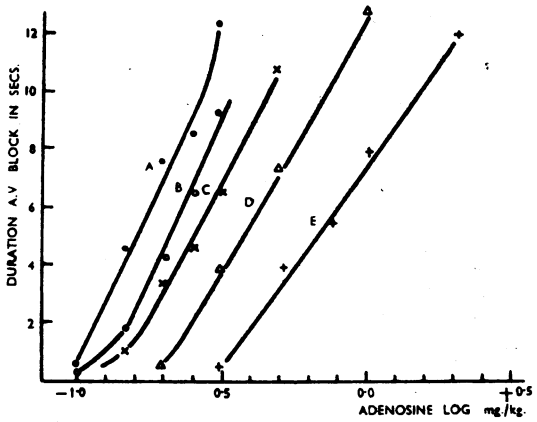


FIG. 4.—"Chronic" antagonism between adenosine and mepacrine HCl in guinea-pigs. Duration of the a.v. block produced by adenosine in guinea-pigs treated by mouth with different doses of mepacrine. Abscissae-dose adenosine as log. mg./kg. Ordinates-duration of block in seconds. A—controls. B—mepacrine HCl, 2 × 5 mg./kg./day. C—mepacrine, 2 × 10 mg./kg./day. D—mepacrine, 2 × 20 mg./kg./day. E—mepacrine, 2 × 50 mg./kg./day.

Quinoline derivatives

Pamaquin given by mouth as the methylene bis-β-hydroxynaphthoic acid salt was tolerated by guinea-pigs in doses up to 20 mg./kg. twice a day for 3½ days. A reduction of the duration of the adenosine block was obtained in guinea-pigs after treatment for 3½ days with 2 × 10 mg./kg. pamaquin salt, equivalent to 6.25 mg./kg. base.

Chloroquine diphosphate was antagonistic to adenosine in doses of from 2 × 5 mg./kg./day. The low toxicity of this substance allowed the investigation of its antagonism in a range of doses from 5 to 50 mg./kg. twice daily. Doses of adenosine that produced blocks of 5 sec. duration in guinea-pigs treated with pamaquin or chloroquine did not show any significant difference between the intensities of the antagonism produced by these two substances.

TABLE III

ANTAGONISM OF ADENOSINE BY ANTIMALARIALS ADMINISTERED ORALLY TWICE DAILY FOR THREE AND A HALF DAYS (7 DOSES)

Compound	Dose (mg./kg.)	Number of animals	Adenosine injected mg./kg. Mean duration of auriculo-ventricular block (seconds) 0.10	0.15	0.2	0.25	0.30	0.50	1.00	Dose which halves sensitivity to adenosine mg./kg.	Minimal dose for full antimalarial activity mg./kg.
Controls (no drug)		40	0.67	4.58	8.12	9.65	12.58	—	—		
Quinine (dihydrochloride) ..	50	3	0.4	1.2	2.48	3.6	5.3	—	—	60	40
	100	6	0.0	0.4	1.6	3.6	4.9	10.15	16.06		
	150	6	0.0	0.9	2.1	3.2	4.4	7.0	11.7		
	200	6	0.0	0.0	0.5	1.0	1.38	2.86	9.4		
Quinidine (base)	50	5	0.0	3.9	5.3	7.5	10.9	—	—	60	40
	100	6	0.0	0.7	1.53	2.57	3.3	—	—		
Hydroquinidine (base)	100	6	0.5	2.1	3.5	6.0	7.5	—	—	100	80
Mepacrine dihydrochloride ..	5	6	0.31	3.01	4.36	6.55	9.23	—	—	14	40
	10	5	0.0	0.45	3.21	—	6.54	15.5	—		
	20	6	—	0.12	1.75	—	3.72	7.15	12.7		
	40	6	—	—	—	—	0.65	3.46	8.2		
Pamaquin methylene bis-β-hydroxy-naphthoate	10	8	0.9	3.4	5.3	7.7	9.5	—	—	9	4
	20	12	0.25	1.86	3.7	4.1	6.88	—	—		
Chloroquine diphosphate ..	5	6	0.0	2.1	3.75	5.9	8.5	—	—	9	5
	10	6	1.5	2.3	3.55	5.05	7.4	11.4	—		
	20	5	0.0	0.0	0.6	2.6	4.0	7.8	13.2		
	50	6	—	—	0.0	0.0	0.0	4.6	7.0		
5735 dihydrochloride	20	6	0.43	1.2	1.7	2.6	4.5	—	—	33	80
	50	6	—	—	0.6	3.4	3.7	6.4	—		
3738 base	50	6	0.0	2.8	5.2	8.05	10.8	—	—	70	Inactive at 400
Paludrine lactate	10	6	0.7	—	6.8	—	11.7	—	—	15	5
	20	6	0.0	2.5	5.4	8.3	9.2	—	—		
4430 acetate	20	6	0.0	1.2	5.1	7.25	12.8	—	—	40	20
	50	6	0.0	1.7	3.9	5.9	7.5	—	—		
5093 hydrochloride	20	6	2.38	4.2	7.34	9.32	12.25	—	—	No action	Inactive at 160
	50	6	1.8	4.2	6.7	9.8	15.9	—	—		

TABLE III (*continued*)

ANTAGONISM OF ADENOSINE BY ANTIMALARIALS ADMINISTERED ORALLY TWICE DAILY FOR THREE AND A HALF DAYS (7 DOSES)

Compound	Dose (mg./kg.)	Number of animals	Adenosine injected mg./kg. 0.10 0.15 0.2 0.25 0.30 0.50 1.00 Mean duration of auriculo-ventricular block (seconds)							Dose which halves sensitivity to adenosine mg./kg.	Minimal dose for full antimalarial activity mg./kg.
3349 base	20	5	0.1	2.0	3.94	5.16	6.56	—	—	20	40
	50	5	0.0	0.16	0.8	2.04	3.58	6.5	—		
	75	4	—	—	0.0	0.0	0.28	1.5	8.5		
	100	6	—	—	—	—	0.0	0.0	0.0		
3672 dihydrochloride	20	6	0.8	2.9	4.6	6.8	10.2	—	—	30	200
	100	6	—	—	0.0	—	1.0	4.2	13.2		
3742 base	20	6	0.12	5.5	8.9	10.8	14.4	—	—	Inactive	Inactive at 200
	50	6	0.0	2.48	4.7	7.2	10.1	—	—		
2666 dihydrochloride	50	6	1.5	4.5	6.6	9.7	13.2	—	—	90	80
	100	6	0.0	1.9	1.1	3.65	7.62	13.9	—		
	200	6	—	0.0	0.0	3.0	4.5	6	—		
3300 dihydrochloride	50	6	0.35	4.16	5.66	7.28	7.4	—	—	75	200
	75	6	—	—	0.8	2.55	6.0	6.56	—		
	100	5	—	—	0.66	—	1.7	3.9	6.74		
4070 base	20	6	0.86	5.4	8.2	10.5	14.5	—	—	60	Inactive at 120
	50	6	—	—	2.2	5.7	7.0	13.4	—		
	100	6	—	—	1.1	—	4.3	8.1	17.72		
3756 base	20	6	0.5	6.26	9.3	10.4	15.2	—	—	No action	Slightly active at 40
	50	6	0.0	2.5	5.5	6.9	9.2	—	—		
4316 dihydrochloride	100	6	0.0	2.1	3.6	6.0	8.2	—	—	100	40
4747 base	50	6	—	4.1	7.2	10.5	11.05	—	—		Trace activity at 240
4419 base	50	6	—	1.8	2.7	4.4	6.2	—	—		80
3448 base	50	6	—	—	0.4	—	2.8	6.1	10.7		100
4746 base	50	6	0.6	3.9	4.5	7.2	9.5	—	—		Trace activity at 400
4420 base	50	6	0.0	0.4	1.2	2.6	5.5	—	—		80
4459 base	50	4	—	—	—	0.27	0.35	4.9	4.0		80
4184 base	50	4	—	—	0.3	1.02	3.1	6.9	10.9		120

The antagonism to adenosine shown by some derivatives of chloroquine such as 5735 and 3738 was weaker than that of the parent compound. The order of intensity of the antagonism shown by these substances was practically parallel to that of their antimalarial activity. Chloroquine and pamaquin, both of which have high antimalarial activity, antagonize adenosine better than 5735, which has low antimalarial activity. 3738, which has hardly any antimalarial action, did not modify the action of adenosine.

Cinchona alkaloids

The cinchona alkaloids studied in this series were quinine, quinidine, and hydroquinidine. In the experiments with quinine it was observed that the antagonism to adenosine was marked in guinea-pigs for 2 hours after the last dose of alkaloid, but in experiments carried out later than that adenosine produced blocks of about the same duration as in the controls. As it is known that quinine is metabolized in the body fairly quickly, it was thought that these differences in the results were due to the rapid disappearance of the drug from the blood. For this reason the dosage schedule for cinchona alkaloids was modified in the following way: The six guinea-pigs used for each dose level received their seventh dose of drug at the usual time, about 9.30 a.m., and three of them were used before 12 noon. The remaining three received an eighth dose at about 1.30 p.m. and were used immediately after. With this modification of the dosage regime the results for both groups were about the same.

Quinine and quinidine were adenosine antagonists in guinea-pigs when doses larger than 50 mg./kg. were administered twice daily. The intensities of the antagonism to adenosine produced by these two alkaloids were practically the same, and it is interesting to note that both are equally active against experimental malaria.

Hydroquinidine, which as an antimalarial is about half as active as quinine, produced a milder action than the latter.

N^1-phenyl-N^5-alkylbiguanides

In the series of drugs of the paludrine type we selected one drug without antimalarial activity (5093), and compared it to 4430 and paludrine itself (4888). The inactive compound was found not to antagonize the action of adenosine even in the maximum tolerated doses.

Paludrine antagonized adenosine when doses from 2 × 10 mg./kg./day were administered to guinea-pigs. Unfortunately it was not possible to increase the dose beyond 2 × 20 mg./kg./day; all guinea-pigs treated with this dose survived this treatment for 4–5 days, but although a small increase of the dose to 2 × 30 mg./kg./day did not kill any animal during the first 1½–2 days it produced a 100 per cent mortality if the treatment was maintained for a longer time. This high toxicity of paludrine, when given repeatedly to guinea-pigs, contrasts with its moderately low toxicity when given in single doses. Similar results have been found by Butler, Davey, and Spinks (1947) in other laboratory animals, and it is probable that they are due to the formation and accumulation of some highly toxic metabolite of paludrine during the period of repeated administration.

The minimum dose of 4430 which antagonized the action of adenosine was 2 × 30 mg./kg./day. This drug has a lower antimalarial activity than paludrine and at the same time it is a weaker antagonist.

Arylguanidinopyrimidines (Curd and Rose, 1946a)

A similar gradation of antagonistic activity was found among the substances of this type, and their antagonistic activity ran parallel to their antimalarial action. 3349 has an antimalarial activity and a capacity to antagonize adenosine similar to those of paludrine. 3672 is a weaker antagonist and a weaker antimalarial, and finally 3742, which has no action on malarial parasites, does not antagonize adenosine.

Anilinopyrimidines (Curd and Rose, 1946b ; Curd, Davies, and Rose, 1946 ; Curd, Davies, Owen, Rose, and Tuey, 1946 ; Curd, Richardson, and Rose, 1946)

The prototype of this group of substances (2666) was the first substance synthesized by Curd and Rose that showed antimalarial activity in chicks. The results of its clinical trials were disappointing; and according to Adams it is not active in human malaria (quoted by Curd, Davey, and Rose, 1945).

This group of substances was the only one in which it was impossible to find any relation between antimalarial activity and adenosine antagonism. Two members of the group, 3300 and 4070, are devoid of antimalarial activity but they were, nevertheless, good antagonists of adenosine. 2666 itself is a substance with a fairly good antimalarial activity, but it inhibited the action of adenosine more intensely than 4316, which is a better antimalarial. On the other hand 3756, which is inactive against experimental malaria, did not show any appreciable antagonism to adenosine.

Alkylpyrimidines (Hull, Lovell, Openshaw, Payman, and Todd, 1946; Hull, Lovell, Openshaw, and Todd, 1947)

Because only small samples of these compounds were available, they were not tested in a full range of doses. For this reason it was neither possible to study the relative order of the intensities of their antagonisms nor to compare these with their antimalarial activities. All of them were tested at 2 × 50 mg./kg./day, which as a rule with most antimalarials produced a measurable antagonism. It is interesting to note that compounds 4747 and 4746, which are inactive against *P. gallinaceum*, did not antagonize the action of adenosine under the conditions of the test. Under the same conditions, all the other compounds tested in this series produced a clear antagonism. The details of the results of these experiments are reproduced in Table III.

Relation between antimalarial activity on chicks and adenosine antagonism

As already stated above, within all groups of substances, except the 2666 type, there is a parallelism between their antimalarial activity and their power to antagonize adenosine, as expressed by the dose which halves the sensitivity of the animals to this substance. In comparing the effects of the substances selected as prototypes for each group it was found that mepacrine was one of the best antagonists of adenosine, in spite of the fact that it is one of the weakest antimalarials in chicks. Among the other substances, however, the best antimalarials are among the best antagonists, and substances with low antimalarial activity are among those which antagonize adenosine feebly. Table IV gives the orders of antimalarial activity and intensity of the antagonism shown by these substances in the "acute" and "chronic" experiments.

TABLE IV

Adenosine antagonism		Antimalarial activity
"Acute"	"Chronic"	
Mepacrine	Pamaquin	Pamaquin
Chloroquine	Chloroquine	Chloroquine
Quinine	Mepacrine	Paludrine
2666	Paludrine	3349
Paludrine	3349	Mepacrine
3349	Quinine	Quinine
	2666	2666

C. Antagonism in the heart-lung preparation

In the experiments reported above it was found that paludrine antagonized adenosine in both "acute" and "chronic" experiments. When the intensity of its "chronic" antagonism was compared with that of other prototype substances it was found that the order of intensity of their antagonism was about the same as that of their antimalarial activity. However, in the "acute" series this parallelism was not maintained and under these conditions paludrine was found among the worst antagonists (Table IV).

Another fact noted was the delayed toxic effect of paludrine when administered repeatedly. A likely explanation of this toxic action may be the production and accumulation of some toxic metabolite during the prolonged administration of paludrine.

It is also possible that the difference in the results obtained with paludrine in the "chronic" and "acute" experiments may be due, not to the drug antagonizing adenosine by itself, but to its being converted during the dosing period into a metabolite which does antagonize adenosine. Even when the paludrine content of the blood was as high as 7–9 mg./l. in the "acute" experiments, the antagonism was not intense, but during the brief period of slow administration of the drug only a small proportion of the injected material could have been metabolized into the hypothetical active substance.

The possibility of such a metabolite being formed was investigated in the heart-lung preparation of the guinea-pig in the following way: e.c.g. records of the action of several small doses of adenosine (from 25 μg.) were taken before and after the addition of paludrine to the blood of the preparation. The blood was then replaced by the same volume of fresh normal blood, which was left to circulate for 15 min.; this "washing out" of the drug was repeated and finally the blood was replaced by blood from guinea-pigs which had been treated orally with 2 × 20 mg./kg. of paludrine for 3½ days. This last change of blood was repeated and 25 ml. of blood from paludrine-treated guinea-pigs were again put in the venous reservoir. A further series of adenosine doses was injected 15 min. after the last change of blood.

With normal blood in the preparation the injection of about 25 μg. adenosine into the venous cannula produced blocks of about 5 sec. duration. The addition of paludrine to the blood of the preparation caused no reduction in the duration of the effects of adenosine, but a significant reduction of these effects was observed when the blood

of paludrine-treated guinea-pigs circulated in the preparation.

The concentration of paludrine in the blood of the treated guinea-pigs and that in the blood to which paludrine had been added was estimated by the method of Spinks and Tottey (1946). In some experiments, such as that reproduced in Table V, the concentration in the blood of the treated animals was smaller than that in the blood to which paludrine had been added, but nevertheless the blocks produced by the same doses of adenosine were shorter when the blood of paludrine-treated animals circulated in the preparation.

TABLE V

ANTAGONISM TO ADENOSINE BY ANTIMALARIALS IN HEART-LUNG PREPARATIONS OF GUINEA-PIGS

Expt. No.	Blood (25 ml.) and drug	Duration of a.v. block in secs. with doses of adenosine, in μg.			
		25	35	50	100
2	Normal blood	1.4	—	9.4	—
	Ditto and mepacrine HCl 0.02 mg.	0.0	—	6.8	—
	Normal blood (after 3 changes)	3.4	—	11.9	—
	Ditto and mepacrine HCl 0.2 mg.	0.0	—	5.0	11.3
13	Normal blood	3.4	—	12.8	—
	Ditto and quinine HCl 0.05 mg.	3.6	—	12.6	—
	Ditto and quinine HCl 0.5 mg.	0.0	—	—	0.0
	Normal blood (after 3 changes)	0.0	—	8.4	14.0
4	Normal blood	0.0	6.2	11.3	—
	Ditto and paludrine (1.027 mg./l.)	—	6.8	10.2	—
	Blood of paludrine-treated guinea-pigs (conc. 0.82 mg./l.) (after 2 changes with blood of paludrine - treated guinea-pigs)	—	2.9	5.6	—

These results were compared with those obtained when mepacrine or quinine was added to the blood of the preparation.

The duration of the heart block produced by adenosine was reduced when mepacrine was added to the blood in concentrations of 80 μg./100 ml. This antagonism was observable so long as blood containing mepacrine circulated in the preparation. When the drug was " washed out " by two or three changes of blood the duration of the action of adenosine returned to normal values. Similar results were obtained with quinine in concentrations of the order of 2 mg./100 ml. Table V gives the results of some of these experiments.

Discussion

The antimalarial action of a number of chemical substances has been compared with their power to antagonize the responses of some mammalian tissues to adenosine. In most of the series of compounds tested a correlation has been observed between the inhibition of responses to adenosine and the antimalarial activity. This may mean that antimalarial action in some way involves interference of the drug with a process, essential to the parasite, in which adenosine or a derivative thereof is concerned.

No idea as to the nature of this hypothetical process can be gathered from the experiments reported here. It is unlikely that the processes in mono-cellular plasmodia in which adenosine or its derivatives are concerned can be closely related to the processes which we have been studying in mammalian tissues.

This study of the antagonism of the action of adenosine in some tissues by antimalarial drugs was undertaken because adenosine forms part of coenzymes, such as, for instance, flavine adenine dinucleotide. It was already known (Madinaveitia, 1946) that the antibacterial action of some antimalarial compounds on *Lactobacillus casei* was antagonized by riboflavine. It has been suggested that this effect is due to the similarity of the spatial configuration of these drugs to that of the vitamin (Curd, Davey, and Rose, 1945). This structural resemblance has also been claimed to be connected with the parasiticidal activity of these drugs (Curd and Rose, 1946a).

On the other hand the alternative hypothesis that an interference with an adenosine-containing enzyme system might result in antimalarial activity has led to the preparation of successful antimalarial compounds (Hull *et al.*, 1946, 1947). A biochemical relationship between adenosine derivatives and antimalarials may be found in the observation (Hellerman, Bovarnick, and Potter, 1946) that adenylic acid and adenosine triphosphate prevent the inhibition which mepacrine causes in the recovery of oxygen uptake by washed *Plasmodium lophurae* in the presence of glucose. The fact that some enzymes contain both adenosine and riboflavine makes both points of view on the mode of action of antimalarial substances compatible.

It is unlikely that *L. casei* uses riboflavine as such in its metabolic processes. It is more probable that the micro-organism builds up some coenzyme from the free riboflavine present in the medium, possibly by combining it through a polyphosphate group with adenosine. It is conceivable that the antagonistic effect of riboflavine in *L. casei* assays is not due to the vitamin itself, but to an adenosine-containing coenzyme into which the free riboflavine is incorporated. The prosthetic group of *d*-amino-acid oxidase is such a flavine adenine dinucleotide (Corran, Green, and Straub, 1939) and is known to antagonize the inhibitory action of mepacrine on the enzymatic deamination of *d*-amino-acids (Wright and Sabine, 1944).

On the other hand it has been shown (Haas, 1944) that mepacrine also inhibits cytochrome reductase by an irreversible reaction with the protein; here the drug has been shown to compete with the prosthetic group of the enzyme, because its action is prevented by the addition of alloxazine mononucleotide. In this coenzyme riboflavine is not combined with adenosine.

All the antimalarial drugs which we have tested have been found to antagonize the particular actions of adenosine which we have chosen to study. From the circumstantial evidence already available and that now presented it appears that some relationship exists between the ability of chemical compounds to interfere with biological processes in which adenosine is concerned and their antimalarial action.

In spite of the fact that the results of the experiments reported above were only semi-quantitative, it was found (*a*) that antimalarial drugs have another action in common—i.e., they antagonize adenosine, and (*b*) that the intensity of this antagonism is proportional to their antimalarial action as measured in chicks infected with *P gallinaceum*.

The relation of the two activities is best seen when the results obtained with a series of homologues are compared. The only exceptions to this rule were the anilinopyrimidines of the 2666 type among which no relation between antimalarial activity and adenosine antagonism could be found. It is noteworthy that although 2666 was found to be active in the chick test, the drug was tried clinically with negative results by Dr. A. R. D. Adams, of the School of Tropical Medicine of Liverpool (cited by Curd, Davey, and Rose, 1945). However, as the chick test is only an indication of possible antimalarial activity in man, and as there is an unpredictable variability in the susceptibility of various species of plasmodia to the same drug (Davey, 1946), it is more than probable that the agreements and disagreements shown in Tables III and IV would have been different if another measurement of activity had been chosen.

In comparing the plasmodicidal activity of the prototype of each group of substances with the intensity of their adenosine antagonism in the "chronic" series, it was found that mepacrine was the best antagonist, although it is not one of the more powerful antimalarials in the chick test. There is, however, a fairly close parallelism between the antagonism and the activity of the other substances.

This relationship is not so close in the "acute" experiments. The most important exception was paludrine: in the "chronic" series it was found among the more intense and in the "acute" series among the weaker antagonists. The difference in the results of the two series could be explained by the assumption that in the "chronic" experiments a metabolite with antagonistic action was formed from paludrine during the period of its administration.

At the end of the 30 min. period of continuous infusion in the "acute" experiments, the paludrine concentration in the blood was sometimes as high as 9 mg./l. In spite of the fact that such high drug concentrations could be obtained only a slight antagonism could be demonstrated. It seems likely that during the infusion period only a small proportion of the drug was metabolized into the hypothetical substance responsible for the antagonism.

It has also been suggested that paludrine itself does not possess plasmodicidal action when examined by a tissue culture method (Hawking, 1947; Hawking and Perry, 1948), but the serum of animals which have been treated with paludrine has a high plasmodicidal activity; from these results Hawking has deduced that the antimalarial activity of paludrine is not due to the drug itself but to some metabolite.

The formation of metabolites with biological actions in the guinea-pig different from those of paludrine has been demonstrated in the heart-lung experiments. In these preparations the addition of small amounts of mepacrine and quinine to the blood reduced the duration of the block produced when adenosine was injected into the venous cannula. If similar amounts of paludrine were added to the blood there was no reduction of the effects of adenosine, but substantial decrease of these effects was observed when blood from paludrine-treated guinea-pigs, possibly containing metabolites of the drug, circulated in the preparation (Table V).

This result cannot be explained by differences in the paludrine content of the blood from the treated guinea-pigs and that of the blood to which paludrine had been added. In some experiments these concentrations as measured by the method of Spinks and Tottey were the same for all practical purposes. (This method consists in the acid hydrolysis of the base, after its extraction with benzene, to *p*-chloroaniline and subsequent diazotization and coupling of the latter. It does not give any indication of the possible *in vivo* changes undergone in the side chain of paludrine, provided that they do not alter the solubilities and rate of hydrolysis to any great extent. How alterations in the benzene ring would affect these results is difficult to predict.)

It is interesting to note that in the acute experiments the antagonism shown by mepacrine was not permanent, and about 90 min. after the infusion of the drug the action of adenosine returned to normal. In heart-lung preparations the antagonistic action of mepacrine and quinine could be even more easily reversed by changing the blood containing drug for fresh normal blood. It appears that the antagonism to adenosine is due mainly to the drug which circulates in the blood and not to that which is fixed in the tissues.

Pamaquin and paludrine, or more probably some metabolite of it, behave with respect to adenosine in the same way as the rest of the antimalarial drugs assayed. This is in contrast to the failure of riboflavine to antagonize their action on *L. casei*, a property common to the other antimalarials assayed. Perhaps paludrine does not show such antagonism because under the *in vitro* conditions of the test its transformation into the substances responsible for its adenosine antagonism and antimalarial activity does not take place. One of the reasons which led Curd and Rose (1946d) to suggest that the antimalarial activity of paludrine was different from that of other types of antimalarials was this failure of riboflavine to antagonize it. In the light of the experimental evidence that paludrine can be converted in the blood of treated animals into some metabolite which does share with other antimalarials the property of antagonizing the action of adenosine, the case for such assumed difference in the mode of antimalarial action is somewhat weakened.

Summary

Antimalarial compounds antagonize the action of adenosine on the guinea-pig heart and on the hen caecum.

A parallelism between the antimalarial activity and the power to antagonize adenosine has been demonstrated in most series of compounds examined.

In heart-lung preparations it has been found that unlike the other antimalarials tested paludrine does not antagonize the action of adenosine. However, the blood of paludrine-treated guinea-pigs did antagonize this action. This is taken as evidence that paludrine is metabolized to another compound with different pharmacological properties.

The considerations which led to the testing of antimalarials as antagonists of adenosine are discussed.

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References

Barsoum, G. S., and Gaddum, J. H. (1935). *J. Physiol.*, **85**, 1.
Basford, F. R., Curd, F. H. S., and Rose, F. L. (1946). *J. chem. Soc.*, 713.
Butler, R., Davey, D. G., and Spinks, A. (1947). *Brit. J. Pharmacol.*, **2**, 181.
Corran, H. S., Green, D. E., and Straub, F. B. (1939). *Biochem. J.*, **33**, 793.
Cruickshank, E. W. E. (1945). *J. Physiol.*, **103**, 417.
Curd, F. H. S., Davey, D. G., and Rose, F. L. (1945). *Ann. trop. Med. Parasit.*, **39**, 139.
Curd, F. H. S., Davies, M. I., Owen, E. C., Rose, F. L., and Tuey, G. A. P. (1946). *J. chem. Soc.*, 370.
Curd, F. H. S., Davies, M. I., and Rose, F. L. (1946). *J. chem. Soc.*, 351.
Curd, F. H. S., and Landquist, J. K. (1948). *J. chem. Soc.*, in preparation.
Curd, F. H. S., Landquist, J. K., and Rose, F. L. (1947). *J. chem. Soc.*, 775.
Curd, F. H. S., Richardson, D. N., and Rose, F. L. (1946). *J. chem. Soc.*, 378.
Curd, F. H. S., and Rose, F. L. (1946a). *J. chem. Soc.*, **343**.
Curd, F. H. S., and Rose, F. L. (1946b). *J. chem. Soc.*, **362**.
Curd, F. H. S., and Rose, F. L. (1946c). *J. chem. Soc.*, 729.
Curd, F. H. S., and Rose, F. L. (1946d). *Nature*, **158**, 707.
Davey, D. G. (1946). *Ann. trop. Med. Parasit.*, **40**, 52.
Drury, A. N., and Seznt-Györgyi, A. (1929). *J. Physiol.*, **68**, 213.
Haas, E. (1944). *J. biol. Chem.*, **155**, 321.
Hawking, F. (1947). *Nature*, **159**, 409.
Hawking, F., and Perry, W. L. M. (1948). *Brit. J. Pharmacol.*, **3**, 320.
Hellerman, L., Bovarnick, M. R., and Potter, C. C. (1946). *Fed. Proc.*, **5**, 400.
Hull, R., Lovell, B. J., Openshaw, H. T., Payman, L. C., and Todd, A. R. (1946). *J. chem. Soc.*, 358.
Hull, R., Lovell, B. J., Openshaw, H. T., and Todd, A. R. (1947). *J. chem. Soc.*, **41**.
Madinaveitia, J. (1946). *Biochem. J.*, **40**, 373.
Spinks, A., and Tottey, M. M. (1946). *Ann. trop. Med. Parasit.*, **40**, 146.
Wright, C. I., and Sabine, J. C. (1944). *J. biol. Chem.*, **155**, 315.