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THE INHIBITION OF ESTERASES BY PALUDRINE

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Many observations on the action of antimalarial drugs on ester-splitting enzymes have been recorded. Rona and Reinicke (1921) have shown that quinine inhibits the esterase which hydrolyses tributyrin in human serum; this enzyme is also inhibited by many other antimalarials, e.g., pamaquin, optochin, and mepacrine (Fulton, 1936). Both quinine and mepacrine act on cholinesterases: quinine has been shown to inhibit the enzyme now generally known as pseudo-cholinesterase (Nachmansohn and Schneemann, 1945); mepacrine inhibits the hydrolysis of acetylcholine by both pseudo- and true cholinesterases (Waelsch and Nachmansohn, 1943).

It therefore seemed interesting to examine the effect of paludrine on esterases. Paludrine differs greatly from the other antimalarials in its chemical constitution and is characterized by a low toxicity. The latter fact made it unlikely that it would strongly inhibit an enzyme so vital as the "true" cholinesterase of the central nervous system and muscles, but it is known that there are other enzymes which will hydrolyse choline esters. We have therefore examined the action of paludrine on all the known cholinesterases and on a number of related enzymes.

MATERIAL AND METHODS

Most of these have been described in the preceding paper (Blaschko, Chou, and Wajda, 1947). In addition we have examined the hydrolysis of acetylcholine by plasma and red-cell haemolysate. The hydrolysis of tributyrin was also studied in human serum and in cat's liver extracts. The cat's liver used for these experiments was washed free from blood *in situ* by perfusion with Locke's fluid.

EXPERIMENTS

1. *Cholinesterases* (Table I)

Two preparations, representative of true cholinesterase, were examined: an extract from the dog's caudate nucleus and a haemolysate of human red cells. Both were inhibited slightly by paludrine. The rate of hydrolysis of acetylcholine ($6\times10^{-3}M$) by the brain extracts was reduced by about 22 per cent in the presence of $10^{-3}M$ paludrine. For the human haemolysate the inhibition was 49 per cent with $10^{-3}M$ paludrine; with $10^{-4}M$ paludrine there was no inhibition.

In the plasma (or serum) of many mammals the enzyme acting on acetylcholine is pseudo-cholinesterase. This enzyme which hydrolyses benzoylcholine as well as acetylcholine was more strongly inhibited by paludrine. In one experiment with human plasma and acetylcholine as substrate the percentage inhibitions were 92 per cent with $10^{-3}M$, and 60 per cent with $10^{-4}M$ paludrine. Similar inhibitions were observed with cat's plasma and horse serum as sources of the enzyme.

TABLE I

ACTION OF PALUDRINE ON CHOLINESTERASES

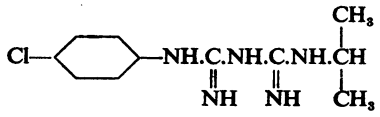
Substrate concentration $6 \times 10^{-3}M$

Species and tissue	Amount of tissue	Substrate used	Percentage inhibition by paludrine $10^{-3}M$	$10^{-4}M$	$10^{-5}M$
Dog's caudate nucleus	4 mg.	acetylcholine	22	—	—
Human red-cell haemolysate	0.1 ml.	„	49	0	—
Human plasma	0.05 ml.	„	92	60	—
Horse serum	0.3 ml.	benzoylcholine	67	35	—
Cat plasma	0.005 ml.	acetylcholine	73	—	—
Guinea-pig liver	12.5 mg.	benzoylcholine	94	76	26
Ox kidney	1,000 mg.	„	0	—	—

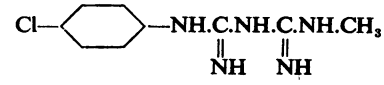
Benzoylcholinesterase hydrolyses benzoylcholine, but not acetylcholine; the enzyme has been found in the liver of the guinea-pig (Sawyer, 1945) and in ox kidney (Gunter, 1946). It is known that the latter organ does not contain pseudo-cholinesterase, and our experiments show that the same is true for the guinea-pig's liver. This was established by comparing the rates of hydrolysis of benzoylcholine with and without eserine, which is a strong inhibitor of pseudo-cholinesterase (Mendel, Mundell, and Rudney, 1943). We find that the rate of hydrolysis of benzoylcholine is the same in the presence or absence of 2×10^{-4} eserine, a concentration which completely inhibits pseudo-cholinesterase.

The affinities of paludrine for the two preparations of benzoylcholinesterase are very different. Whereas the ox kidney preparation was not inhibited by $10^{-3}M$ paludrine, this concentration rendered the enzyme from guinea-pig's liver almost completely inactive.

It seemed interesting to compare the effect of paludrine on benzoylcholinesterase with that of a related compound which has no antimalarial action, at least on *P. Gallinaceum* in chicks (Curd and Rose, 1946). Such a compound is the N_5-methyl homologue of paludrine, the substance M5093. In this substance the *iso*propyl group of paludrine is replaced by a methyl group.



Paludrine



M5093

The anti-benzoylcholinesterase activity of M5093 is very much less than that of paludrine. In one experiment we compared the inhibition with paludrine and M5093, both in $10^{-3}M$ concentrations, on the benzoylcholinesterase of guinea-pig's liver. The inhibitions were:

with paludrine 91 per cent; with M5093 42 per cent.

In another experiment the inhibitions were:

with $4 \times 10^{-5} M$ paludrine 49 per cent; with 10^{-3} M5093 41 per cent.

The latter experiment shows that the N-methyl homologue was less active as an inhibitor when present in a concentration 25 times that of paludrine.

M5093 had no inhibitory action on the true cholinesterase from dog's brain in a molar concentration of 10^{-3}.

2. *Tributyrinesterases* (Table II)

Rona and his collaborators have shown that the effect of quinine on the enzymic hydrolysis of tributyrin differs for preparations obtained from different organs: the tributyrinesterase of serum is strongly inhibited by quinine (Rona and Reinicke, 1921), but the esterase from liver is resistant to quinine (Rona and Pavlovic, 1922).

Both paludrine and mepacrine behave similarly to quinine. In the presence of $10^{-3} M$ paludrine the tributyrinesterase of human serum was almost completely inhibited and with $10^{-4} M$ paludrine the inhibition was 82 per cent. With $10^{-3} M$ mepacrine the inhibition was 92 per cent. On the other hand, with the preparation from cat's liver, the rate of hydrolysis of tributyrin was not affected in the presence of $10^{-3} M$ paludrine and of $10^{-3} M$ mepacrine.

It is interesting that the three antimalarial substances, so very different in their chemical constitution, should resemble each other so closely in their affinities for this enzyme, but it must be pointed out that the compound M5093, which is without antimalarial activity, behaves in a similar fashion. In a concentration of $10^{-3} M$ it was without inhibitory action on the enzyme from cat's liver, but with human serum the inhibition was 72 per cent.

TABLE II

INHIBITION OF TRIBUTYRINESTERASES

Substrate concentration 0.015 *M*

Species	Tissue	Amount of tissue	Inhibitor	Percentage inhibition at different concentrations		
				$10^{-3} M$	$10^{-4} M$	$10^{-5} M$
Man	Serum	0.2 ml.	paludrine	94	81	24
Cat	Liver	0.667 mg.	,,	2	—	—
Rabbit	Pancreas	20 mg.	,,	0	—	—
Rabbit	Kidney	200 mg.	,,	0	—	—
Man	Serum	0.2 ml.	mepacrine	92	87	60
Cat	Liver	0.667 mg.	,,	0	0	0
Man	Serum	0.2 ml.	M5093	73	36	8
Cat	Liver	0.667 mg.	,,	0	—	—

The hydrolysis of tributyrin in rabbit's kidney and pancreas was not inhibited by $10^{-3} M$ paludrine.

3. *Other esterases* (Table III)

Paludrine had no effect on the hydrolysis of methyl butyrate by rabbit's pancreas. It had a slight inhibitory action on the tropinesterase of rabbit's serum: with $10^{-3} M$ paludrine the inhibition was 48 per cent. We have also examined the tropacocainesterase of horse serum described by Glick and Glaubach (1941); this enzyme was not inhibited by paludrine.

TABLE III

ACTION OF PALUDRINE ON OTHER ESTERASES

Paludrine concentration $10^{-3}M$

Species and tissue	Amount of tissue used	Substrate used	Percentage inhibition
Rabbit pancreas	20 mg.	0.015 *M* methylbutyrate	0
Rabbit serum*	0.15 ml.	1 g./100 ml. atropine sulphate	48
Horse serum	0.3 ml.	0.01 *M* tropacocaine hydrochloride	0

* The serum was from a rabbit which contained tropinesterase.

DISCUSSION

The mechanism of antimalarial activity is a matter of speculation, but it seems likely that the therapeutic action is due to the drugs interfering with metabolic reactions of vital importance to the parasites; such interference is most easily thought of as an inhibition of enzymic reactions. Plasmodial enzymes have not yet been available for study, but it has long been known that some antimalarials will inhibit certain mammalian enzymes. Paludrine shares this property with such well-known antimalarials as quinine and mepacrine, but also displays some differences; it has, for example, a very low affinity for one of the physiologically most important enzymes, namely the true cholinesterase. Its most powerful inhibitory action in our experiments was on the benzoylcholinesterase of guinea-pig's liver, an enzyme so far characterized by a substrate which has not yet been shown to occur in animal tissues.

There is no general parallelism between antimalarial and anti-esterase properties, but the fact that so many antimalarials are at the same time inhibitors of esterases makes the suggestion that these substances act by inhibiting an esterase of importance in the life cycle of the malaria parasite worthy of investigation.

The pattern of affinities of paludrine for the cholinesterases is similar to that of the substances examined in the preceding paper. Like cocaine, trasentin 6H, and lachesine, paludrine has little affinity for true cholinesterase; pseudocholinesterase is more strongly inhibited and there is a strong inhibition of the benzoylcholinesterase of guinea-pig's liver, whereas the benzoylcholinesterase of the ox kidney is not inhibited. So far as true and pseudo-cholinesterase are concerned, it has been shown that many substances have a greater affinity for the latter enzyme, e.g., di*iso*propyl fluorophosphonate (Hawkins and Mendel, 1947), quinine (Nachmansohn and Schneemann, 1945), and the aromatic amino alcohols of the type Ar-CHOH.CH_2NR_2 recently studied by Wright (1946). It is

interesting that the enzyme specifically connected with the metabolism of acetylcholine in nerve and muscle should be much less readily inhibited.

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SUMMARY

1. Paludrine has little affinity for true cholinesterase.

2. It inhibits pseudo-cholinesterase more strongly.

3. It is an inhibitor of the benzoylcholinesterase of guinea-pig's liver, but not of the benzoylcholinesterase of ox kidney.

4. Paludrine, like quinine and mepacrine, has little affinity for the tributyrinesterase of cat's liver, but does inhibit the tributyrinesterase of human serum.

5. The N_5-methyl homologue of paludrine has a much lower affinity for the benzoylcholinesterase of guinea-pig's liver than paludrine itself.

6. The guinea-pig's liver does not contain pseudo-cholinesterase.

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