DISCUSSION

Intact animal preparations.

In vivo experiments using spinal vagotomised preparations as well as the anaesthetised animals revealed that chloroquine and amodiaquin augment certain of the cardiovascular responses to injected adrenaline and noradrenaline. The augmentation was moderate in magnitude and developed fully only after an interval of time. Amongst the other vasoactive agents studied, tyramine was the only agent the pressor action of which was augmented by the 4-aminoquinolines. These observations indicate that the augmenting effect of the 4-aminoquinolines cannot be satisfactorily explained on the basis of an alteration in cardiovascular reflexes or on the basis of a generalised change, either drug induced or fortuitous, in the sensitivity of the preparations. Furthermore, in vitro experiments (described in Part II) revealed that chloroquine and amodiaquin did have no augmenting effect on responses to catecholamines mediated through the α-or β-adrenergic receptors (3). The peripheral tissue sensitisation, therefore, cannot be an explanation for the augmenting effect of the 4-aminoquinolines observed in intact animal experiments.
After an acute intravenous injection of reserpine, the responses to various sympathomimetic amines have been found to be augmented (see, 31, for many references). This augmentation develops after a latent period, at a time when the levels of circulating catecholamines are raised (168,195). The augmentation has been ascribed (238) to 'the additive effect of endogenous noradrenaline (increased in the proximity of receptors because of the releasing action of reserpine) to the action of injected sympathomimetic amines'. In reserpine pretreated animals, the augmenting effect of chloroquine and amodiaquin was reduced, indicating that intact amine stores in the tissues are primarily essential for the full augmenting effect of the 4-aminoquinolines. It may be possible that these compounds augment the amine pressor responses, at least in part, through an endogenous release of catecholamines, that is through the mechanism which probably explains the augmenting effect of acute intravenous administration of reserpine. This proposal would explain the latent period after which the augmentation develops fully, and also the augmentation exclusively of sympathomimetic amines.

At least for chloroquine the proposal probably finds support in the observation that chloroquine caused a brief rise in blood pressure in spinal cats and that
this rise is remarkably less in reserpine pretreated cats. It was also found that chloroquine and amodiaquine elicit a brisk hypertensive responses in dogs pretreated with methylamphetamine, the responses which are mediated through an endogenous release of catecholamine (Part III). It is of interest that in this respect chloroquine and amodiaquine resemble with reserpine (31).

Circulating catecholamines are taken up, bound and retained at or near sympathetic nerve ending (see,43, for references). This appears to be one of the important 'sites of loss' (248) responsible for terminating the biological action of catecholamines (164). The first step in the two stage uptake mechanism is transfer of the amine across the cell membrane. Agents like cocaine and probably hexamethonium (125,193,245) block the uptake at this stage and make more of the amines available to act at receptor site thereby augmenting the overt biological effect. In animals pretreated with cocaine or hexamethonium to produce a blockade of the amine uptake mechanism, chloroquine had little augmenting effect on the amine responses. This suggests that as with cocaine or hexamethonium, the augmenting effect of chloroquine was, in part, due to blockade of amine uptake. While cocaine blocks the liberation of catecholamines due to indirectly acting sympathomimetic agents (102,116,236), hexamethonium does
not exhibit this action (178). It is, thus possible that cocaine reduced the augmenting effect of chloroquine by reducing the efficiency of this agent in liberating catecholamines as well as in preventing the uptake of the injected amines. Hexamethonium, which probably blocks the uptake of catecholamines by tissues, would be expected to stabilize the catecholamines liberated by chloroquine and thus enhance further the potentiating effect of chloroquine. Though this was not observed in studies on the potentiating effect of chloroquine, the pressor responses caused by a brisk catecholamine release due to the 4-aminoquinolines in methylamphetamine pretreated animals were found to be augmented by hexamethonium (see, Part III).

On the other hand, amodiaquin does not appear to act by blocking the amine uptake as in animals pretreated with cocaine or hexamethonium, amodiaquin did augment the amine responses. Moreover, augmentation through a cocaine-like action could have been seen in reserpinized animals (103).

A greater augmentation of catecholamine pressor responses by amodiaquin in cocainized dogs could have been due to one or more of the following factors: (a) While in control (untreated) dogs amodiaquin reduced tachycardia due to adrenaline and enhanced bradycardia due to noradrenaline, it did not exert such an action in
cocainised dogs. This could have contributed towards better pressor responses to catecholamines. (b) It has been reported (184) that cocaine reduces the blocking effect of several adrenergic blocking agents. Doses of cocaine used in these experiments could have reduced adrenergic blocking action of amodiaquin and proportionately augmented the amine pressor responses. (c) If the assertion that amodiaquin liberates catecholamines from the stores and thereby augments the amine pressor responses is true, the amines liberated by amodiaquin would be further stabilised by cocaine and would further enhance the amine pressor responses.

Cocaine, which causes subsensitivity to 'indirectly' acting sympathomimetic agents, could be expected to reduce the catecholamine liberation due to amodiaquin as well and hence the augmenting effect of this agent. The finding that amodiaquin is even a better sensitising agent in cocaine pretreated animals does not accord with this view. Recent investigations indicate that cocaine prevents the access of indirectly acting sympathomimetic agents to amine storage sites rather than obstructing the process of amine release (see,194, for discussion). It could be possible that cocaine does not interfere with the migration and subsequent action of amodiaquin on the tissue amine stores. It is interesting to note in this connection that cocaine does not reduce
the depletion of cardiac catecholamines by guanethidine (23) though it prevents the pressor action of guanethidine which is probably due to release of catecholamines from the stores (116).

Though the augmenting effect of chloroquine and amodiaquin on the amine pressor responses was reduced by nialamide, the augmentation cannot be explained by proposing a monoamine oxidase-inhibitory action for chloroquine or amodiaquin. Though it has been suggested (35) that inhibition of this enzyme by drugs can account for their augmenting effect on the catecholamine responses, the overall evidence (238) does not support this proposal. Acting through such a mechanism, the 4-aminoquinolines would be expected to augment the effect of tyramine to a much greater extent than the effect of catecholamines, as monoamine oxidase is of much greater importance in catalysis of tyramine. Such a differential augmentation of the amines after phenoxyprazine is evident in published work (218). The results do not bring forth such a distinction after chloroquine or amodiaquin.

Inhibition of monoamine oxidase, irrespective of the chemical structure of the inhibitors, antagonises catecholamines liberating action of many agents (9, 23, 100, 204). Nialamide could have reduced such an action exerted by chloroquine and amodiaquin. This might explain why the
magnitude of the amine pressor responses was not much augmented by the 4-aminoquinolines in nialamide pre-treated animals.

The depressant action of chloroquine on central nervous system (70) was evident in cats chronically treated with chloroquine. However these animals were considerably less sensitive to catecholamines and probably to tyramine and angiotensin. This peripheral cardiovascular subsensitivity cannot be readily explained. It is probably pertinent to point out that dogs chronically treated with certain 8-aminoquinolines exhibited profound signs of central depression and a specific inhibition of sympathetic reflex function (138). In contrast to supersensitivity following acute intravenous administration of amodiaquin, the supersensitivity following chronic administration of small amounts of this agent was considerably greater in magnitude than that observed in acute experiments and was probably a part of generalised cardiovascular sensitization.

Ocular toxicity of chronically administered chloroquine in man and in animals is well documented (5, 58,136,171,200,211). It has been suggested (136) that vascular spasm underlies the chloroquine retinopathy. Considerable cardiovascular subsensitivity which was observed at least in cats chronically treated with chloroquine does not appear to support this view.
Isolated tissue preparations.

Chloroquine and amodiaquin inhibited the stimulant action of catecholamines on the rabbit aortic strip and the rat seminal vesicle, the preparations which have a preponderance of $\alpha$-adrenergic receptors. The reversible and surmountable nature of this inhibition and the shift of the dose/response curves for the amines to the right without an appreciable change in their slopes suggest that the blockade is competitive in nature (10).

The blocking action of chloroquine and of amodiaquin could be ascribed to the local anaesthetic action of these agents (149,181). This explanation, however, does not appear to be convincing in view of the observation that other stimulants of these experimental preparations were not uniformly inhibited by concentrations of the 4-aminoquinoline which reduced the stimulant effect of the catecholamines. Moreover, in some of the intact animal experiments (see above) larger doses of amodiaquin were found to inhibit the pressor responses to catecholamines probably through a partial blockade of $\alpha$-adrenergic receptors.

Larger concentrations of chloroquine and amodiaquin reduced the pilocarpine induced spasm of the rabbit tracheal chain preparations. This could have been due to the anticholinergic effect of these agents; such
an action of chloroquine has already been reported (1). In concentrations which do not grossly affect the pilo-
carpine induced spasm chloroquine augmented the inhibi-
tory action of catecholamines on the tracheal chains. Amodiaquin, however, did not have such an effect. The augmentation induced by chloroquine was also moderate, transient and rather inconsistent. On the other hand, there was no evidence for any sensitizing effect of either of the compounds in experiments using isolated heart preparations. The augmentation of the positive chronotropic effect of catecholamines on heart seen in the intact animal experiments cannot, therefore, be ascribed to a peripheral effect, viz. the sensitization of $\beta$-adrenergic receptors. In fact both chloroquine and amodiaquin reduced the positive chronotropic and inotropic effect of the catecholamines on isolated rabbit atria and perfused hearts of the frog, signifying a partial blockade of the $\beta$-adrenergic receptors.

The ileum of rabbit and of guinea-pig contains both the $\alpha$- and $\beta$-adrenergic receptors (3). The inhibitory effect of catecholamines on these preparations was considerably reduced by amodiaquin. This substantiates the conclusion that amodiaquin can exert a blocking effect on adrenergic receptors of both types. The study of chloro-
quine in similar experiments was however inconclusive, as this drug caused a profound relaxation of the tone of ilea.
Pressor response to chloroquine and amodiaquin in methy lamphetamine pretreated dogs.

In dogs pretreated with methy lamphetamine, amodiaquin and chloroquine produced qualitatively similar, characteristic pressor responses. Tolazoline and dibozane, which block the α-adrenergic receptors severely reduced these responses. Furthermore, administration of pronethalol, a specific β-adrenergic blocking agent, totally abolished the pressor responses. Thus, amodiaquin and chloroquine appear to act through an adrenergic mechanism to evoke pressor responses.

Administration of hexamethonium, and other ganglion blocking agents given in doses sufficient to block the pressor effect of dimethylphenylpiperazinium did not reduce but augmented the pressor effect of amodiaquin and chloroquine. Furthermore, administration of atropine in doses blocking the pressor effect of MeN-A-343 had no effect on the pressor action of the 4-aminoquinolines. These results make it improbable that the pressor effects of amodiaquin and chloroquine are due to ganglionic or central adrenergic excitation. Pressor responses to physostigmine due to the latter effect have been shown to be susceptible to blockade by atropine and by mecamylamine (115).

Burn and Rand (36) suggested that tyramine acts through the release of noradrenaline; this hypothesis
explains the subsensitivity of reserpine pretreated animals and of sympathetically denervated structures to tyramine (see, 194, for references). The concept has its strong support in the finding (36, 38, 190) that an infusion of noradrenaline or its precursors restores the action of tyramine in reserpine pretreated animals. Here it was seen that a prior treatment of the animals with reserpine, which causes subsensitivity to agents like tyramine by depleting tissue catecholamine stores (36, 219, 237, 57, 190), also reduced the pressor responses to the 4-aminoquinolines. This suggest that like tyramine, amodiaquin and chloroquine induce pressor effects through a release of catecholamines from tissue stores.

It was not possible to restore the pressor responses to amodiaquin and chloroquine in reserpineized animals by giving a prior infusion of noradrenaline though the pressor effect of methylamphetamine which was given as pretreatment was restored. Fawas (99) reported that after noradrenaline infusion in reserpineized animals, tyramine evoked pressor response immediately after the infusion but it had little pressor effect after 30 min. The failure to restore the pressor responses of the 4-aminoquinolines given 30 to 40 min after the noradrenaline infusion, as in case of tyramine, might have been due to instability of the repleted tissue amine stores in the post-infusion period.
The results obtained by using the isolated stomach strips of reserpine pretreated rats bathed in blood can be explained by proposing that amodiaquin and chloroquine release the amines in vicinity of the receptor sites but not in general circulation. This action is similar to that of certain sympathomimetic amines like tyramine (244). A rise in tone of the blood bathed stomach strip after the administration of methylnlamphetamine observed in the experiments could have been due to the activation of tryptamine receptors by methylnlamphetamine as proposed by Vane (244). The initial relaxation of the stomach strips in response to amodiaquin or chloroquine was not seen on a second exposure of the strips to these drugs. This effect could not be due to a direct action of these drugs on the smooth muscle of the preparation but was most probably due to a local release of amines from the tissue stores persisting in spite of adequate reserpine pretreatment of the rats.

The results obtained in these experiments further make it unlikely that the 4-aminoquinolines release catecholamines through a nicotine-like action, that is through a depolarisation of the nerve terminal or the chromaffin cell; in these experiments the adrenal medulla of the dogs was undisturbed. The observations with the use of dimethylphenylpipperazinium suggest that the amines
released through a nicotine-like action could have been promptly detected by the stomach strips which exhibited a high degree of sensitivity to the circulating catecholamines. Furthermore, small doses of P-286 which reduced the pressor effects of small doses of dimethylphenylpiperazinium, did not much reduce the pressor effects of the 4-aminoquinolines. It thus appears that adrenal medulla does not participate to any major extent in the amine release due to amodiaquin and chloroquine. In this respect again the compounds appear to resemble tyramine as most of the workers have not been able to demonstrate the amine release from medullary or extramedullary chromaffin tissue (244, 234, 196).

If amodiaquin and chloroquine exert their pressor effects by releasing catecholamines from the tissue stores as has been proposed, the augmentation of the pressor effects of 4-aminoquinolines after the ganglion blocking agents which has been observed could partly be explained on the basis of the known potentiating effect of ganglion blocking agents on responses of tissues, in vivo and in vitro, to catecholamines (182, 245, 183, 154, 252).