STUDIES ON THE PHARMACOLOGY OF SOME ANTIMALARIAL DRUGS

A THESIS

presented by

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This thesis is dedicated to:

MY FATHER

for leading his children into intellectual pursuits

MY MOTHER

for her sympathetic approach to my problems

MY WIFE - ADEDAYO

for her eternal patience, love and deep understanding

MY CHILDREN

for making everything worthwhile.

SUMMARY

Pharmacological studies of the antimalarial drugs chloroquine, primaquine, quinine, proguanil and pyrimethamine have been made. These studies have shown that the compounds possess a wide spectrum of pharmacological actions in common on many tissues and organ-systems. In most cases, their effects were biphasic, consisting of an initial stimulation followed by a more permanent inhibitory phase.

All five antimalarial compounds, at low concentrations, reduced the base-line tone (tension) of all smooth muscles studied, and augmented electrically-induced contractions of the chick oesophagus, vas deferens and central ear artery preparations. Higher concentrations of the drugs dose-dependently contracted gastrointestinal smooth muscles and inhibited the spontaneous, myogenic rhythmic contractions of intestinal muscles, uterine strips and portal veins *in vitro* in a dose-dependent manner. In the same dose range they inhibited the electrically-evoked contractions of the chick oesophagus, vas deferens and central ear artery preparations.

The drugs relaxed tracheal chain preparations contracted with acetylcholine (in the presence of physostigmine), carbachol, histamine and 5-hydroxytryptamine. *In vitro*, all five compounds antagonised the actions of standard spasmogens in all preparations examined. This spasmolytic

effect of the drugs has been shown to be non-specific in nature.

All the five compounds, in very low doses, augmented the action of acetylcholine on frog rectus abdominis and chick biventer-cervicis muscles. High concentrations of the drugs themselves caused dose-related sustained contractions of the muscles in vitro. In similar concentrations they inhibited, or abolished, the actions of acetylcholine, carbachol, nicotine and potassium chloride. In some cases, low concentrations of the compounds, especially quinine, chloroquine and primaquine, augmented electrically-induced twitches of the chick biventer and rat hemi-diaphragm muscles in vitro, and of the soleus and tibialis anterior muscles of the cat in vivo. High doses of the compounds, themselves inhibited the twitches in a dose-related manner, and augmented the effects of neuromuscular blocking agents on the preparations. The drugs also inhibited the tetanic as well as the intra-arterially injected acetylcholine-induced contractions of the tibialis anterior muscle in vivo. All the five compounds possessed anticholinesterase activity.

In isolated cardiac muscle, all the five drugs studied increased the refractory period and caused negative inotropic and chronotropic responses. However, low concentrations of the quinoline compounds (primaquine, chloroquine and quinine) induced slight but measurable transient positive

inotropic and chronotropic effects in the heart. Intravenous injections of each of the five compounds into anaesthetized cats produced similar cardiovascular changes. These changes consisted of dose-dependent reductions in systemic and pulmonary arterial pressures, left ventricular pressure, left ventricular dP/dt max; and heart rate. Other changes consisted of dose-related increases in right atrial and left ventricular end-diastolic pressures, P-R interval and QRS complex duration. All the compounds inhibited or abolished the pressor effects of intravenous noradrenaline on the cardiovascular system.

CONTENTS

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SECTION 1

GENERAL INTRODUCTION

MALARIA

The word 'malaria' is derived from two Italian words "mala" and "aria" meaning "bad air". Today however, malaria is recognized as a disease caused by infection with protozoan parasites assigned to the genus Plasmodium, and is characterized by intermittent fever, anaemia and splenic enlargement.

It has long been established that malaria in man is caused by four species of Plasmodium (protozoan parasites) and in other animals, birds and reptiles, by many other species. The four species of plasmodia known to infect man, and for which man is the natural host, are: Plasmodium vivas, Plasmodium falciparum, Plasmodium malariae, and Plasmodium ovale. The first three of these are widely distributed and occur most frequently in tropical and sub-tropical countries, whilst the last is more common in cooler regions. Each of the four human plasmodial species produces a specific malarial disease which is named according to its pathogen respectively as: vivax malaria (often referred to as either benign tertian, simple intermittent fever, or the tertians), falciparum malaria (also known as either malignant tertian, aestivo-autumnal, sub-tertian, malignant bilious, tropical and pernicious malaria, or congestive remittent fever) malariae malaria (sometimes called quartan fever or quartan ague), and ovale malaria (usually described as ovale tertian). The infections are labelled 'tertian' or 'quartan' because the fever tends to re-occur every third or fourth day,

although some variation in time intervals may be observed. The disease caused by infection with malarial parasite is characterized by successive chills, periodic fevers and convulsions. Other clinical signs include anorexia, jaundice, pain and muscular weakness.

All species of Plasmodium have two hosts, a vertebrate and a mosquito that acts as both vector and definitive host. Mosquitoes of the genus Anopheles are the vectors for human malaria while mosquitoes of the genera Aedes, Culex and Culiseta as well as Anopheles are the vectors for the plasmodia infecting other vertebrates. In the vertebrate host, all species of plasmodia have a predilection for life in the erythrocyte in which they metabolize haemoglobin with the formation of a pigment, haemozoin or haematin, as a by-product and the liberation of toxins or malarial 'poison'.

1.1 Life cycle of human plasmodium

The life cycle of human plasmodium is illustrated in Figure 1 and consists of two distinct phases denoted by stages in the mosquito and stages in man respectively. The sexual phase of the life cycle begins when a female anopheles mosquito bites an individual and ingests blood containing the malarial parasite in the gametocyte stage. In the stomach of the mosquito, the sexual phase of development called sporogony occurs. The male and female gametocytes form gametes. An ookinete is formed by fertilization and this penetrates the stomach wall. Outside the stomach, an

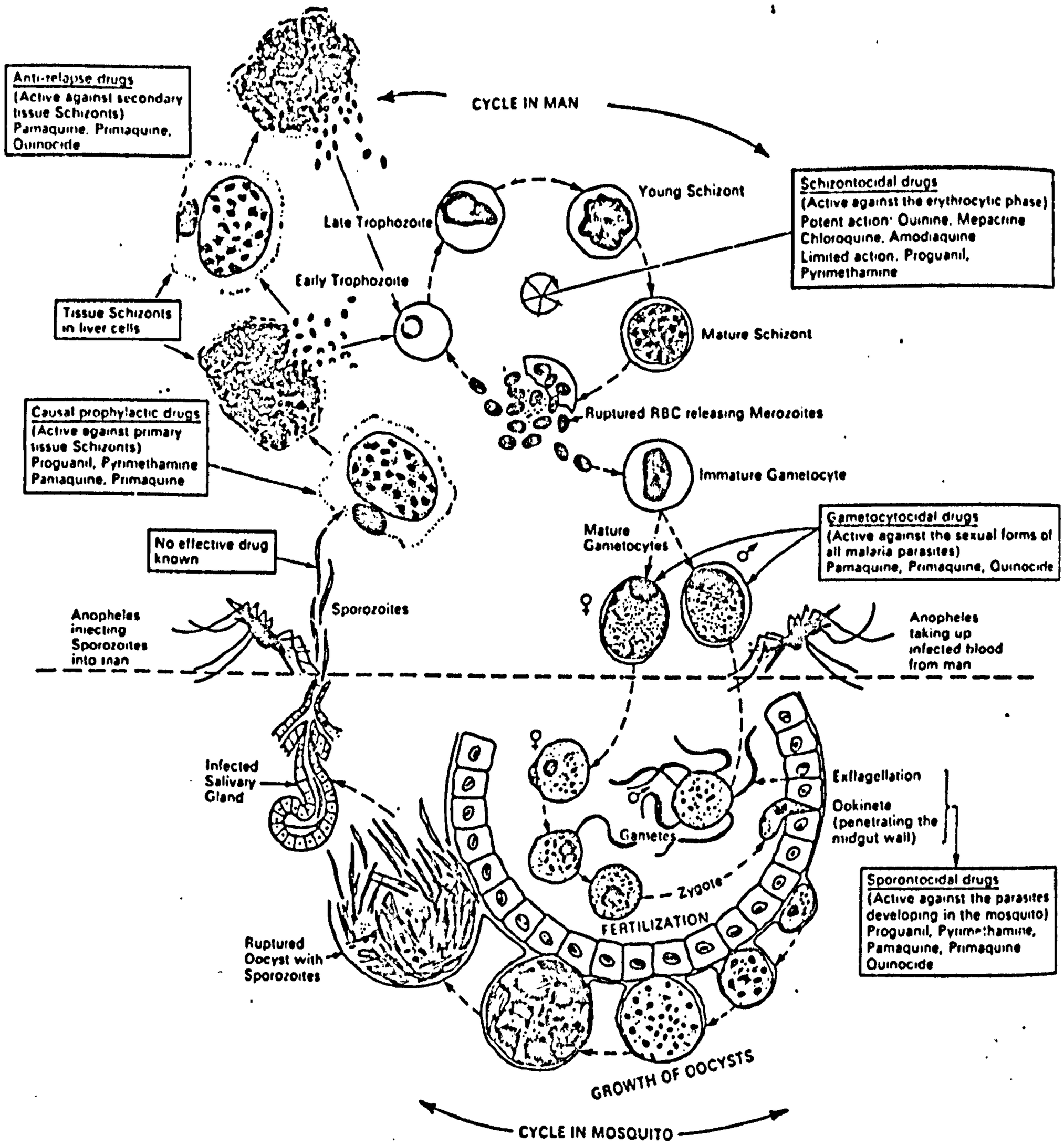


FIGURE 1

CLASSIFICATION OF ANTIMALARIAL DRUGS IN RELATION TO THE LIFE-CYCLE OF MALARIAL PARASITE

oocyst is formed which produces sporozoites that are released by the rupturing of the oocyst. The sporozoites travel to the salivary glands of the mosquito, from which they may be transferred to an uninfected individual host by the bite of the mosquito when it starts its blood meal. Injected sporozoites disappear rapidly from the blood stream of the new host, entering the parenchyma cells of the liver and, perhaps, some other tissues. The parasite now begins the asexual phase of development called schizogony. In this pre-erythrocytic (primary exoerythrocytic) stage, the parasite grows and divides to form a schizont. The schizont segments to form many merozoites, which causes the rupturing of the cell, and the merozoites enter the blood stream. The merozoites invade the red blood cells, beginning the erythrocytic stage. Within the red blood cells, the merozoites become trophozoites, and multiplication by schizogony occurs. The schizonts that are formed from the trophozoites divide into two merozoites and, thus, continuously increase the number of merozoites available to invade more red blood cells, so that finally, the number of rupturing cells is sufficiently great to initiate the clinical symptoms of the disease. This asexual cycle continues until chemotherapy is initiated, immunity is developed, or death occurs. The continuous invasion and subsequent eruption of erythrocytes lead to the development of another significant symptom of malaria, anaemia. It is the chronic anaemia of the victim that contributes to the malaise and the general lassitude of the people in

malarious countries (White 1971).

At any time, but particularly when normal reproduction of the erythrocytes becomes unfavourable, some of the trophozoites from the erythrocyte stage develop into male (micro-) or female (macro-) gametocytes that circulate in the blood to become available for ingestion by another mosquito. Thus, the life cycle is complete. Some species of Plasmodium, notably P. vivax (but not P. falciparum), are capable of existing in para-erythrocytic (secondary exoerythrocytic) forms that have a variety of patterns but always pass through schizont stages. By this development, the parasite may enter into a dormant state in the tissues of the host during which time it may appear that the infection has been overcome. However, some time later the parasite may return to the blood stream and thus cause a relapse.

1.2 Chemotherapy of malaria

Numerous excellent authoritative reviews on various aspects of malaria chemotherapy have been published since 1945 (for references, see Elslager 1969). On the basis of chemotherapy, antimalarial drugs may be classified into five major different types depending on which stage of the life-cycle of the organism is affected (Figure 1).

(1) Sporozoitocides:

These are antimalarial drugs that are capable of killing the sporozoites as soon as they are introduced into the blood stream by the bite of a female anopheles mosquito. Such drugs would be most desirable, since they would be truly causal prophylactics capable of preventing the development of the disease. Unfortunately, very few compounds with such chemotherapeutic properties have been found.

(2) Exoerythrocytic Schizontocides:

These are drugs capable of killing the parasites in their schizont stage, in either the primary or the secondary exoerythrocytic form. Such drugs, sometimes called "tissue schizontocides", may be said to be curative, because they are capable of eradicating the parasites before they enter the red blood cells or while they are dormant in the host. A favourable effect on relapse rate results. Only a few drugs have been found that possess such activity to a significant degree.

(3) Erythrocytic Schizontocides:

These are antimalarial drugs that are capable of inhibiting the development of schizonts during the erythrocytic stage of the parasite's life-cycle. Usually, such drugs keep the number of the blood forms of the organism at a level below that necessary to precipitate the clinical symptoms of the disease. Such drugs, sometimes called "suppressives" or "clinical prophylactics", are also known as "blood schizontocides". Many of the widely used antimalarials exhibit this kind of activity.

(4) Gametocides:

These are drugs that are capable of killing the parasites in their gametocyte stage. Such drugs help to prevent the spread of the disease, since the vector mosquitoes do not become infected. A few antimalarial drugs possess such activity.

(5) Sporontocides:

These are drugs that are capable of preventing sporogony in the vector mosquito by their effect on the gametocytes in the blood of the vertebrate host. All sporontocidal drugs show activity as exo-erythrocytic schizontocides as well.

The ideal drug would be one that exhibited all five types of activity against all four species of human plasmodia. Unfortunately however, no such broad-spectrum antimalarial compound has yet been found.

In the evaluation and use of antimalarial drugs, certain concepts and definitions are useful. For example, drug action on schizonts, erythrocytic or exo-erythrocytic phase is referred to as SCHIZONTICIDAL; on gametocytes as GAMETOCIDAL; and on sporozoites as SPORONTOCIDAL.

CLINICAL CURE implies that acute symptoms and signs of the disease have been relieved so that the patient appears to have recovered, RADICAL CURE, whether occurring naturally or after medication, implies clinical cure plus elimination of plasmodia from the blood and tissues so that relapses are not possible; SUPPRESSION implies the prevention of

clinical symptoms by action on the plasmodia and it may be TEMPORARY, ie. present only while the drug is effective in the plasma, or PERMANENT, in which case no attack supervenes after suppressive medication has been stopped; SUPPRESSIVE CURE is a radical cure brought about while the patient is receiving suppressive rather than therapeutic medication; DRUG PROPHYLAXIS is the use of chemotherapeutic agents(s) to protect individuals from the attacks and manifestations of the disease; such protection may be in the form of INDIVIDUAL DRUG PROPHYLAXIS, or communal, ie. COLLECTIVE DRUG PROPHYLAXIS. When a drug acts on sporozoites or on pre-erythrocytic plasmodia to prevent further development in the body, the result is TRUE CAUSAL PROPHYLAXIS. The term PRESUMPTIVE TREATMENT has been used to describe the administration of an antimalarial agent in a single dose to presumptive cases of malaria before the result of blood smear examination becomes available. Presumptive treatment is an important procedure in the surveillance period of a malaria eradication programme.

The drugs most effective in bringing an acute attack of malaria to a speedy end, by acting on the asexual plasmodia, are quinine, mepacrine and the 4-aminoquinolines - chloroquine and amodiaquine. On the other hand, the drugs most useful in effecting a radical cure of vivax infections, once the primary attack has been halted, are the 8-aminoquinolines - pamaquine and primaquine. Lastly, the drugs most useful for chemoprophylaxis are: chloroquine, amodia-

quine, proguanil and pyrimethamine.

1.3 Development of active synthetic antimalarial compounds

The most important classes of synthetic antimalarial drugs known today are:

(1) Cinchona alkaloids
(2) 4-Aminoquinolines
(3) 8-Aminoquinolines
(4) 9-Aminoacridines
(5) Biguanides
(6) Pyrimidines
(7) Sulphones and Sulphonamides

Out of all these major groups, only a few are therapeutically important and will be discussed.

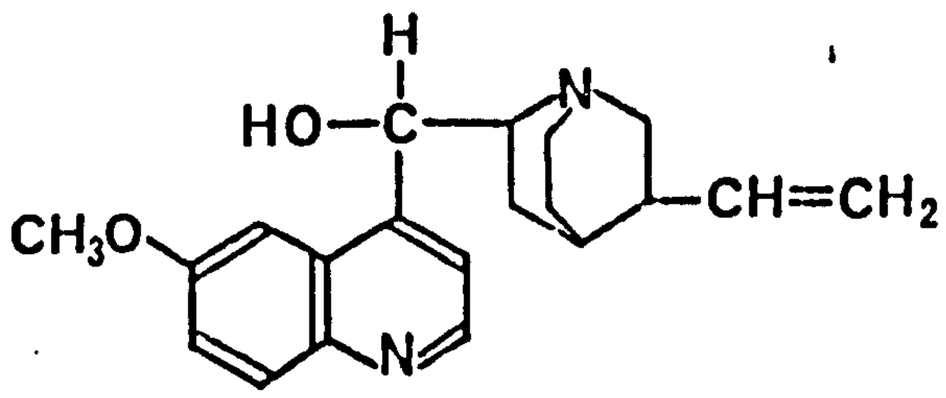
Cinchona alkaloids

The crude drug (cinchona bark) contains numerous alkaloids, of which quinine, quinidine, cinchonine and cinchonidine are the most abundant and important. Although each member of this quartet has some therapeutic effect on malaria, quinine has been the most useful.

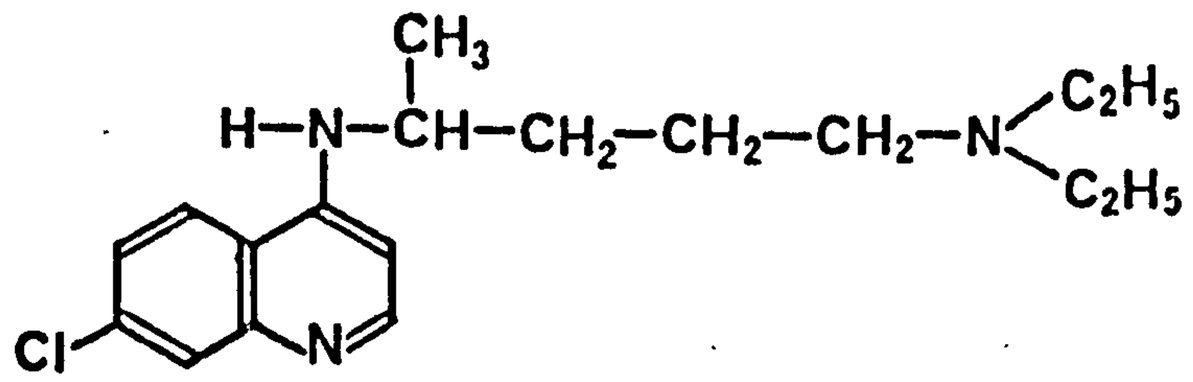
Quinine

Chemically, quinine isolated by Pelletier and Caventou in 1820 (Duran-Reynals 1946) is a quinoline compound of complex molecular structure (Figure 2) and first synthetisized by Woodward and Doering in 1944. Quinine is a general proto-

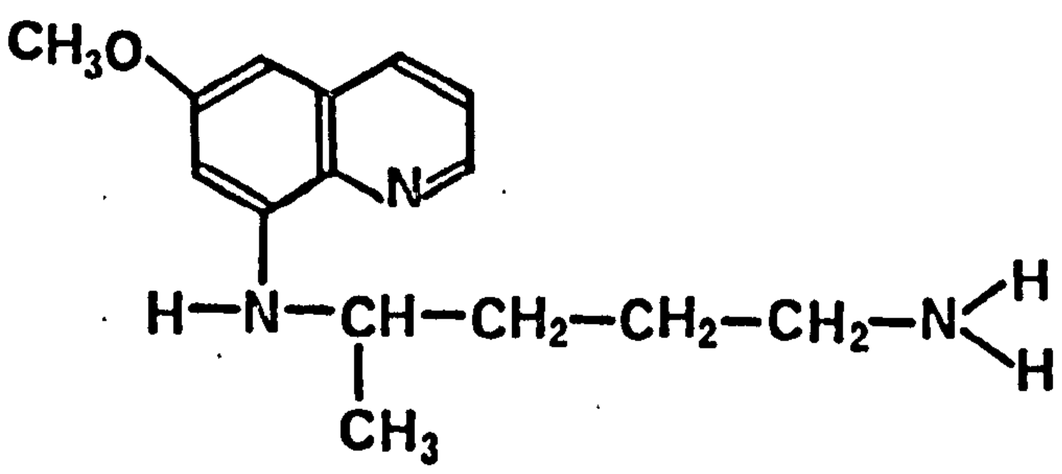
Quinine



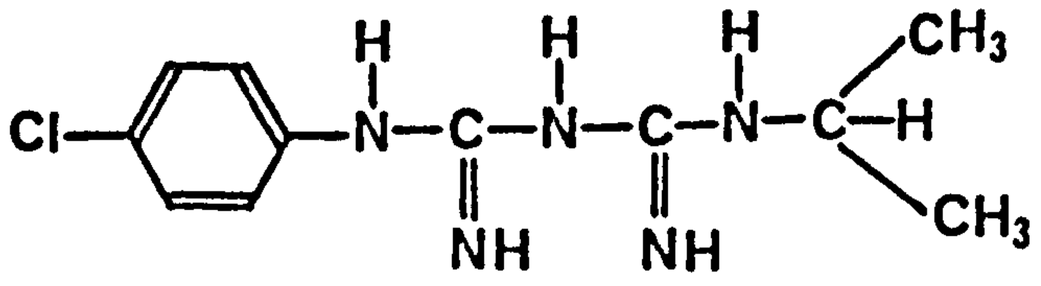
Chloroquine



Primaquine



Proguanil



Pyrimethamine

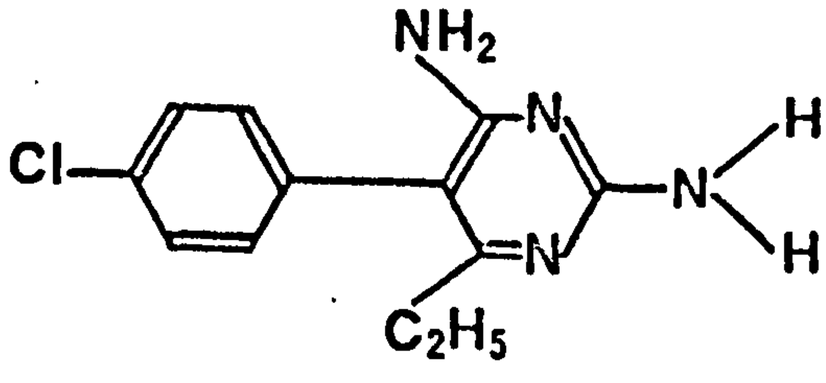


FIGURE 2

STRUCTURAL FORMULAE OF THE ANTIMALARIAL COMPOUNDS STUDIED

plasmic poison. By virtue of its toxicity to cells, it is a local irritant. Intramuscular injections of the drug, for example, may cause pain and sloughing whilst careless intravenous injections may thrombose veins. In high concentrations (not attained in clinical treatment) quinine haemolyzes red blood cells. After oral administration, quinine is absorbed in the small intestine and becomes concentrated in plasma and cellular elements of blood. It has been suggested that the reticulo-endothelial cells remove approximately two-thirds or more of quinine in the blood and take it to the liver, kidneys and to the muscle tissues where it is destroyed. The rest is eliminated in urine, except for small amounts secreted in the bile, saliva, or milk of lactating mothers.

Toxicity

The chief toxic manifestations of quinine are grouped together under the term "cinchonism". This syndrone may include one or more of the following: tinnitus, slight deafness, dizziness, amblyopia, photophobia, diplopia, vertigo, headache, gastric distress, nausea, vomiting, diarrhoea, fever, sweating, flushed skin, and urticarial rash. Sometimes, facial oedema, slight mental depression, and syncope occur. Some individuals are hypersensitive to quinine. When such toxic effects appear in the course of standard malaria therapy, they are usually mild and clear up completely, but when they are due to overdosage, they may result in permanent injury to the retinal and auditory

ganglion cells, or to the optic nerve. Therapy should however be terminated when the signs and symptoms of "cinchonism" appear during the course of normal malaria treatment with quinine.

Chloroquine and other 4-aminoquinolines

The synthesis of 4-aminoquinolines for antimalarial studies was first undertaken by Russian and German workers just prior to World War II. The general direction that research had been taking may be seen by the German report in 1942 (Schönhöfer 1942) that 4-, 6- and 8-aminoquinolines gave antimalarials when properly subsitituted. The first 4-aminoquinoline synthesized in Germany in 1937 (Coatney 1963) was named 'Resochin'. This compound is officially known now in the United Kingdom, United States and many other parts of the world as chloroquine. (Other names by which chloroquine is known are: 'Aralen', 'Avloclor', Nivaquine B' and 'Tanakan'). Strangely "Resochin" was discarded at first as being too toxic and Sontochin was synthesized. This compound which differs from chloroquine only in having an extra methyl group on the quinoline nucleus, was the second 4-aminoquinoline and it was being tested in the German Army when samples were obtained in North Africa by the Allies in 1943. Sontochin (also known as: 'Santochin','sontoquine', Nivaquine A', 'Nivaquine C') and a series of other 4-aminoquinolines were then synthesized in the United States and tested in clinical studies. Of all the 4-aminoquinolines, however, chloroquine and amodiaquine ('Cam-aqi', 'Camoquin',

'Flavoquine', or 'Miaquine') were found to be the best for malaria therapy and prophylaxis, and are now in common use.

Toxicity

Chloroquine and amodiaquine can be administered orally or parenterally. The compounds are rapidly and almost completely absorbed from the gastro-intestinal tract and are localized in various tissues from which they are slowly metabolized and excreted.

Chloroquine and amodiaquine have a lower therapeutic index and higher safety margin than quinine. Although the toxicity of 4-aminoquinolines is quite low in the usual antimalarial regimen, both acute and chronic toxic reactions may develop. Acute side effects include nausea, vomiting, anorexia, abdominal cramps, diarrhoea, headache, dizziness, pruritus and urticaria, convulsion and blurring of vision. Usually, such symptoms are completely reversible on reduction of the dose or complete withdrawal of the drug. Toxic effects that are found less frequently are leucopenia, tinnitus and deafness. Long term administration or high dosages may have serious effects on the eyes, and opthalmological examinations should be carefully carried out. More serious toxicity has been reported after prolonged dosing in the treatment of skin diseases. For example, a fatal agranulocytosis was reported after 0.2 g of amodiaquine administered daily for eight weeks. Severe leucopenia and thrombocytopenia have also occurred after long courses of chloroquine in the treat-

ment of skin diseases (Russel, West, Manwell and Macdonald 1963). Rare cases of bluish-grey to black pigmentation have been reported in individuals who have taken amodiaquine prophylactically for a prolonged period of time. For instance, in certain cases where three tablets of 'camoquin' (containing 0.2 g amodiaquine base per tablet) had been taken weekly for a year (Russel et al, 1963). The discoloration was noticed especially in the nail beds of fingers and toes, and on the nose, lips and palate. The pigmentation was in some areas diffuse but in others was in the form of macules or irregular patches, 1 to 10 mm in diameter. It has been recommended that chemotherapy should be stopped when toxic signs and symptoms appear.

Primaquine and other 8-aminoquinolines

Shortage of quinine in Germany during the First World War directed attention to the need for synthetic antimalarials. Starting with Guttman and Ehrlich's observation that methylene blue has some inhibitory effect on plasmodia, Schulemann, Schonhofer and Wingler synthesized, and Roehl tested, several compounds, finally selecting in 1925, pamaquine (plasmochin) as the best. This drug was the first truly successful synthetic antimalarial compound. Pamaquine (Wiselogle 1946; Loeb, Clark, Coatney, Coggeshall, Dieuaide, Dochez, Hakansson, Marshall, Marvel, McCoy, Sapero, Sebrell, Shannon and Carden 1946) was the first of the synthetic 8-aminoquinolines, and it is variously called 'Aminoquin', 'Beprochin', 'Gamefar', 'Plasmochin', 'Plasmoquine',

'Plasmocide', and 'Praequine'. This drug is being superseded by primaquine (Wiselogle 1946; Edgcomb, Arnold, Yount, Alving and Eichelberger 1950) synthesized in the United States in 1945, which has a similar action to pamaquine but a lower toxicity.

Toxicity

It has become increasingly clear that there is only a narrow margin of safety between therapeutically effective and seriously toxic doses of pamaquine, primaquine and other 8-aminoquinolines. Consequently, most observers have ceased to use these compounds routinely in the treatment and prophylaxis of malaria (Russel et al, 1963).

The toxic effects of the 8-aminoquinolines are principally on the central nervous and haemopoietic systems. Occasionally, anorexia, abdominal pain, vomiting and cyanosis may be produced. The toxic effects related to the blood system are more common; haemolytic anaemia (particularly in dark-skinned people), leucopenia and methemoglobinemia are the usual findings.

Symptoms of pamaquine toxicity frequently seen after minimal therapeutically effective dosages include: abdominal pain, nausea, vomiting, headache, dizziness, and drowsiness. Haemoglobinuria, acute haemolytic anaemia, granulocytopenia, cyanosis, circulatory collapse, jaundice, or acute yellow atrophy of the liver are less common but very disturbing

side effects of pamaquine.

The tendency for haemolytic reaction following primaquine administration has been shown by Alving, Kellermeyer, Tarlov, Schrier, and Carson (1958) to be linked with a defect of glucose-6-phosphate-dehydrogenase enzyme in the erythrocytes of susceptible persons. It is an hereditary characteristic most commonly found in dark-skinned people in equatorial countries. There is some overlap between therapeutic and toxic doses of pamaquine in such people, but there is usually a slight margin between the two in the case of primaquine, with which toxic effects are therefore much less frequent. It has, however, been shown that acute intravascular haemolysis may occur in such people after the daily administration of 30 mg. primaquine. The haemolysis is self-limited, as only the older red blood cells are destroyed. In susceptible individuals, primaquine is haemolytic at all dosages but the haemolysis is usually not of clinical significance if the daily adult dose of 15 mg. base is not exceeded. Some observers recommend that primaquine should not be given to infants under six months of age (Russel et al, 1963).

Proguanil and other biguanides

The development of the biguanides as antimalarials began in the mid 1940's as a result of a research programme of some British scientists (Curd, Davey and Rose 1945) who had observed the activity of some sulphonamide drugs, particularly

sulphadiazine, against malaria infections. It was then thought that the incorporation of certain dialkylaminoalkyl chains onto the pyrimidine ring might lead to significant antimalarial compounds. Although some of their pyrimidine derivatives were active, their studies lead them to some open models including certain biguanides. These compounds showed definite activity against plasmodia, and subsequent chemical modifications led to the production of the compound now known as proguanil by Adams, Maegraith, King, Townshead, Davey and Havard in 1945. Almost at the same time, this team of workers synthesized chlorproguanil in England. However, the dichloro-compound, chlorproguanil, is more toxic than proguanil itself, and for this reason is less frequently used in clinical practice. Proguanil is the British Pharmacopoeia name for the biguanide known officially in the United States as chlorguanide. Other names for proguanil are: 'Bigumal', 'Chloriguane', 'Diguanyl', 'Guanatol', 'Paludrine', 'Palusil', and 'Tirian'.

It has been established that the active forms of biguanides are their metabolic products (Crowther and Levi 1953). For proguanil, this is 4, 6-diamino-1-p-chlorophenyl-1, 2-dihydro-2, 2-dimethyl-1,-3,-5-triazine (sometimes called 'cyclo-guanil'). Because these metabolic products are eliminated so rapidly, they are not useful per se in the treatment of human malarias although they are about ten times as active as their precursors. However, a repository preparation of the metabolite of proguanil (cycloguanil pamoate) has

become available and has achieved spectacular success as an antimalarial agent with a prolonged duration of activity.

The biguanides are usually administered orally. They are absorbed from the gastro-intestinal tract very quickly, but not as rapidly as quinine or chloroquine. They are concentrated in the liver, lungs, spleen and the kidney but appear not to cross the blood-brain barrier. They are metabolized and eliminated very rapidly, principally in the urine. As a result, frequent administration of these drugs is necessary.

Toxicity

The toxic manifestations of biguanides are very mild in man. Some gastro-intestinal disturbances may occur if the drugs are taken on an empty stomach but not if they are taken after meals. With excessive doses (1 g. of proguanil) some renal disorders such as haematuria and albuminuria may develop.

Pyrimethamine and pyrimidines

Following the observations made in the 1940's that some 2, 4-diaminopyrimidines are capable of interfering with the utilization of folic acid by Lactobacillus casei, a property also shown by proguanil, these compounds received intensive study as potential antimalarials. It was noted that certain 2, 4-diamino-5-phenoxypyrimidines possessed a structural resemblance to proguanil, and a series of such compounds

was synthesized and found to possess good antimalarial action. Subsequently, a large series of the compounds was prepared and tested for activity. The best in the series of compounds was the one that became known as pyrimethamine (Falco, Goodwin, Hitchings, Rollo and Russell, 1951).

Pyrimethamine, also known as 'Daraprim' or 'Malocide', is the most powerful malaria suppressive agent known. Except for the metabolic products of the biguanides, pyrimethamine is also the most active antimalarial drug developed for clinical use (Russel et al, 1963). The drug is always administered orally in the form of the free base, a relatively tasteless powder. It is slowly but completely absorbed from the gastro-intestinal tract. It is localized in the liver, lungs, kidneys and the spleen, and excreted through the urine, chiefly in matabolized form.

Toxicity

The toxicity of pyrimethamine is very low and when administered in suitable doses, it rarely gives trouble. However, over-dosage may lead to depression of cell growth by inhibition of folic acid activity. Several fatalities have been reported in children who ate pyrimethamine tablets as a sweet. The early signs and symptoms in these children included convulsions, fever, and sweating (Russel et al, 1963).

1.4 Aims of the study reported in this thesis

In recent years, the toxic and unwanted side-effects of antimalarial drugs have become more prominent and widespread. These have included gastro-intestinal cramps, skeletal muscle weakness, and cardiovascular impairment - for details, see Toxicity under each compound in the present section, and Introduction to each of the sections 2, 3, and 4 . Unfortunately however, the pharmacological mechanisms by which the compounds produce these effects are ill-understood. Since millions of people living in malarious countries still depend mainly on antimalarial drugs for the management, prevention and treatment of malaria disease, it becomes necessary to investigate fully the pharmacological actions of the compounds on various tissues and organ-systems. It was also thought that such studies would probably provide rational explanations for some of the aforementioned well-known toxic and side-effects of the compounds. Furthermore, since there has apparently been no previous comparative study of the pharmacological actions of these drugs, it was thought to be of interest to examine the effects of these agents in a parallel manner on some tissues and organ-systems of experimental animals. In order to fulfil these objectives therefore, the pharmacological effects of chloroquine, primaquine, quinine, proguanil and pyrimethamine were studied on smooth, skeletal and cardiac muscles and also on the cardiovascular system of some laboratory animals.

SECTION 2

SMOOTH MUSCLE PHARMACOLOGY OF THE ANTIMALARIAL DRUGS

INTRODUCTION

2.1 Effects of antimalarial drugs on intestinal smooth muscle

There are several reports in the literature describing non-specific and spasmolytic effects of antimalarial drugs in intestinal smooth muscle. Keogh and Shaw (1943, 1944) found that quinine relaxed the rat intestine and reduced responses of the muscle to acetylcholine, barium, adrenaline and potassium. Proguanil depressed the rabbit small intestine and reduced responses of the guinea-pig ileum to histamine (Chen and Anderson 1947; Vane 1949) and acetycholine (Vane 1949). In anaesthetized cats, proguanil lowered the tone of intestinal muscle and diminished the response to vagal stimulation (Vane 1949). Chloroquine reduced contractions produced by acetylcholine, 5-hydroxytryptamine or histamine in the guinea-pig ileum (Agarwal and Deshmanker 1963; Deshpande, Sharma and Dashputra 1963; Olatunde 1970). The pA_2 values of chloroquine against histamine, acetylcholine and 5-hydroxytryptamine were determined on the guinea-pig isolated ileum by Olatunde (1970) who concluded that this drug exerts a direct spasmolytic action on smooth muscle rather than a specific receptor antagonism. The quinolyl piperazine antimalarial agent, WR 4809, was found to produce a parallel shift to the right of dose/response curves to bethanechol, histamine, potassium chloride and 5-hydroxytryptamine in the guinea-pig ileum (Burks 1972). Using Finkleman's preparation,

Achari, Banerji and Kapoor (1972) found that low concentrations of quinine and quinidine (2.5 - 5.0 µG/ml) blocked the effects of sympathetic stimulation but not the noradrenaline response. Apart from quinine, this blockade was not reversible. These workers further observed that quinine potentiated the inhibitory response to noradrenaline, and the preparation always became more sensitive to noradrenaline after washing out quinidine from the tissue.

2.2 Effects of antimalarial compounds on uterine smooth muscle

Since the earliest days of its use as an antimalarial agent, it has been repeatedly emphasized that care should be exercised when administering quinine to pregnant women. Manson-Bahr (1954) stated that although large doses of quinine might sometimes cause miscarriage, pregnancy should not debar the use of the drug. He further observed that prophylactic doses of quinine did not interfere with menstruation, conception or pregnancy. It was similarly observed that mepacrine (quinacrine) and plasmoquine were better tolerated by pregnant women. Several clinical reports are now available which support the safe use of chloroquine in pregnancy. The most striking ones include the reports of Goldsmith (1946); Merwin and Winkelmann (1962); Dziubinski, Winkelmann and Wilson (1962); Stone Jr. (1962), and Klumpp (1965). All these investigators found that chloroquine had no harmful effect in pregnancy on either the mother or the foetus.

Morwin and Winkelmann (1962) further claimed that chloroquine prevented miscarriages and/or threatening abortions, facilitated the formation and production of viable infants and aided normal delivery in women. There have been isolated reports and speculations, however, that some antimalarial compounds might constitute a potential danger to pregnant women. Foremost among such reports is the editorial comment of JAMA a few years ago on 'The Placental Barrier and Drugs' (JAMA 1964, 190, 392) which made reference to a paper by Hart and Naunton (1964) reporting on the effects of chloroquine phosphate given to a woman during four of her seven pregnancies. The editorial concluded that "chloroquine is yet another drug that has been found to pass the placental barrier with resultant damage to the foetus". Goodman and Gilman (1975) have also stated that "quinine and related alkaloids possess oxytocic activity and might induce abortion". Experiments in laboratory animals have suggested a biphasic action for most of the antimalarial drugs on the uterus and again call into question their safety in pregnancy. The stimulant action of quinine on the uterus was demonstrated by Kurdinowski and Kehrer as early as 1906 and this was quoted by Sollmann (1948). Gunn and Russell (1946) showed that human uterine strips *in vitro* were either unaffected, or inhibited, by quinine which also antagonized the augmentary effect of adrenaline on uterine contractions. Sollmann (1948) described the action of quinine on the uterus, both excised and *in situ*, as stimulant in moderate

doses and depressant in large doses. Chen and Anderson (1947) found that proguanil inhibited the isolated uterus of rabbit and stimulated guinea-pig uterus. Joseph and Jindal (1957) observed that relatively low doses of quinine, chloroquine and amodiaquine stimulated uterine strips of rats and guinea-pigs whilst large doses of the compounds relaxed uterine muscle. These workers further observed that in fairly high doses, quinine, proguanil and chloroquine antagonized oxytocin. Gracia, Miyares, Reyes-Diaz, and Sainz (1968) reported that chloroquine inhibited the contractions of the rat isolated uterus evoked by acetylcholine, 5-hydroxytryptamine, angiotensin, vasopressin, oxytocin and barium chloride. Abdel-Aziz, Karrar, and Idris (1971) studied the effects of the antimalarial compounds quinine, chloroquine, chloroguanil and mepacrine on the rat isolated spontaneously contracting uterus under ovarian hormones treatment and during pregnancy. They reported that in animals pretreated with oestrogen, the action of quinine on isolated uteri was inconsistent; chloroquine increased the tone and frequency of uterine contractions, while chloroguanil and mepacrine were predominantly inhibitory. Progesterone treatment decreased the sensitivity of the rat uterus to the oxytocic action of chloroquine and to the inhibitory action of chloroguanil, but changed the inhibitory effect of mepacrine into a stimulant action. All four antimalarial agents inhibited uterine strips taken from animals in early and late pregnancy, and the inhibitory action of quinine was

associated with an increase in frequency of contractions (Abdel-Aziz et al). More recently, Kurantsin-Mills and Chinyanga (1974) also showed that low concentrations of chloroquine sulphate (36.7 - 146.8 nM) exhibited oxytocic properties on the oestrogen-primed guinea-pig isolated uterus, while higher doses of the drug depressed the contractile tension and frequency of contractions of the isolated uterus. They further observed, as did Garcia et al, (1968), that the stimulant effect of oxytocin on this tissue was inhibited by high concentrations of chloroquine sulphate.

2.3 Effects of antimalarials on tracheo-bronchial smooth muscle

The use of certain antimalarial compounds in the clinical management of bronchial asthma is now becoming more widespread. Of the various classes of antimalarial agents, the aminoquinolines appear to be the most useful therapeutically. The first aminoquinoline used for the treatment of bronchial asthma was quinetholate (phthalamaquin or aureoquin). Geschikter (1953, 1954, 1955) reported that this compound: (1) relaxed the isolated guinea-pig tracheal chain and antagonized bronchospasm induced by histamine and acetylcholine, and (2) relieved bronchial asthma in patients in an oral dose of 50 mg. Blanc (1962) confirmed the favourable effects of quinetholate in seven out of 15 patients. In a double-blind study, Young, Murray, Carr and Harden (1965) recorded that quinetholate produced a delayed improvement

in ventilatory function in some, but not all, patients. The use of chloroquine in asthmatic patients was first reported by Engeset (1957). A daily dose of 100 - 250 mg administered orally for two weeks caused an amelioration of the respiratory difficulties in 75% of 32 patients. Sylvio de Camargo (1958), apparently unaware of Engeset's report, described his experiences with 20 patients whose condition improved under chloroquine treatment. Favourable results have also been reported by Krammer (1960), Stern, Zucker, Sherman and Florio (1960), Juul-Møller (1961), De Aguilar (1961, 1962), Benda and Mosse (1962), Kimura, Moritani, Tsuchida and Matsuura (1962), Jyo, Katsuya, Shikauchi and Takahashi (1963), Tannenbaum and Smith (1966).

Several workers have investigated the effects of various antimalarial drugs on isolated tracheal chains and on bronchospasm induced by histamine in laboratory animals. In guinea-pigs, Vane (1949) showed that intravenous injections of proguanil (1 - 20 mg/kg) had in themselves no effect but enhanced broncho-constriction caused by histamine. Vane (1949) also found that, in anaesthetized rabbits, proguanil (8 - 20 mg/kg) reduced respiration. In anaesthetized or pithed cats Chen and Anderson (1947) found that proguanil increased the respiratory rate but diminished the depth of respiration. Aviado, Inoh and Cho (1968) showed that chloroguanide increased pulmonary resistance in anaesthetized dogs. In 1958, Benda and Miravet reported the anti-histamine action of chloroquine and mepacrine on guinea-pig lungs.

Deshpande, Sharma and Dashputra (1963) studied the action of three structurally different antimalarials, namely quinine, mepacrine and chloroquine, on various smooth muscle preparations. These workers found that all the antimalarial drugs studied effectively inhibited the broncho-constrictor action of histamine on guinea-pig isolated tracheal chain preparation. Agarwal and Deshmankar (1963) found that chloroquine blocked the anaphylactic contraction of sensitized guinea-pig tracheal chain induced by addition of the antigen. Since allergic reactions are mediated partly through histamine release from tissues, Agarwal, Deshmankar and Bhargava (1963) studied the tissue levels of histamine after chronic administration of chloroquine in rats and found that treatment of male rats with chloroquine (5 mg/kg) administered daily for three weeks resulted in a 50% reduction of the histamine content of the lung. They concluded that the histamine-depleting action of chloroquine in the lungs might possibly account for its value in bronchial asthma. However, chloroquine has been shown to release histamine in the rat, although not in humans, cat or rabbit (Lecomte 1955). Furthermore, Cohn (1965) has reported that chloroquine inhibited the methylation of histamine in the male rat. In rabbits, Aviado, Sadavongvivad and Cambar (1970) showed that chloroquine had no important effect on the histamine content of the lung.

In 1969, Aviado and Bellet showed that chloroquine (0.5 mg/kg) caused a rise in airways resistance in anaesthetized dogs.

Administration of 1 or 2 mg/kg of the drug produced a variable response in airways resistance. No important changes in pulmonary compliance was observed and this was interpreted to mean that the elasticity of the lung was not influenced by the drug. The workers therefore concluded by ascribing a lack of bronchodilator effect to chloroquine.

In rabbits, Aviado, Sadavongvivad and Cambar (1970) found that chloroquine did not influence the anaphylactic response to egg albumin and did not alter the content of histamine, serotonin and catecholamines in the lungs. Although intravenous injections of chloroquine reduced the broncho-constrictor effect of histamine, this antagonism would appear to be unlike that of standard anti-histaminic agents which are ineffective against bronchial asthma (Aviado et al, 1970). Quinetholate, like chloroquine, antagonized the effect of histamine on the lung in rabbits. Ayitey-Smith and Boye (1974) studied the effect of chloroquine on histamine-induced bronchial asthma in guinea-pigs in vivo and found that chloroquine, in the dose of 20 mg/kg intra-peritoneally, markedly protected animals against fatal asphyxia caused by the broncho-constrictor effect of histamine. The results obtained convinced the workers that chloroquine conferred some protection against histamine-induced broncho-constriction, and the observation that chloroquine afforded protection against the histamine reaction several days after discontinuing the drug suggested that chloroquine not only accumulated in the lungs (Berliner, Earle, Taggart, Zubrod, Welch, Conan, Bauman, Scudder and

Shannon 1948; Grundmann, Vrublovsky and Mikulikova 1970), but also that it might be retained in sufficient quantity to afford a long-lasting protection.

2.4 Effects of antimalarial agents on vascular smooth muscle

The literature on the effects of antimalarial drugs on the activity of the vascular smooth muscles is relatively meagre. Molitor (1941) showed that quinine, mepacrine and pamaquine produced vasodilatation. Proguanil was found to induce vasodilatation in the perfused hind-leg of dog and cat by Vane (1949) and this vasodilatation was partially blocked by antihistaminic agents. Burn and Dutta (1948) showed that quinidine reversed the constrictor action of adrenaline on the rabbit ear artery and the compound has been shown to reduce pressor responses to adrenaline (Nelson 1928; Hiatt 1950; Lu 1951), perhaps by blockade of alpha-(α-) adrenergic receptors Conn and Luchi (1964). Schmid, Nelson, Mark, Heistad and Abboud (1974) found that quinidine caused vasodilatation and markedly reduced constrictor responses to intravenous noradrenaline. The authors interpreted their results as indicating that quinidine interfered solectively with vaso-constrictor stimuli which activate alpha-(α-) adrenergic receptors and suggested that this mechanism, as well as a direct vasodilator effect, might contribute to the hypotension produced by the drug.

2.5 Aims of this study

The studies reported in this Section were carried out in order to examine the following:

(1) To systematically study the effects of anti-malarial drugs (representatives of the major classes of these agents) on a range of smooth muscles. The tissues used included some of those that have not been previously reported in the literature, eg. the portal vein, vas deferens, and chick oesophagus.

(2) To elucidate the mechanism of the already well-documented effects of the antimalarial agents on smooth muscles, for example, their non-specific spasmolytic effect on guinea-pig ileum, inhibition and/or stimulation of the isolated uterine strips, and induction of gastro-intestinal tract cramps;

(3) To examine the hypothesis

(a) that the action of antimalarials on the uterus involves prostaglandins, and,

(b) that the spasmolytic effects of the drugs on the guinea-pig isolated ileum is linked with membrane stabilization;

(4) To examine if some of the side-effects of the compounds (for example, gastro-intestinal disturbances) or some of the unusual therapeutic applications of the drugs (eg. management and/or treatment of asthma) could be explained by an action on smooth muscle.

METHODS

All the tissue preparations were set up in 10 ml organ baths and the bathing solution was continuously aerated with 5% carbon-dioxide in oxygen. Unless otherwise stated, the tissues were suspended in Krebs-Henseleit solution of the following composition: NaCl 118 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2.52 mM, KCl 4.7 mM, $MgCl_2$ 1.2 mM, NaH_2PO_4 1.28 mM and glucose 5.55 mM. The solution was maintained at temperatures ranging from 32° to 37°C, depending on the tissue. The preparations were allowed to equilibrate for a period of 30 - 60 minutes under an applied tension of 0.5 - 1.0 g before they were exposed to drugs. A drug contact time of 30 - 60 seconds was allowed followed by 2 - 3 washes. Isometric contractions were recorded using a Devices force-displacement transducer (type UF1), pre-amplifier (model M2P) and a two-channel pen recorder (model M2R). Isotonic contractions were recorded on moving kymograph smoked paper with a frontal writing lever which produced an 8 - 12 fold magnification.

2.6 Statistical treatment of results

For all the studies reported in this thesis, statistical significance of the difference between the means of control and drug-treated preparations was determined using the "student t test". Statistically significant differences were assumed to be present if the calculated value for "t" was less than the tabulated value for "t" at 0.05 level of P.

All results were expressed as the mean ± standard error (of the mean).

2.7 Guinea-pig isolated ileum

Male and female guinea-pigs of the Dunkin Hartley strain weighing between 350 and 700 g were used. The animals were killed by a blow on the back of the head and bled out. The ileum was removed and segments of 3 - 4 cm long were cut from the distal end having discarded approximately 10 - 12 cm of the portion nearest to the ileocaecal junction. Each piece of the tissue was suspended in Krebs-Henseleit solution maintained at a temperature of 37°C. A tension of 0.5 g was applied to the tissue and isotonic contractions were recorded on smoked kymograph paper.

2.8 Guinea-pig isolated longitudinal muscle strips (Paton and Aboo Zar 1968)

A length of the small intestine was removed after discarding approximately 10 - 12 cm of the part nearest to the ileo-caecal junction. The lumen of the ileum was flushed with 25 ml of Krebs-Henseleit solution and a piece of the ileum, approximately 20 - 30 cm long, was stretched on glass pipette of 7 mm diameter firmly clamped on a table. The mesentery was cut away. To obtain longitudinal muscle strips, a piece of cotton wool, wet with Krebs-Henseleit solution, was used to stroke the muscle tangentially away from the mesenteric attachment at one end of the tissue. The

longitudinal muscle layer was separated at this point and pulled up slightly from the underlying circular muscle. The longitudinal muscle was then tied with a thread and by gentle upward retraction, was stripped off from the whole muscle length. The strip was kept moist throughout this procedure. On examination, the strip was found to contain the Auerbach's plexus in a variable length of its proximal end and to be plexus-free in most of its distal region. A completely plexus-free strip was selected and cut off from the plexus-retaining portion with fine scissors.

The innervated and denervated strips were cut into small pieces of approximately 3 - 4 cm in length and were set up separately in Krebs-Henseleit solution and maintained at a temperature of 37°C. A tension of 0.5 g was applied to the tissue and isotonic contractions were recorded on smoked kymograph paper.

2.9 Rabbit isolated duodenum (Finkleman 1930)

Rabbits of either sex weighing between 1.5 and 4.0 kg were used. The animals were killed by a blow on the back of the head. The duodenum was then removed and segments of approximately 3 - 4 cm long were suspended in Krebs-Henseleit solution, the temperature of which was maintained at 37°C. A tension of 2 - 3 g was applied to the tissue. The periarterial nerves in the mesentery were stimulated with rectangular pulses of 0.3 - 1.0 m sec duration at a

frequency of 30 - 50 Hz and a supramaximal voltage (40 - 60 volts) delivered by an SRI stimulator through platinum ring electrodes. The stimulation usually lasted for 20 seconds and was repeated every 5 - 10 minutes. Drugs were sequentially added between electrical stimulations. The pendular movements of the tissue were recorded either isometrically or isotonically. No qualitative differences were observed between the results obtained with the two types of recording; and therefore, no distinction has been made between them in describing the results.

2.10 Chick isolated oesophagus (Bowman and Everett 1964)

Chicks (aged between 3 and 15 days after hatching) were starved overnight and killed by ether inhalation. The upper oesophagus as far as the crop was removed together with as much as possible of the right parasympathetic nerve trunk which runs along the course of the jugular vein. The preparation (approximately 3 - 4 cm in length) was suspended in Krebs-Henseleit solution maintained at a temperature of 32°C. A tension of 1 - 2 g was applied to the tissue. The muscle was indirectly stimulated through the nerve trunk by means of bipolar platinum ring electrodes. Maximal contractions were produced with trains of stimuli of 0.5 - 1.0 m sec duration at 10 - 30 Hz and supramaximal voltages (60 - 100 volts) delivered from an SRI square wave stimulator. The stimulation lasted for 10 - 20 seconds and was repeated, where necessary, at intervals of 5 - 10 minutes. In some preparations drugs were added non-cumulatively in between

electrical stimulations. Contractions of the tissue were recorded isotonically on smoked kymograph paper.

2.11 Guinea-pig isolated tracheal chain (Akcasu 1959; Foster 1960)

Adult guinea-pigs of either sex weighing between 400 and 800 g were killed by a blow on the back of the head and bled out. The whole trachea was removed and cut into 6 - 8 approximately equal rings. Each ring was cut open through the cartilage and three or four of the open rings were tied together to form a chain. The preparation was set up in Krebs-Henseleit solution at a temperature of 37°C. A tension of 0.5 - 1.0 g was applied to the tissue. The preparation was contracted with standard agonists and relaxed with cumulative additions of relaxant drugs under study when the effect of the agonists had reached a plateau. After maximal relaxation, the preparation was washed two or three times and left to recover for 15 - 20 minutes. The contractions and relaxations were recorded isometrically.

2.12 Guinea-pig and rat isolated uteri (de Jalon, Bayo and de Jalon 1945)

Young female guinea-pig weighing between 200 and 300 g and female albino rats (120 - 200 g) were used. The animals were divided into two categories as follows:

(1) Oestrogen-treated guinea-pigs and rats

All animals in this group were injected with stilboestrol (0.1 mg/kg body weight) sub-cutaneously 20 - 24 hours before use. Vaginal smears were taken immediately before sacrifice in order to ascertain that the animals were in oestrus.

(2) Pregnant guinea-pigs and rats

Mated guinea-pigs and rats were examined daily for the presence of a cervical plug. The day on which cervical plug was first found was taken as 'day one' of pregnancy. Early pregnancy was regarded as days one to eight, and late pregnancy was taken to be from day 16 to day 20.

The animals were killed by a blow on the back of the head and bled out. The entire uterus was carefully removed and the two horns were trimmed. Segments of approximately equal lengths (about 2 - 3 cm) were removed from the horns by cutting off one or both ends. The preparations were suspended in de Jalon's solution of composition (g/l): NaCl 9.0, KCl 0.4, $NaHCO_3$ 0.5, $CaCl_2$ 0.07 and glucose 0.5. The bath solution was maintained at a temperature of $32^{o}C$ and the tissues were subjected to an applied tension of 0.5 - 1.0 g. Drugs were added either cumulatively or sequentially. An interval of 3 - 10 minutes (sometimes up to 20 minutes) was allowed between successive single doses. Contractions were recorded both isometrically and isotonically

by the methods previously described.

2.13 Guinea-pig and rat isolated vasa deferentia (Hukovic 1961)

Adult male guinea-pigs (400 - 800 g) and male Wistar rats (300 - 600 g) were killed by a blow on the back of the head and bled out. The vasa deferentia were removed free from mesentery and connective tissue. In most of the guinea-pigs (42) and the rats (45), vasa deferentia with hypogastric nerves attached were isolated for nerve stimulation as described by Hukovic (1961).

The tissues (12 ± 3 mm long and 117 ± 10 mg in weight) were mounted in 10 ml organ baths containing Krebs-Henseleit solution maintained at a temperature of 32°C and continuously gassed with a mixture of 95% O_2 and 5% CO_2. Paired vasa deferentia from the same animal were set up in all cases. The preparations were allowed to equilibrate for 60 - 90 minutes under an applied tension of 0.5 - 1.0 g before they were exposed to electrical stimulation and/or drugs. Hypogastric nerve stimulation was performed by electrical field stimulation through bipolar platinum ring electrodes (1.0 - 1.5 mm wide) applied to the nerve. The nerves were stimulated with rectangular wave pulses of 0.3 - 1.0 m sec duration at a frequency of 5 - 60 Hz and with supramaximal voltages of 10 - 50 volts from SRI stimulators.

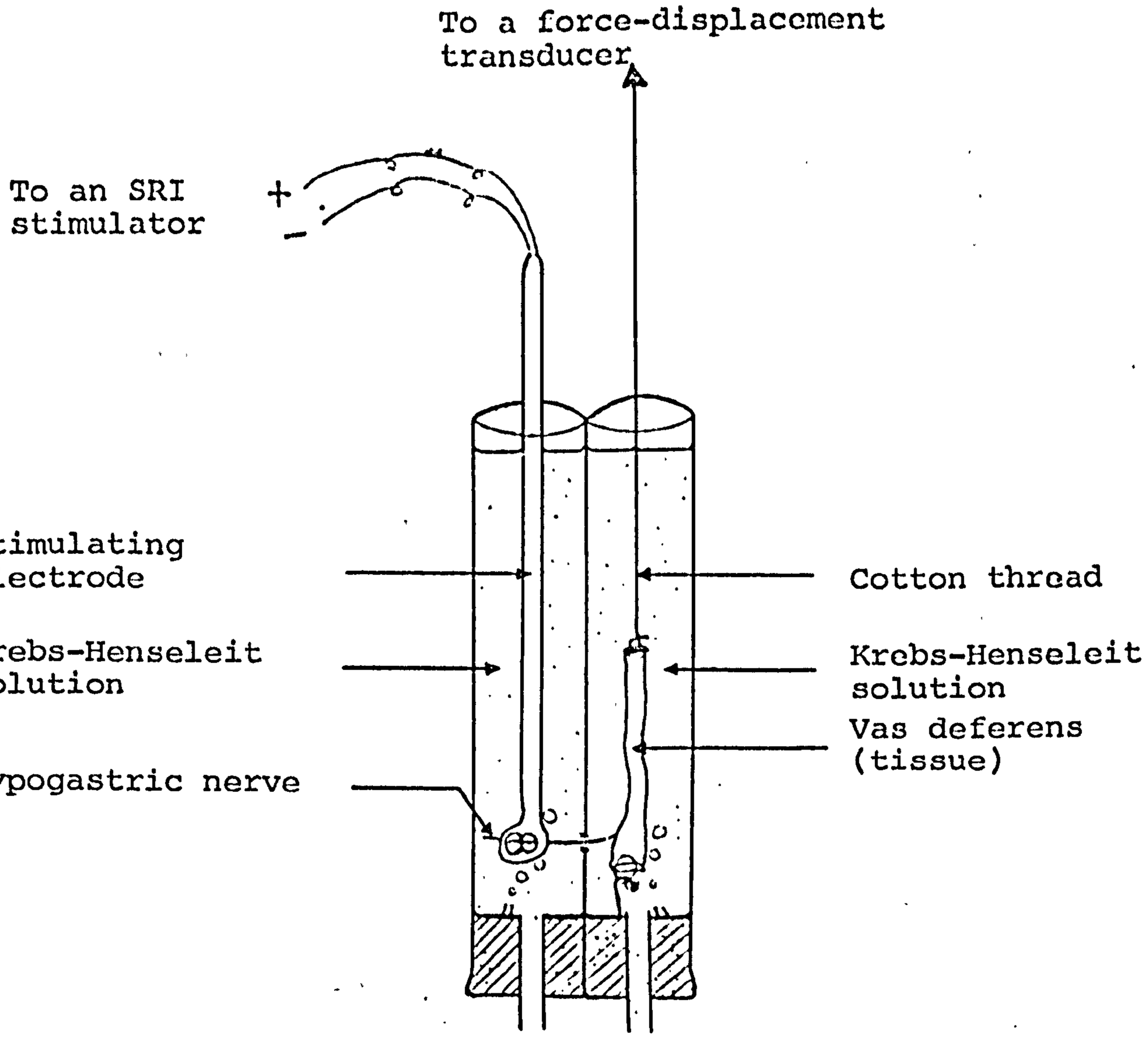


FIGURE 3

Diagrammatic representation of the experimental set-up for the 'two-chambered organ bath' experiments. The hole between the two chambers was sealed up with paraffin wax such that leakage (and subsequent dilution of the content of one chamber by that of the other) was prevented.

Some of these preparations were set up in a 'two-chambered organ bath'. Figure 3 shows the diagramatic representation of the experimental set-up. Although the muscle was still stimulated through the hypogastric nerve, this device made it possible to 'separate' the nerve from the muscle and to administer drugs to the 'nerve' and 'muscle' portions separately in turn.

Drugs were applied either singly or cumulatively. A contact time of 30 - 60 seconds was allowed for each drug before the tissue was electrically stimulated and then washed out 2 - 3 times. In some cases, the tissues were removed from guinea-pigs and rats pretreated with reserpine (5 mg/kg body weight injected intraperitoneally) 18 - 24 hours before sacrifice.

Contractions of the tissue evoked by electrical stimulation and/or exogenously added drugs were recorded either isometrically or isotonically. No qualitative differences were observed between the results obtained with the two types of recording and hence no distinction has been made between them in describing the results.

2.14 Rabbit isolated perfused central ear artery

Rabbits of either sex weighing between 1.5 and 4 kg were used. The animals were killed either by a blow on the head and bled out, or by deep pentobarbitone sodium anaesthesia (45 - 60 mg/kg i.v). The method used in these experiments

was a modification of the ones described by de la Lande and Harvey (1965), de la Lande and Rand (1965), and de la Lande, Frewin and Waterson (1967). The central ear arteries close to the base of each ear were exposed and cleaned free from adherent unwanted connective extraneous and fatty tissues as completely as possible. These central arteries (30 - 50 mm long) were removed and cannulated at the proximal wider ends with fine polythene tubing or with glass cannulae; the narrower distal ends were left free. The isolated arteries were perfused with Krebs-Henseleit solution at a constant flow rate of 5 - 10 ml/minute by means of Watson-Marlow H.R. Flow Inducer (type MHRE 200). The perfusion solution was maintained at 37°C. Perfusion pressure was measured with Elcomatic pressure transducer (type EM 750) and recorded on a George Washington Oscillograph (type 400 M D/2). It was 20 to 30 mm Hg in the absence of drugs or electrical stimulation. The preparations were allowed to equilibrate for a period of 45 - 60 minutes before intraluminal drug administration or extraluminal periarterial nerve stimulation was commenced.

Drugs were added intraluminally into the perfusion fluid through a 3-way tap located above the proximal end of the artery. The procedure used for electrical stimulation of the periarterial nerves was adopted from the one described by de la Lande and Rand (1965). Platinum ring electrodes were arranged around the proximal end of each artery just below the area where the perfusion cannula lay within the

artery. The nerves were extraluminally stimulated with SRI stimulators delivering rectangular wave pulses of 0.4 - 1.0 mS duration at a frequency of 10 - 30 Hz and a supra-maximal voltage of 30 - 50 volts.

Constrictions of the arteries in response to added drugs and electrical stimulation were recorded as a rise in perfusion pressure. The change in perfusion pressure was measured and dose-response curves to intraluminally applied drugs, and in some experiments, frequency-response curves, were plotted using the same preparation. Doses of the drugs and electrical stimulations were repeated after the previous responses had returned to control levels.

2.15 Rat isolated portal vein

Wistar albino rats of both sexes weighing between 200 and 600 g were killed by a sharp blow on the head and bled out. The abdomen were opened by a mid-line incision and the intestines were pulled aside. The portal veins, with *in situ* lengths of approximately 20 mm each, were carefully cleaned free of connective extraneous and fatty tissues and then removed. The isolated portal veins were suspended in 10 ml organ baths containing Krebs-Henseleit solution maintained at a temperature of 37°C and continuously aerated with a mixture of 5% carbon-dioxide in oxygen. Paired preparations were always set up. The tissues were allowed to equilibrate for a period of 30 - 60 minutes under an applied tension of 0.5 - 0.75 g before they were

exposed to drugs.

Drugs were applied either cumulatively or non-cumulatively. The spontaneous myogenic contractions and the drug-induced responses of the tissues were recorded isometrically.

2.16 Determination of pA2 values

An agonist induces a stimulus which sets in motion a chain of events or reactions leading from the occupation of the receptors, by the agonist, to the effect. A second drug may interfere with this chain of events by interacting with receptors which are different from those for the agonists. This can result in a non-competitive sensitization or inhibition of the biological response to the agonist (Ariens, Simonis and de Groot 1955; Ariens, van Rossum and Simonis 1957).

In the case of a non-competitive antagonism, the presence of the antagonist results in a decrease in the slope and in the maximum response to the agonist. This non-competitive antagonistic action is insurmountable. Non-competitive antagonists can be evaluated on the basis of PD'_x values. If the maximum response to the agonist Ea_m is reduced to a response Ea in the presence of the antagonist, then the PD'_x value is equal to the negative logarithm of the molar concentration of the antagonist for which $x = Ea_m/Ea$ (Ariens, Simonis and van Rossum 1964a). In the present experiments (on guinea-pig isolated ileum preparations) log

dose/response curves to the agonists (acetylcholine and histamine) were obtained in the absence, and then in the presence, of increasing concentrations of the antagonists chloroquine, primaquine, quinine, proguanil and pyrimethamine. Percentage inhibition of the maximum response to the agonists (ordinate) was plotted against the negative logarithm of the molar concentration of the antagonists (abscissa). The PD'_2 value is equal to the abscissa value corresponding to 50% inhibition.

If log dose/response curves for an agonist with intrinsic activity equal to one are plotted in the presence of constant concentrations of a competitive antagonist (acting on the same receptors) with intrinsic activity equal to zero, then the presence of the antagonist results in a parallel shift of the log dose/response curve for the agonist to higher concentrations (Ariens, Simonis and van Rossum 1964). The competition between the agonist and the antagonist can be overcome by increasing the dose of the agonist. The antagonism is surmountable. Competitive antagonists are best evaluated on the basis of pA_x values as introduced by Schild (1947, 1949). The pA_x value is the negative logarithm of the molar concentration of the antagonist that reduces the effect of a multiple dose (x) of the agonist to that of a single dose acting alone. In the present experiments, cumulative log dose/response curves (on guinea-pig tracheal chain) and log dose/response curves (on guinea-pig ileum) to the agonists (acetylcholine and histamine) were obtained

in the absence and in the presence of the antagonists (antimalarial drugs). Isoboles were constructed by plotting the negative logarithm of the molar concentration of antagonist (abscissa) against the logarithm of the (dose ratio - 1) where the dose ratio is equal to the concentration of agonist required to produce 50% of the maximum response in the presence of the antagonist/the concentration of agonist required to produce 50% of the maximum response in the absence of the antagonist. The point where the isobole intersects the abscissa is the pA_2 value of the antagonist against the agonist.

In all experiments, unless otherwise stated, various concentrations of the different antagonists (antimalarial drugs) were used against varying doses of the agonists (acetylcholine and histamine). A concentration of an antagonist was added to the organ bath containing the isolated tissue preparation (guinea-pig tracheal chain or guinea-pig ileum) 3 or 5 minutes before the first dose of the agonist was applied to the tissue, and the subsequent doses of the agonist required for a complete dose/response curve were added either cumulatively (in the case of guinea-pig tracheal chain) or singly (in guinea-pig ileum) in the continued presence of the antagonist. In the guinea-pig ileum, each dose of the agonist was allowed to act on the tissue for 30 seconds only (both in the absence and in the presence of the antagonist).

RESULTS

The results obtained in the present studies demonstrate that all five antimalarial compounds studied have a number of pharmacological actions in common.

2.17 Guinea-pig isolated ileum

In all the preparations, concentrations of the quinoline antimalarial agents (chloroquine, primaquine and quinine) in the range of 7.5×10^{-9} to 1.0×10^{-4}M reduced, in a dose-dependent manner, the base-line tension (tone). At the same dose levels, proguanil and pyrimethamine (non-quinoline antimalarials) produced comparatively less relaxation. Higher doses of the quinoline compounds (2.5×10^{-4} - 1.0×10^{-2}M) and of the non-quinoline drugs (7.5×10^{-4} - 1.0×10^{-2}M) caused dose-related contractions of the isolated ileum. These contractions were compared with those evoked by acetylcholine and nicotine. The dose-response curves are plotted in Figure 4 from which it can be seen that the antimalarial drugs were about 10^4 and 10^3 times less potent than acetylcholine and nicotine respectively, and that the order of potency of the antimalarials was: primaquine $\geqslant$ chloroquine $>$ quinine $>$ proguanil $\geqslant$ pyrimethamine.

The spasmogenic effect of the antimalarials was found to increase with a rise in organ bath temperature and decrease with a reduction in temperature (Figure 5). The spasmogenic effect of physostigmine was also found to vary with the organ bath temperature whilst responses to acetylcholine

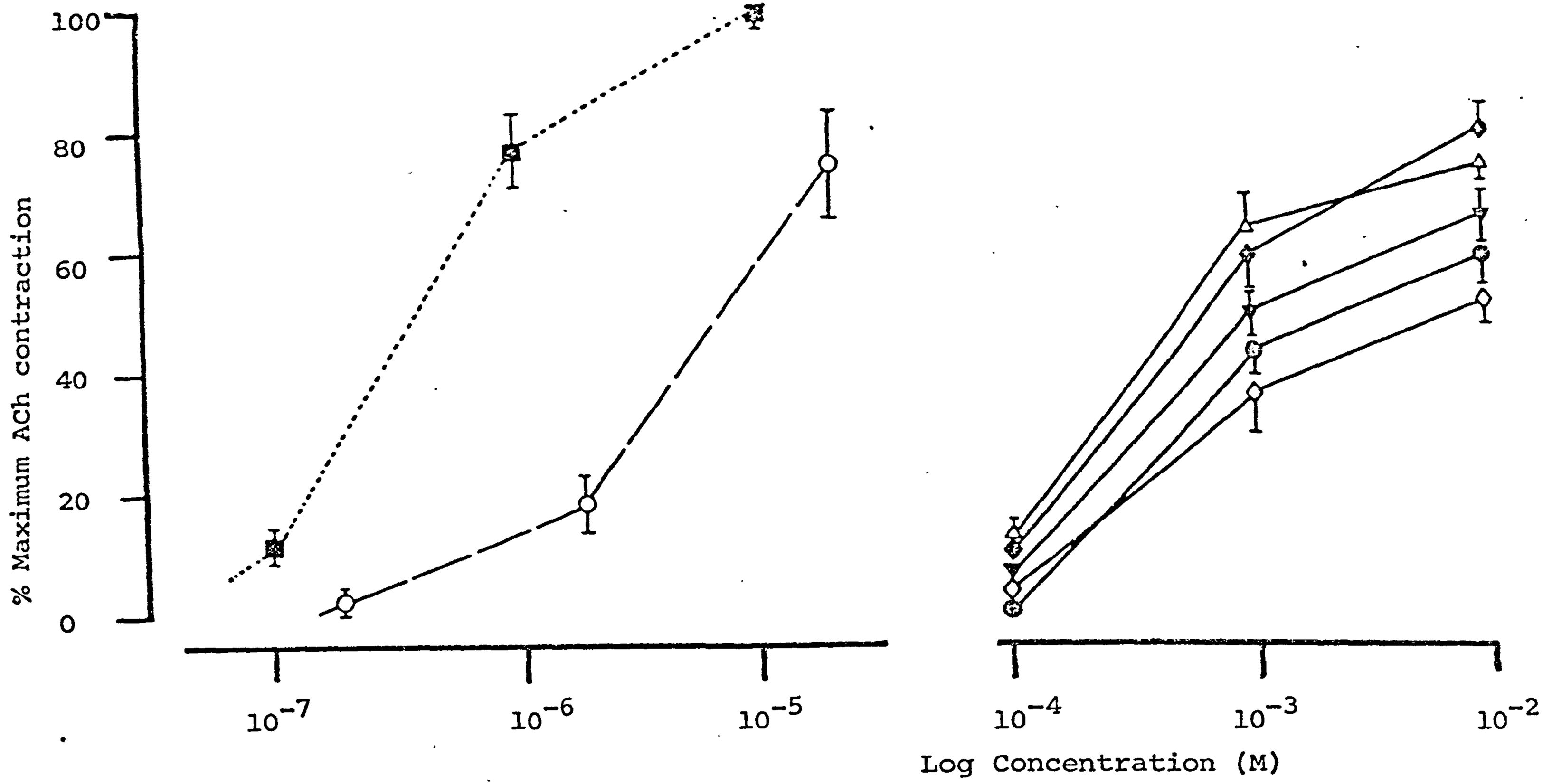


FIGURE 4

Guinea-pig isolated ileum. Mean log dose/response curves to acetylcholine (ACh, ■), nicotine (○), chloroquine (◆), primaquine (△), quinine (▼), proguanil (●) and pyrimethamine (◇). Each point represents mean of 5 - 8 observations, and the vertical bars denote s.e. of means.

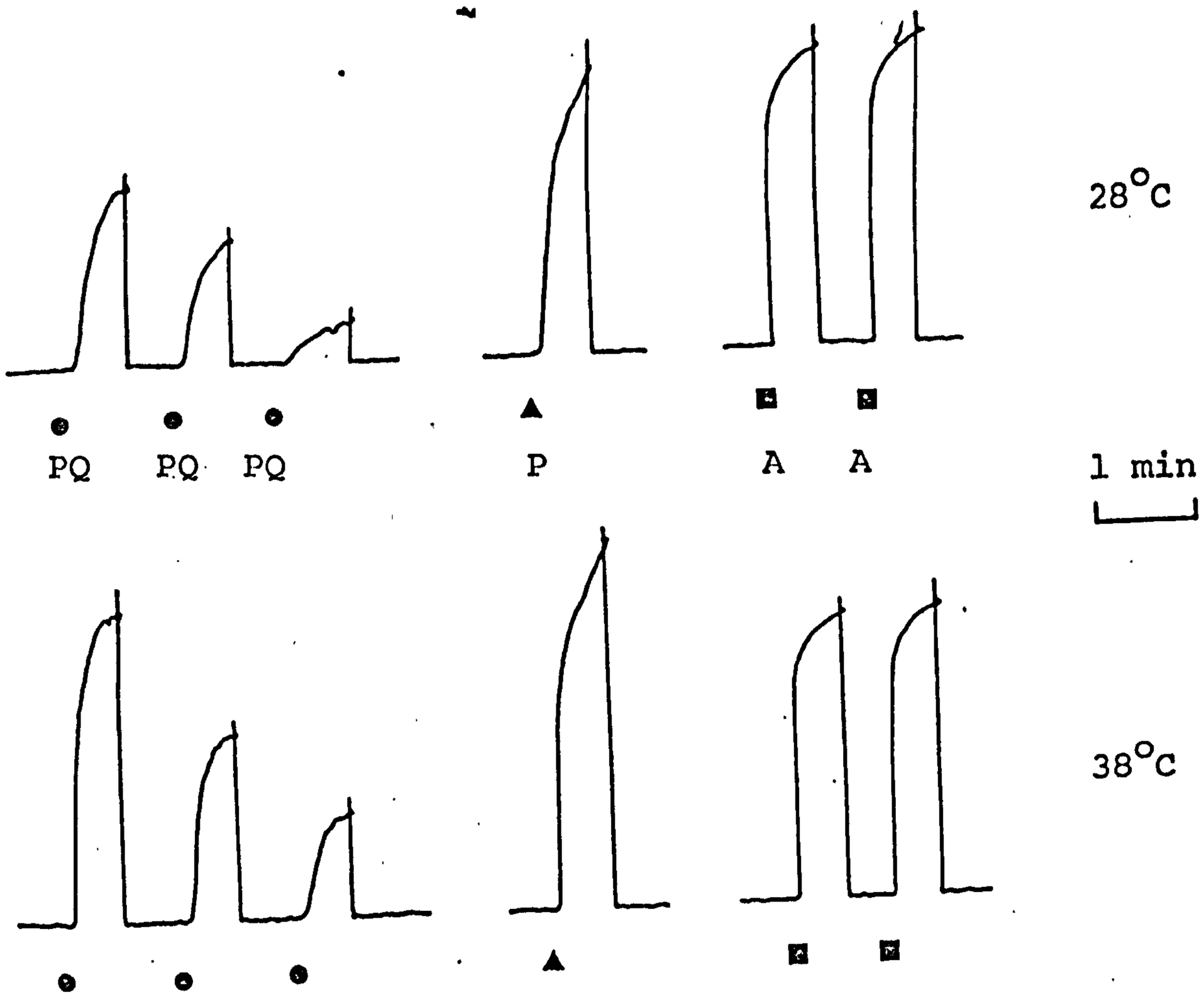


FIGURE 5

Guinea-pig isolated ileum. Effects of primaquine (PQ, 1.0×10^{-3}M), physostigmine (P, 5.0×10^{-5}M), and acetylcholine (A, 1.0×10^{-7}M) at 28°C (upper trace) and 38°C (lower trace). The spasmogenic effects of primaquine (PQ) and physostigmine (P) increased with rise in the organ bath temperature.

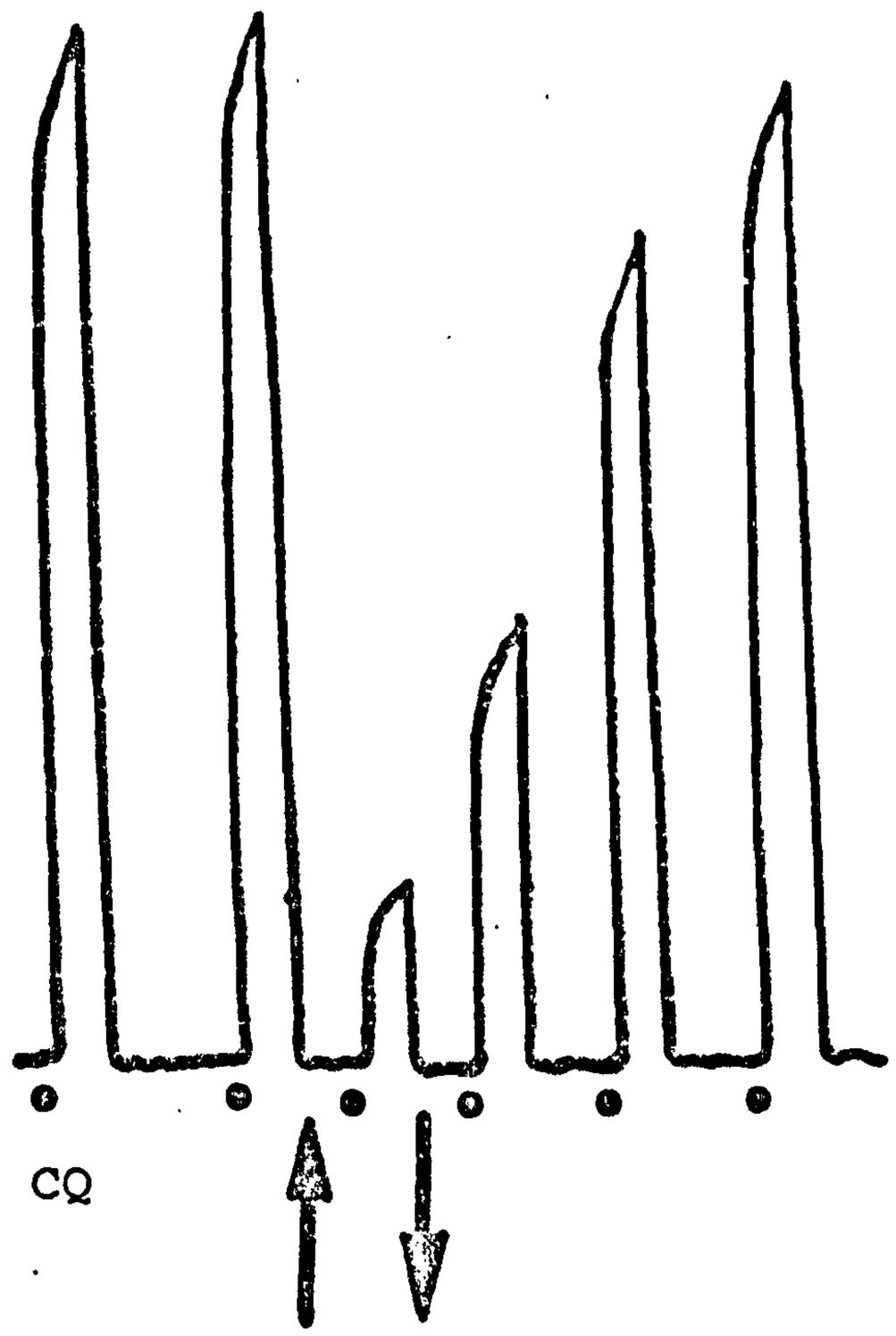


FIGURE 6

Guinea-pig isolated ileum. Effect of atropine (AT, 1.0×10^{-5}M) on contractions of the guinea-pig ileum induced by chloroquine (CQ, (●) 2.0×10^{-3}M). Atropine was added at the upward arrow and washed out at the downward arrow. To minimise the tachyphylactic effect of chloroquine, doses of the drug were administered every 30 minutes following 3 washings.

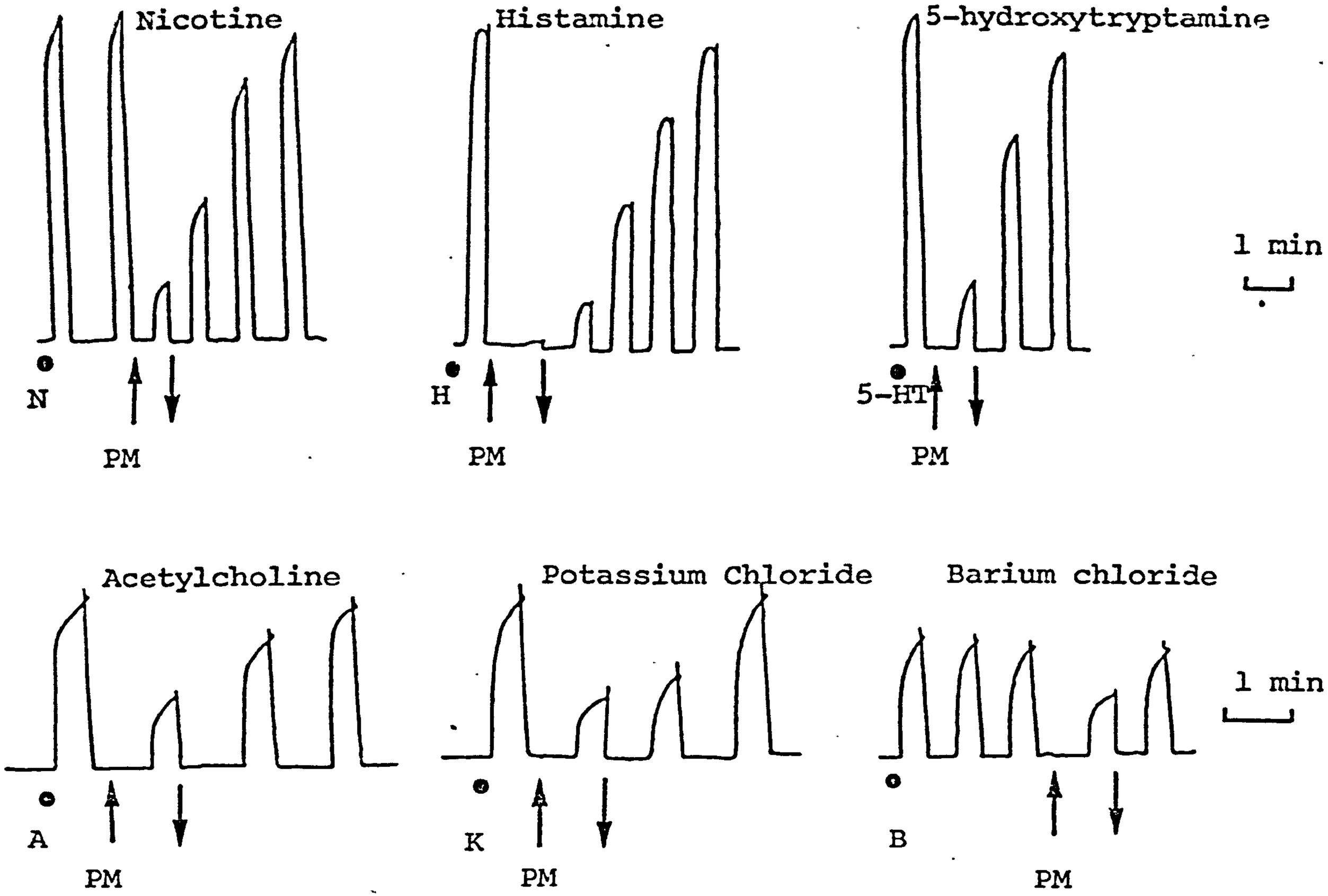


FIGURE 7

Guinea-pig isolated ileum. Effect of pyrimethamine (PM) on the spasmogenic actions of nicotine (N, ● 1.0 x 10^{-6}M), histamine (H, (●), 1.5 x 10^{-6}M), 5-hydroxytryptamine (5HT, (●) 2.5 x 10^{-6}M), acetylcholine (A, (●) 5.0 x 10^{-8}M), potassium chloride (K, (●) 20 mM) and barium chloride (B, (●) 35 mM). Pyrimethamine (2.5 x 10^{-6}M) was added at the upward arrow and washed out at the downward arrow.

were unaffected (Figure 5). Tachyphylaxis was observed with successive doses of antimalarial drugs (Figure 5).

The effects of the antimalarials on the guinea-pig isolated ileum were not modified by atropine ($1.0 - 2.5 \times 10^{-6}$M), mepyramine ($1.0 - 2.5 \times 10^{-6}$M), hexamethonium ($1.0 - 2.5 \times 10^{-6}$M) or methysergide ($1.0 - 2.5 \times 10^{-6}$M) which inhibited, or abolished, the responses of the tissue to acetylcholine ($1.0 \times 10^{-7} - 2.5 \times 10^{-5}$M), histamine ($1.5 \times 10^{-7} - 2.5 \times 10^{-5}$M), nicotine ($2.5 \times 10^{-6} - 7.5 \times 10^{-5}$M) and 5-hydroxytryptamine ($2.5 \times 10^{-6} - 7.5 \times 10^{-5}$M) respectively by 73 - 100% of the control values. However, relatively high concentrations of atropine ($5.5 \times 10^{-6} - 5.0 \times 10^{-5}$M) dose-dependently inhibited the contractions of the tissue induced by all five antimalarials (Figure 6).

Apart from producing contractions, all the five antimalarial compounds ($7.5 \times 10^{-6} - 1.0 \times 10^{-3}$M) inhibited the responses of the isolated ileum to acetylcholine, histamine, nicotine, 5-hydroxytryptamine, potassium chloride and barium chloride (Figure 7). This inhibitory effect was produced by concentrations of the antimalarial compounds too small to contract the ileum. The spasmolytic actions of the antimalarials were most marked against histamine and least effective against potassium and barium. "pA_2" values were calculated for the antimalarials against acetylcholine and histamine. The values obtained have been compared with published figures in Table 1.

TABLE 1

Antagonist	Agonist	"pA_2"	n = 4
Chloroquine	Acetylcholine	4.1	(3.8)
Primaquine		4.3	
Quinine		3.8	
Proguanil		3.3	
Pyrimethamine		3.0	
Atropine		8.8	
Chloroquine	Histamine	8.3	(9.1)
Primaquine		8.1	
Quinine		7.7	
Proguanil		5.9	
Pyrimethamine		6.2	
Mepyramine		9.0	

"pA_2" values obtained for the antimalarial drugs, atropine and mepyramine against acetylcholine and histamine (3-min contact time) on guinea-pig isolated ileum. Figures in the brackets are published values (Olatunde, 1970).

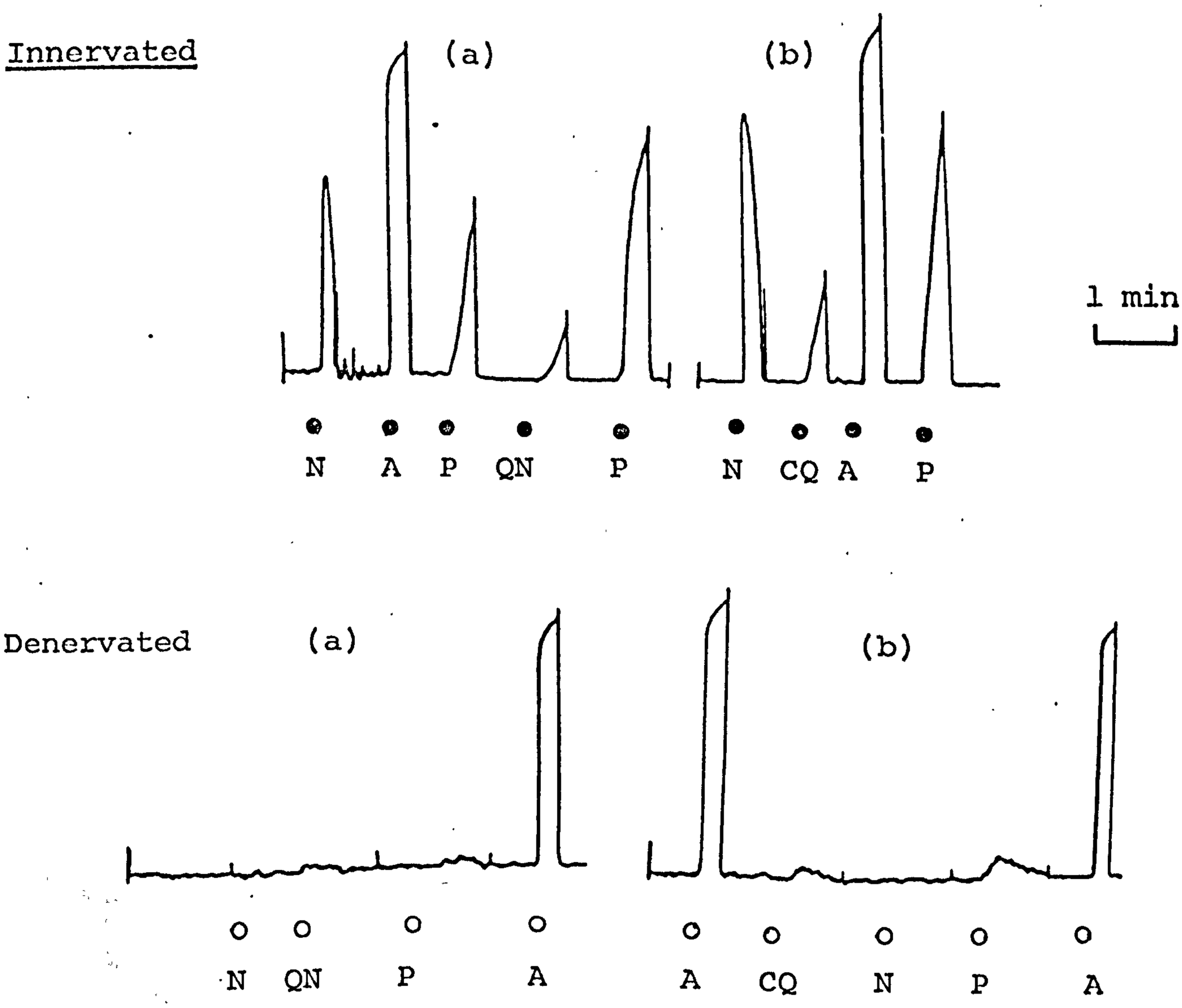


FIGURE 8

Guinea-pig isolated longitudinal muscle strips. Effects of nicotine (N), acetylcholine (A), physostigmine (P), quinine (QN) and chloroquine (CQ) on the innervated (upper traces, (a) and (b)); and denervated (lower traces, (c) and (d)) strips of guinea-pig ileum.

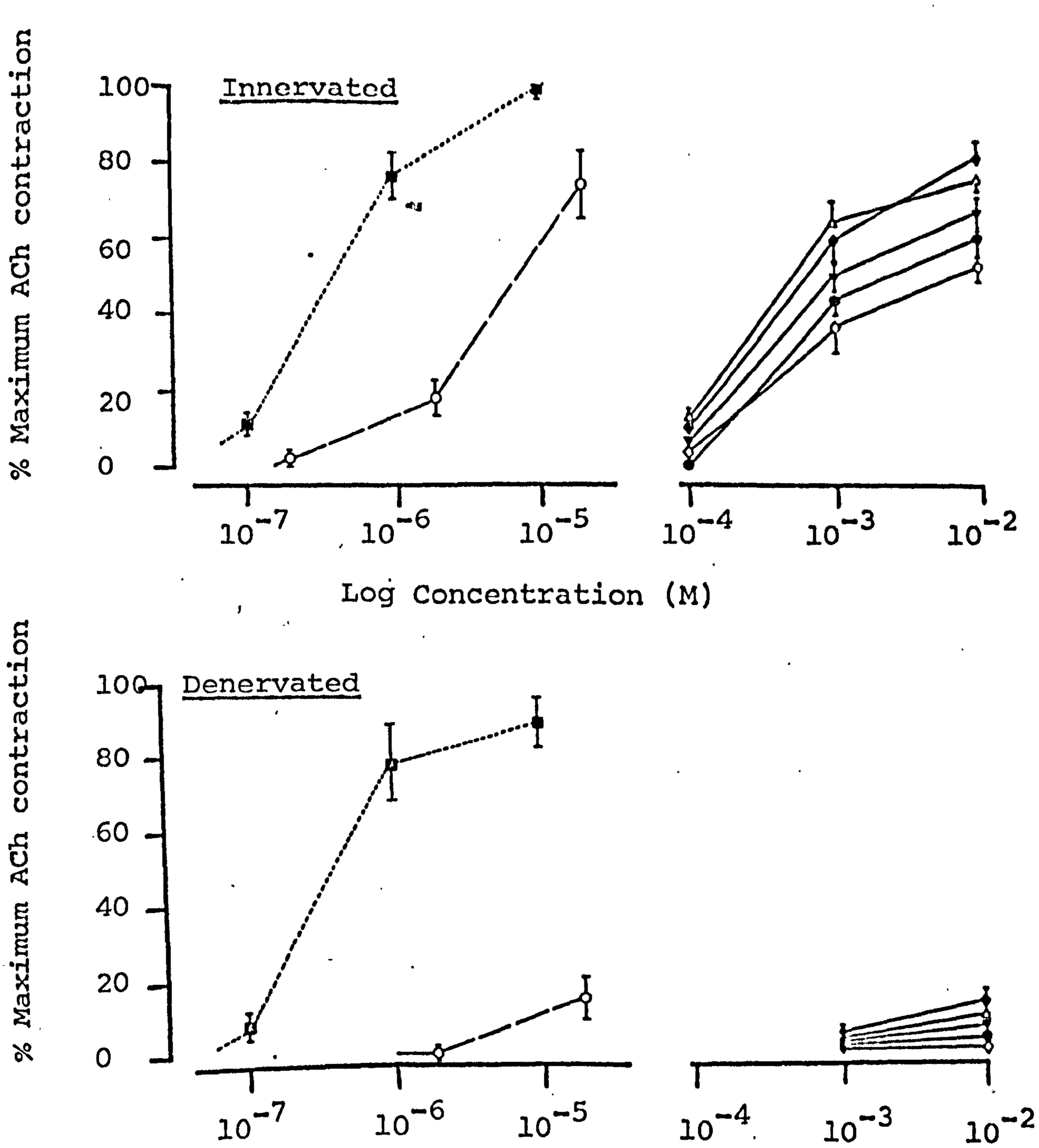


FIGURE 9

Guinea-pig isolated longitudinal muscle strips. Mean log dose/response curves to acetylcholine, ACh (■), nicotine (○), chloroquine (◆), primaquine (△), quinine (▼), proguanil (●) and pyrimethamine (◇) on innervated (upper panel) and denervated (lower panel) strips. Each point represents the mean of 5 - 10 observations, and the vertical bars denote s.e. of means.

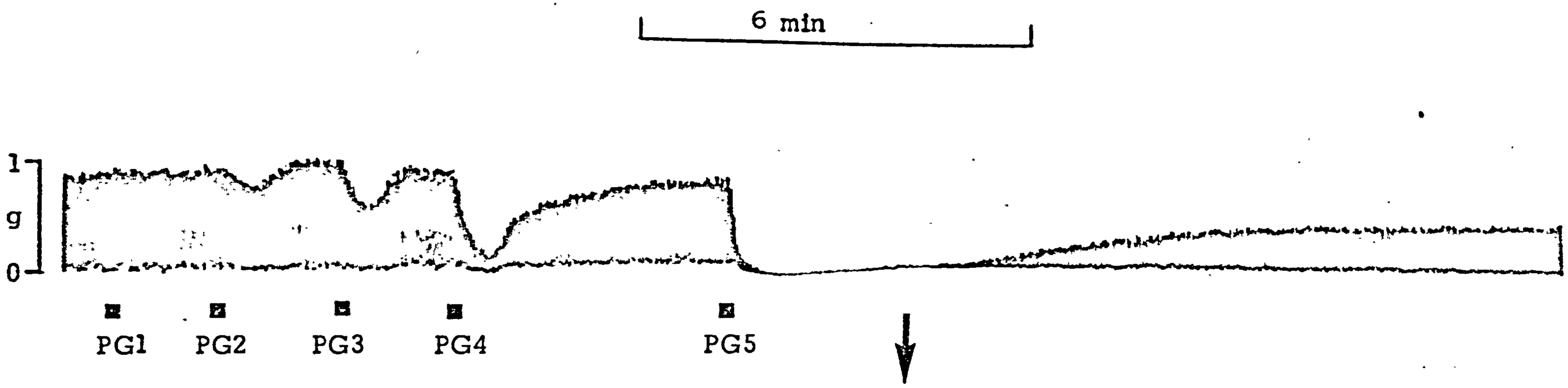


FIGURE 10

Rabbit isolated duodenum.

Effect of proquanil (PG) on spontaneous rhythmic contractions of the rabbit isolated duodenum. PG1, PG2, PG3, PG4 and PG5 denote proquanil 5.0×10^{-6} M, 5.0×10^{-5} M, 1.0×10^{-4} M, 1.0×10^{-3} M and 1.0×10^{-2} M respectively. The last dose of proquanil was washed out at the downward arrow.

2.18 Guinea-pig isolated longitudinal muscle strips - innervated and denervated

Relatively low to medium concentrations of all five antimalarial compounds ($7.5 \times 10^{-9} - 1.0 \times 10^{-4}$M) produced dose-related reductions of the base-line tensions of both the innervated and denervated muscle strips as they did in the intact isolated ileum. All the five antimalarials ($7.5 \times 10^{-4} - 2.0 \times 10^{-2}$M) caused dose-dependent contractions of the innervated muscle strips, resembling those obtained in the intact ileum since they were found to be temperature-dependent, tachyphylactic and resistant to the actions of atropine, mepyramine, hexamethonium and methysergide ($1.0 - 2.5 \times 10^{-6}$M). In contrast, the denervated muscle preparations were largely unresponsive to antimalarial agents (Figure 8). Acetylcholine and histamine produced dose-related contractions of both the innervated and denervated muscle strips, although they were much less marked in the latter. Nicotine and physostigmine, like the antimalarials, only contracted the innervated muscle strips (Figures 8 and 9).

2.19 Rabbit isolated duodenum

All five antimalarial drugs relaxed the isolated duodenum at all dose levels used ($1.0 \times 10^{-6} - 1.0 \times 10^{-2}$M). Spontaneous myogenic activity was also inhibited in a dose-dependent manner by the compounds (Figure 10). The order of potency of the drugs was: primaquine $\geq$ chloroquine $>$

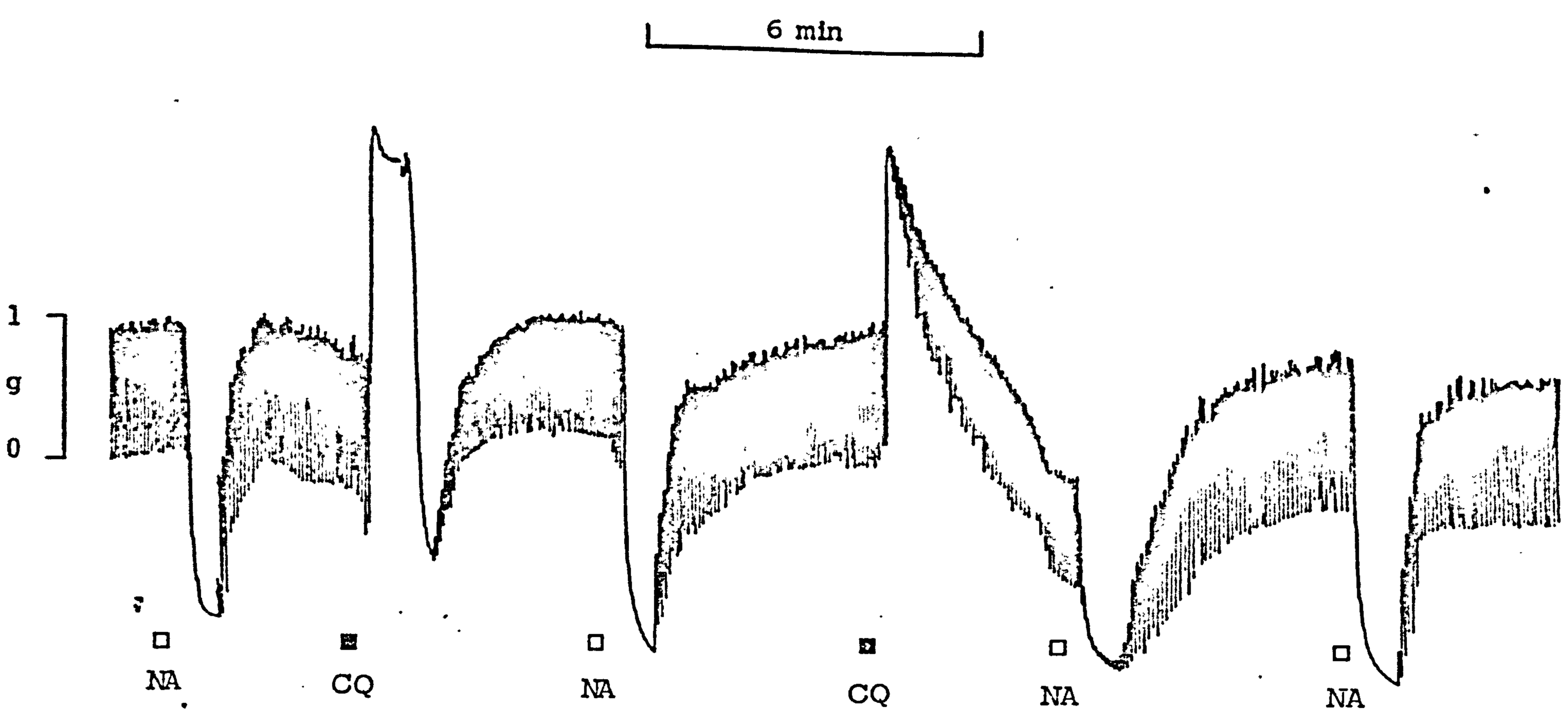


FIGURE 11

Rabbit isolated duodenum.

Effects of chloroquine (CQ, 7.5×10^{-4} M) and noradrenaline (NA, 7.5×10^{-7} M) on spontaneous activity of rabbit isolated duodenum. Chloroquine (CQ, 7.5×10^{-4} M) contracted the tissue and antagonised the inhibitory effect of exogenous noradrenaline (NA, 7.5×10^{-7} M).

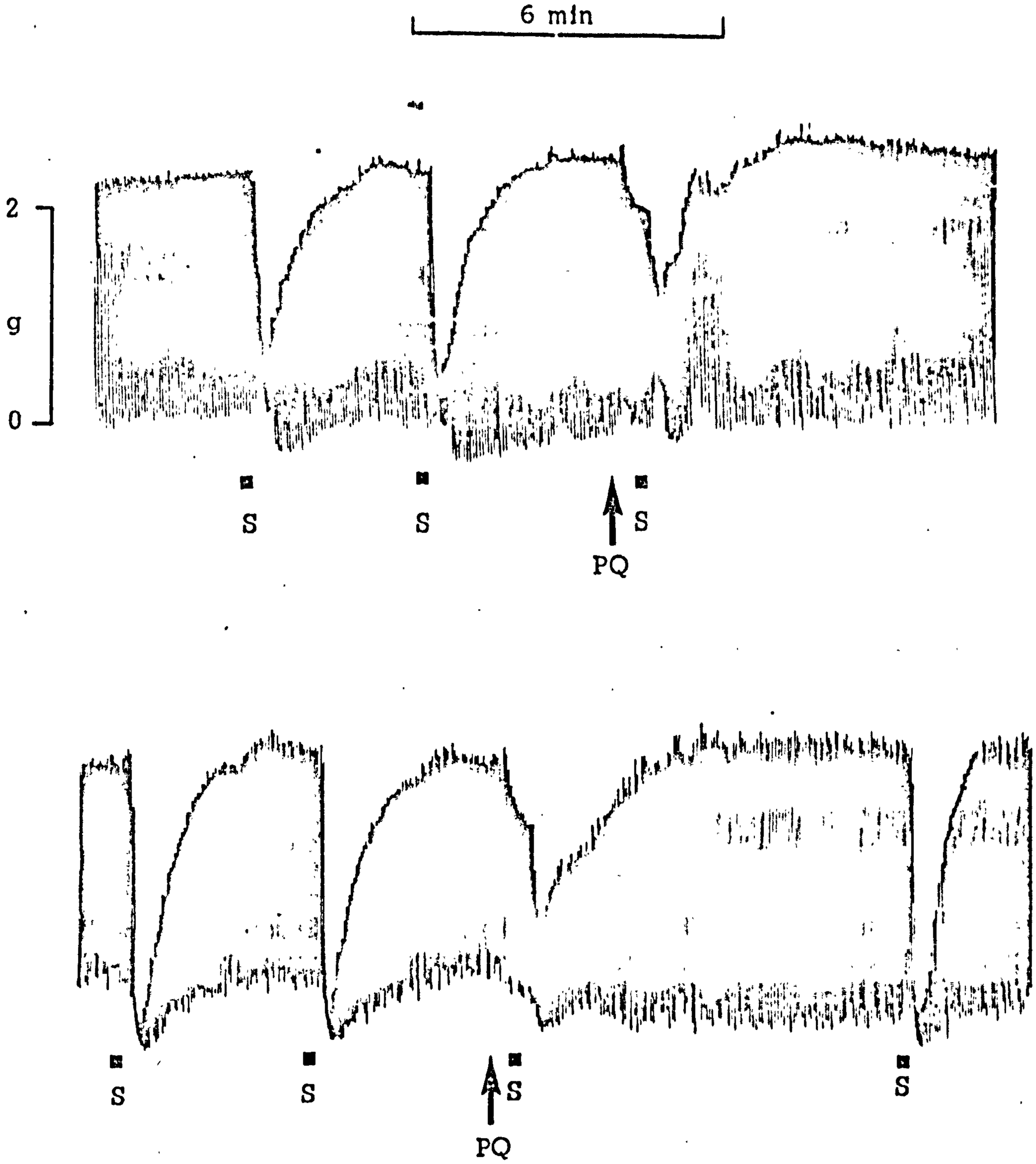


FIGURE 12

Rabbit isolated duodenum. Effect of primaquine (PQ, 2.5×10^{-4} M) on the inhibitory responses of rabbit isolated duodenum to sub-maximal electrical stimulation (S, at 40 Hz).

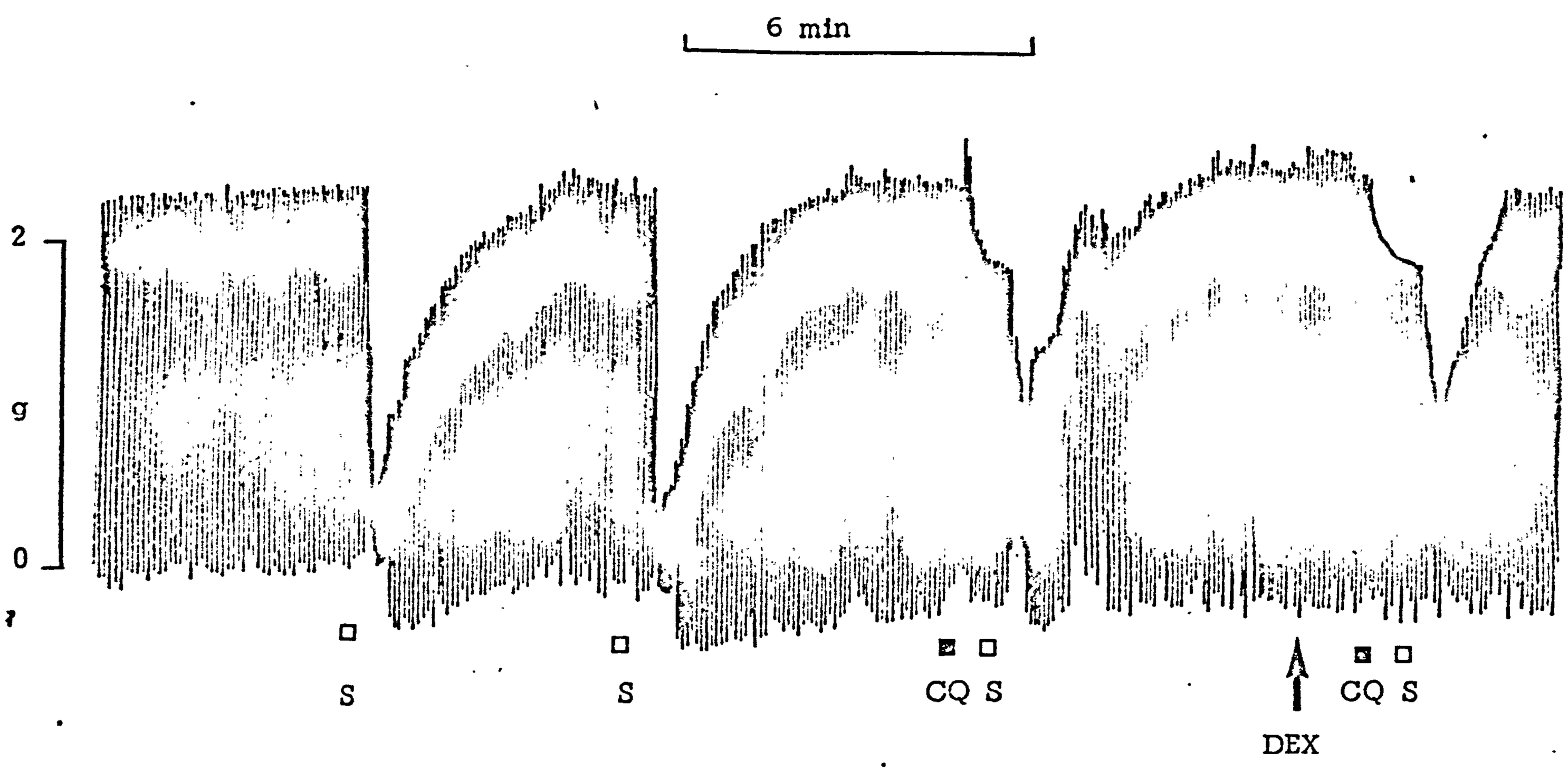


FIGURE 13

Rabbit isolated duodenum (Finkleman preparation). Effect of chloroquine (CQ, 2.5×10^{-4} M) on the inhibitory responses of the rabbit isolated duodenum to sub-maximal electrical stimulation (S, at 40 Hz). The inhibitory effect of chloroquine was not reversed by dexamphetamine (DEX, 7.5×10^{-6} M, at the upward arrow).

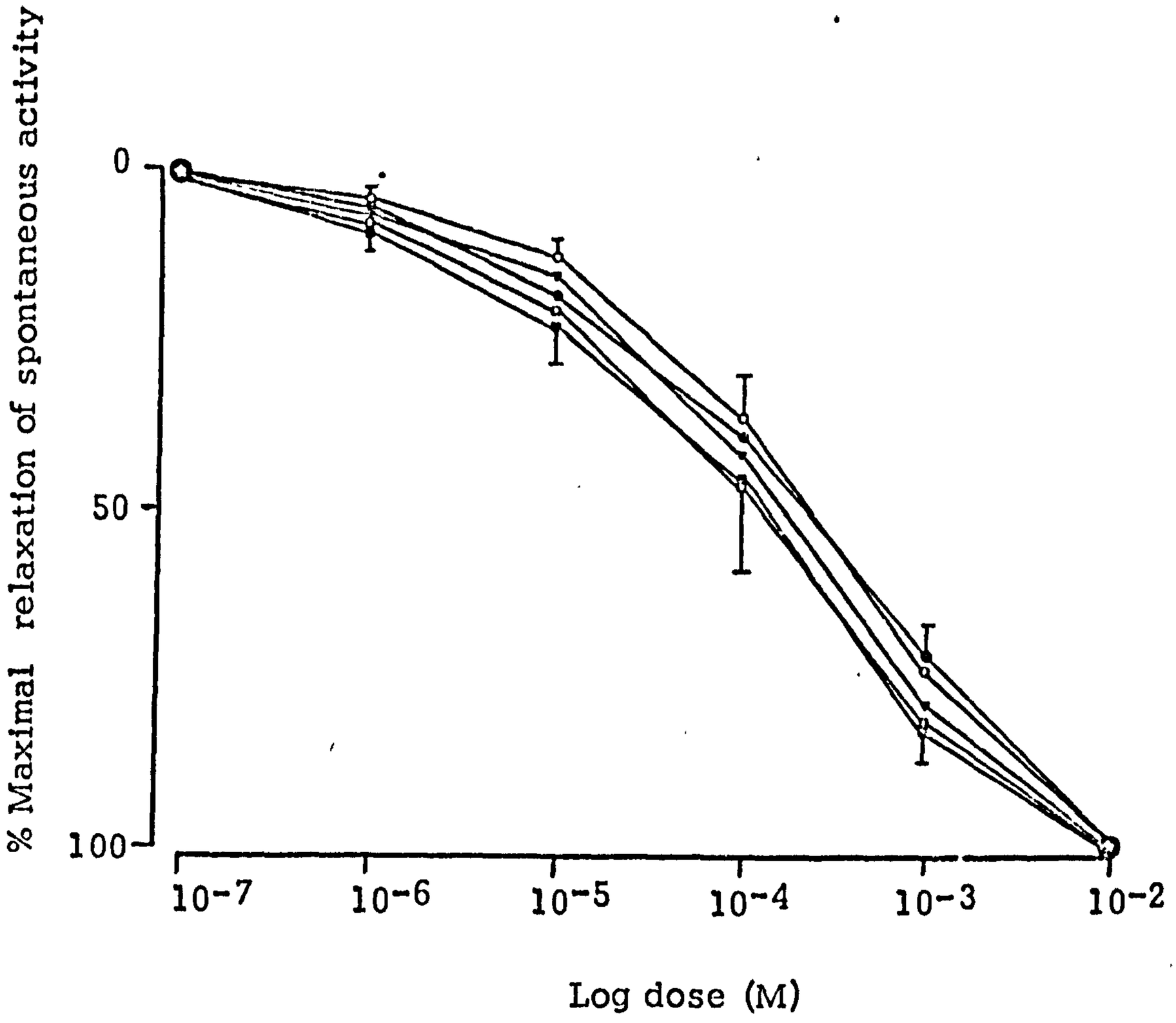


FIGURE 14

Rabbit isolated duodenum.

Mean dose/response curves to primaquine (■), chloroquine (□), quinine (▼), proguanil (●) and pyrimethamine (o) on rabbit isolated spontaneously contracting duodenum. Each point is the mean of 4 - 9 observations. The vertical bars represent s.e. of means.

quinine ≧ proguanil ≧ pyrimethamine. The inhibitory effects of pyrimethamine on this tissue but not those of proguanil or the quinoline antimalarials, were readily reversed by washing out (Figure 10).

In most of the preparations, relatively moderate to high concentrations of chloroquine, quinine and primaquine (5.0×10^{-4} - 2.0×10^{-3}M), but not proguanil or pyrimethamine, caused an initial contraction of the tissue before relaxing it (Figure 11). This initial contractile effect was found to be inhibited by a lowered organ bath temperature but was unaffected by atropine ($1 - 2.5 \times 10^{-6}$M), mepyramine ($1 - 2.5 \times 10^{-6}$M), hexamethonium ($1 - 2.5 \times 10^{-6}$M) or methysergide ($1 - 2.5 \times 10^{-6}$M).

All the antimalarials studied (7.5×10^{-7} - 1.0×10^{-4}M) dose-dependently potentiated the inhibitory effect of exogenously added noradrenaline. However, in higher concentrations (2.5×10^{-4}M) the noradrenaline responses (Figure 11) and the effect of perivascular nerve stimulation were inhibited (Figure 12). This effect, unlike that of guanethidine, was not reversed by dexamphetamine ($2.5 - 7.5 \times 10^{-6}$M, see Figure 13). The inhibitory effects of the antimalarial compounds on the isolated rabbit duodenum are summarized in Figure 14.

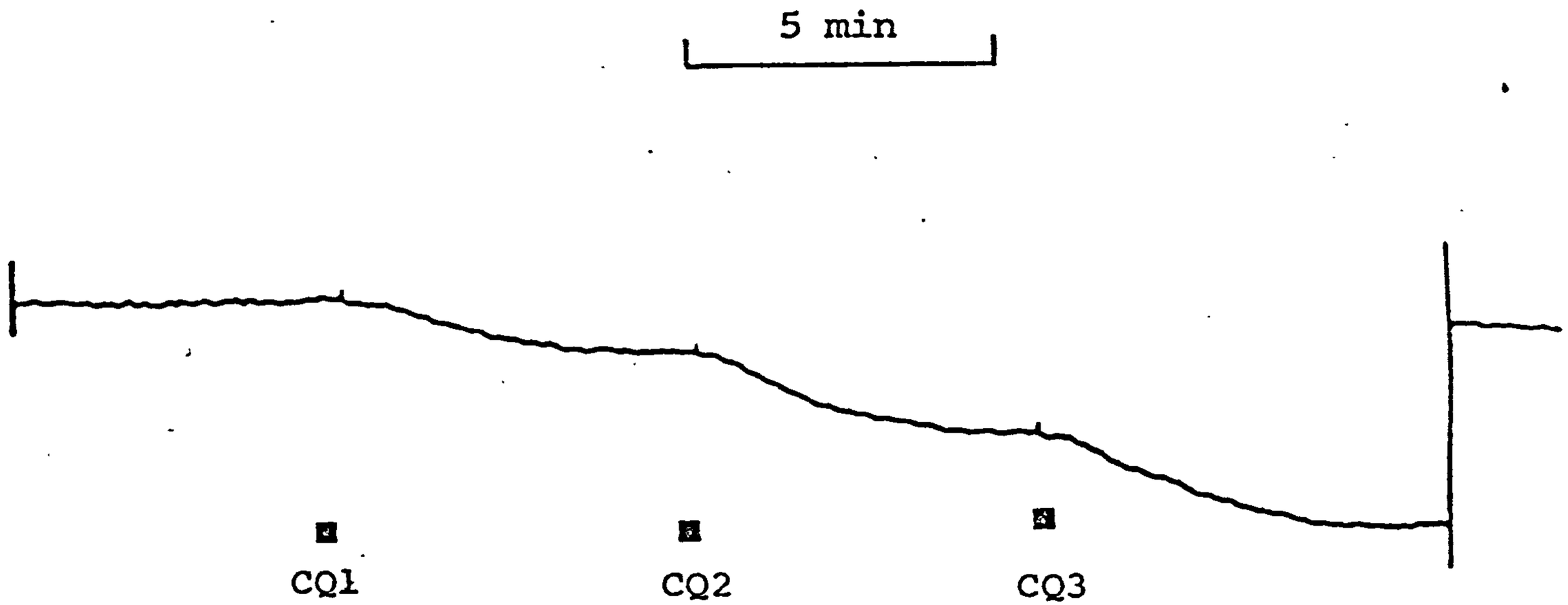


FIGURE 15

Chick isolated oesophagus. Effect of chloroquine (CQ) on the basal tone of the chick oesophagus. CQ1, CQ2 and CQ3 represent chloroquine 10^{-8}M, 10^{-7}M and 10^{-6}M respectively.

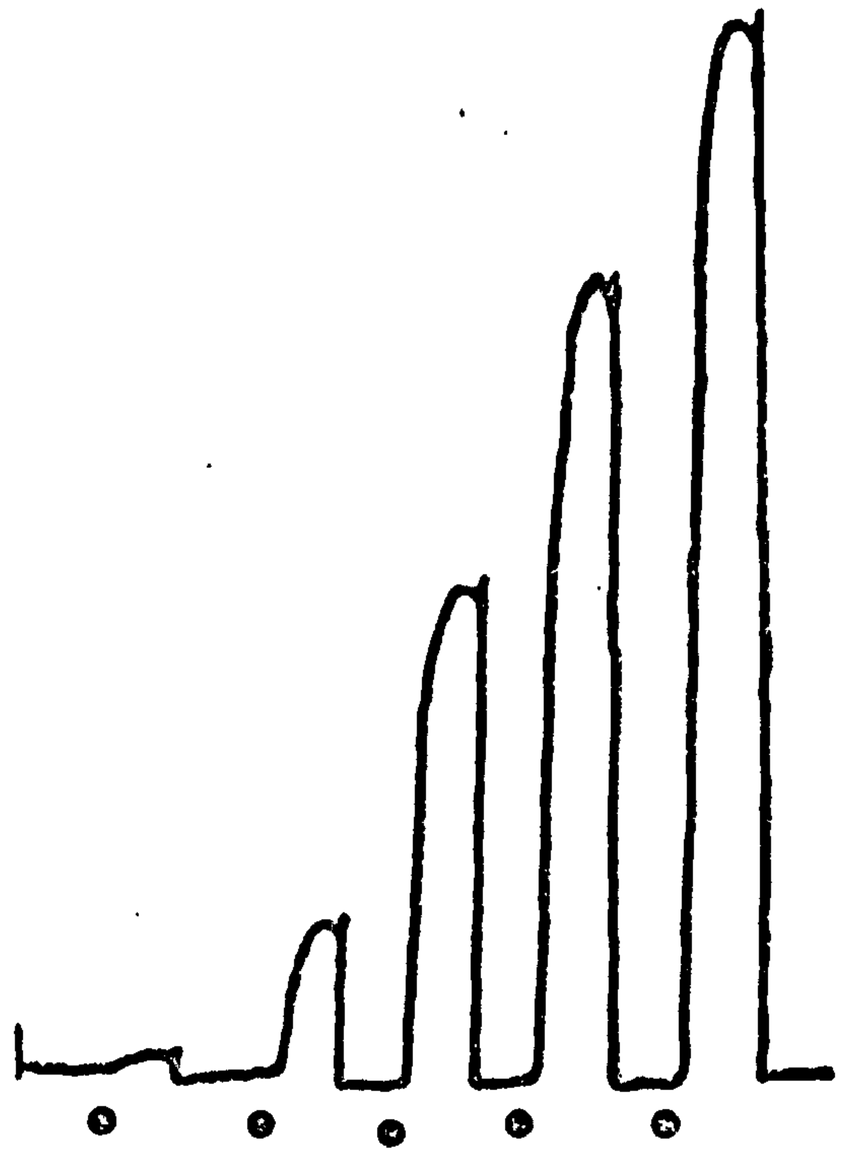


FIGURE 16

Chick isolated oesophagus. Dose/response curve constructed to quinine (QN) on the isolated oesophagus. Doses were added at 30 minute intervals, allowed to act for 40 seconds and then washed out 3 times. QN1, QN2, QN3, QN4 and QN5 represent quinine 7.5×10^{-4}M, 1.0×10^{-3}M, 2.5×10^{-3}M, 5.0×10^{-3}M and 7.5×10^{-3} respectively.

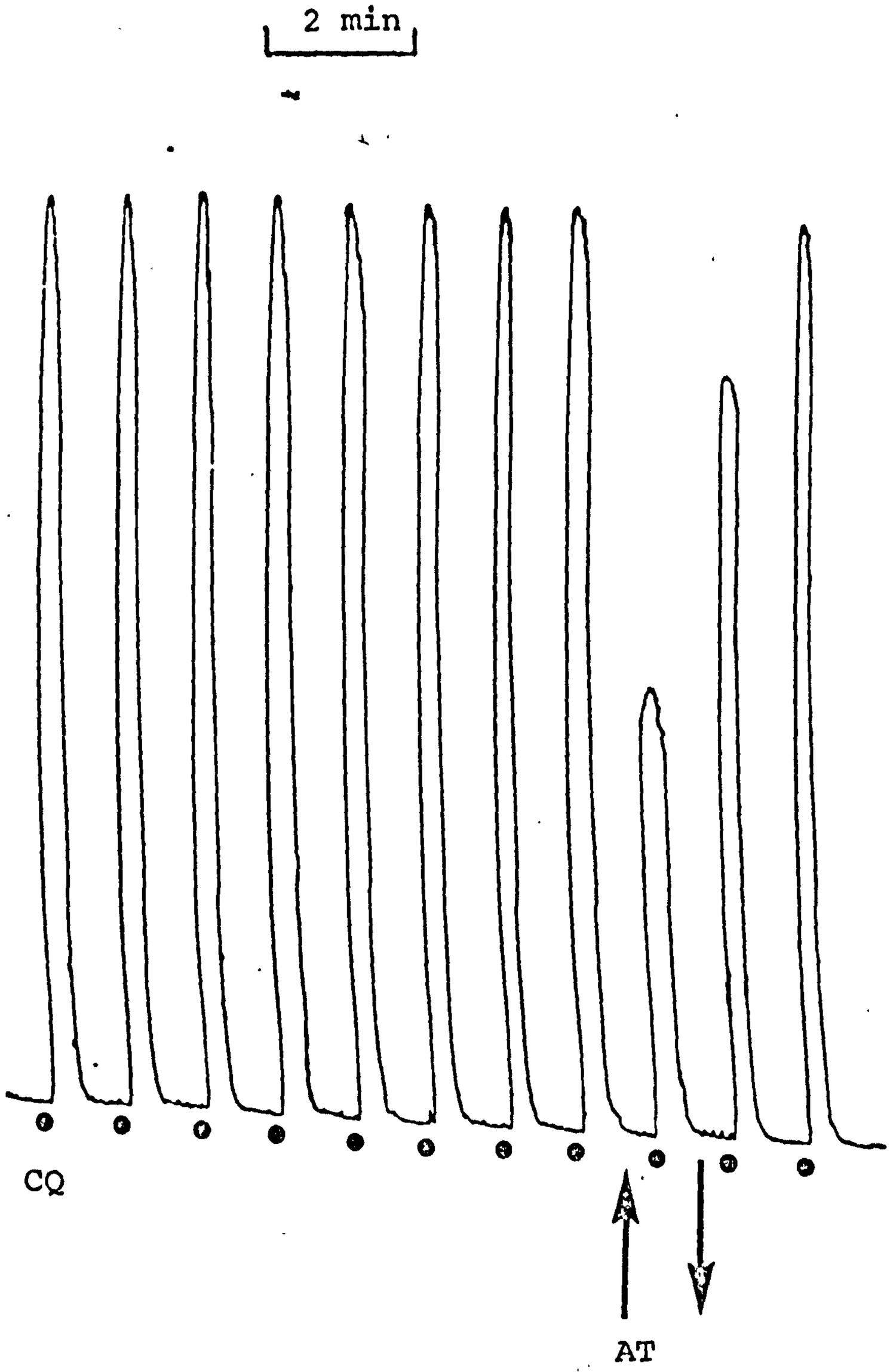


FIGURE 17

Chick isolated oesophagus. The effect of atropine (AT, 1.0×10^{-5}M) on the contractions of the chick oesophagus induced by chloroquine (CQ, ● , 5.0×10^{-3}M). The same doses of chloroquine were repeated at 40 minute intervals and allowed to act for 30 seconds. At the upward arrow, atropine (AT) was added to the bath and washed off at the downward arrow.

2.20 Chick isolated oesophagus

All the antimalarial compounds studied, in low doses (7.5×10^{-8} - 2.5×10^{-4}M), caused dose-dependent reductions of the base-line tension of the chick isolated oesophagus (Figure 15). However, higher concentrations ($\geqslant 7.5 \times 10^{-4}$M) produced dose-related contractions of the tissue (Figure 16). These contractions were augmented by an elevated organ bath temperature. The contractions were resistant to the actions of atropine (1 - 2.5×10^{-6}M), mepyramine (1 - 2.5×10^{-6}M), hexamethonium (1 - 2.5×10^{-6}M) and methysergide (1 - 2.5×10^{-6}M), but were inhibited by high doses of atropine (7.5×10^{-6} - 5.0×10^{-5}M). Figure 17 illustrates a typical trace. In some preparations, low concentrations of all the antimalarial agents studied (2.5×10^{-8} - 2.5×10^{-5}M) augmented contractions of the tissue induced by acetylcholine (1.0×10^{-7} - 2.5×10^{-5}M) but inhibited contractions evoked by histamine (1.0×10^{-7} - 2.5×10^{-5}M), nicotine (2.5×10^{-6} - 7.5×10^{-5}M), and 5-hydroxytryptamine (2.5×10^{-6} - 7.5×10^{-5}M). These results are similar to those obtained on the guinea-pig ileum. However, higher concentrations of all the antimalarials (2.5×10^{-4} - 2.5×10^{-3}M) inhibited, in a dose-dependent manner, the contractions induced by acetylcholine, histamine, nicotine, 5-hydroxytryptamine, potassium and barium chloride.

In most of the preparations, low concentrations of all five compounds studied (5.0×10^{-7} - 2.5×10^{-5}M) augmented the

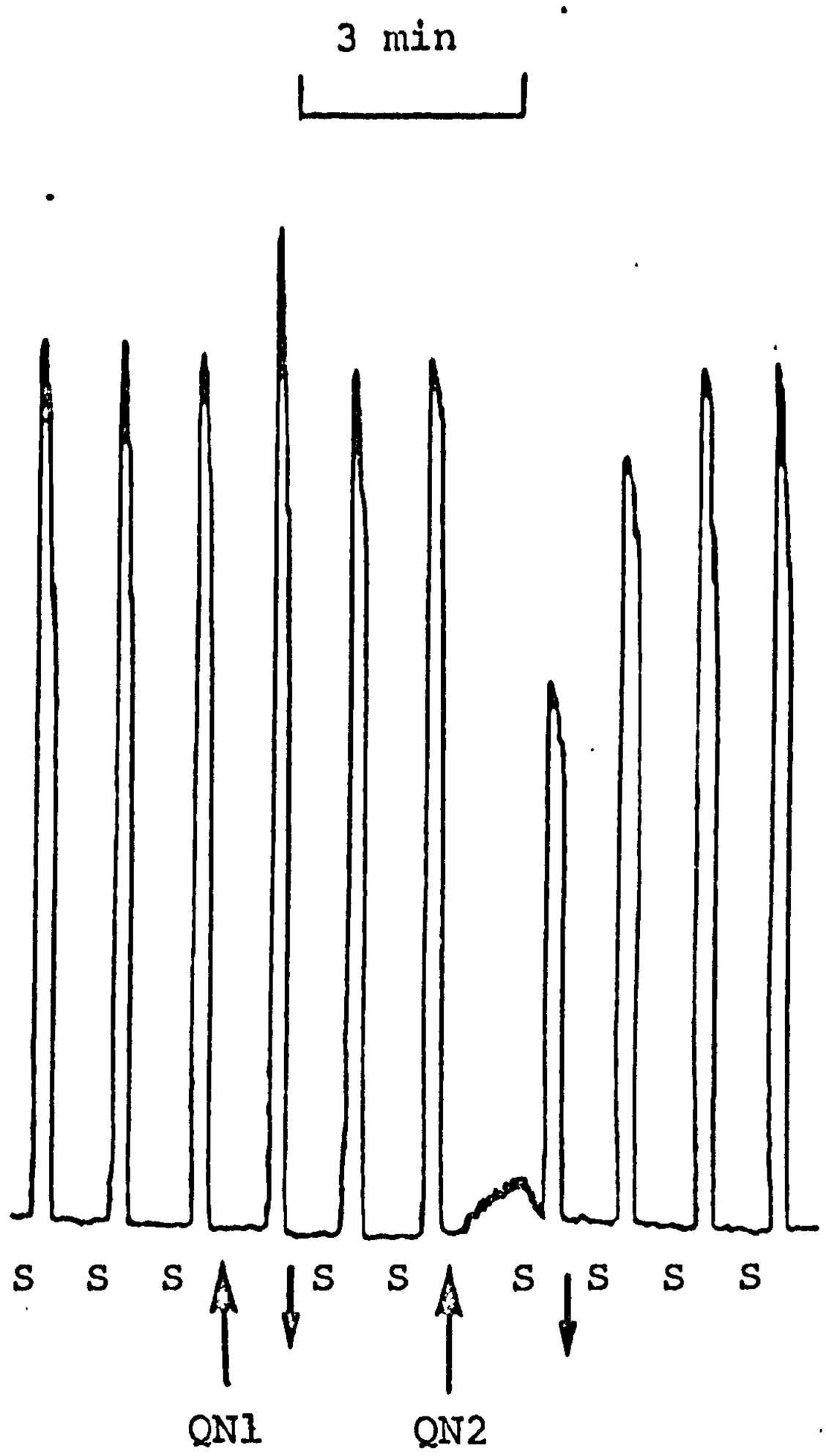


FIGURE 18

Chick isolated oesophagus. Effects of quinine (QN) on the responses of the tissue to electrical stimulation (S, at 20 Hz). QN1 and QN2 denote quinine, 10^{-6}M and 10^{-3}M respectively. Quinine was added to the bath at the upward arrow and washed out at the downward arrow.

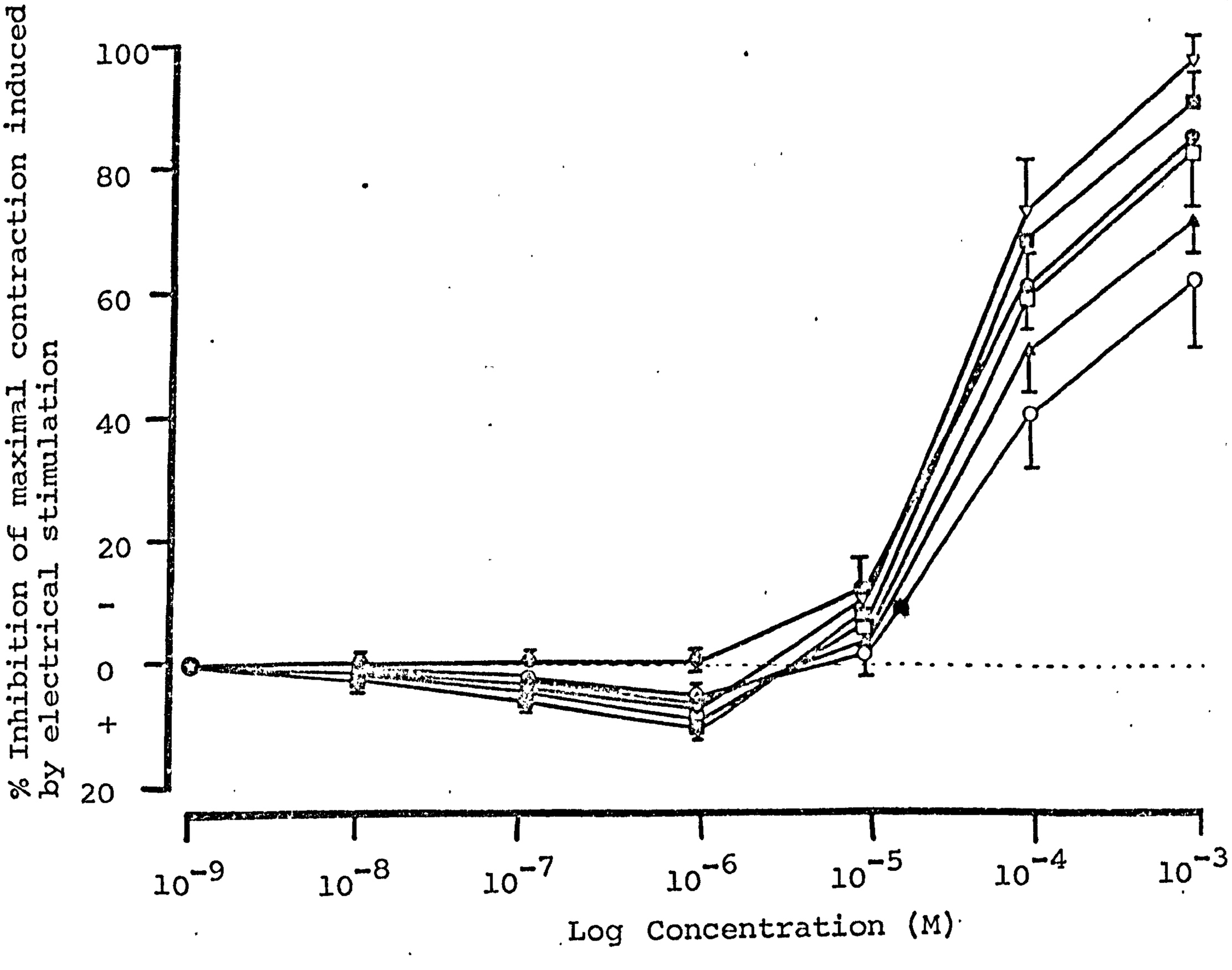


FIGURE 19

Chick oesophagus. Mean log dose/response curves constructed to primaquine (▽), chloroquine (■), quinine (□), proguanil (▲), pyrimethamine (○) and lignocaine (●). Each point is the mean of 5 - 8 observations. Vertical bars denote s.e. of means.

contractions evoked by electrical stimulation of the vagus. In contrast, higher concentrations (7.5×10^{-5} - 2.5×10^{-3}M) inhibited the electrically-induced contractions (Figures 18 and 19).

2.21 Guinea-pig tracheal chain

The tracheal smooth muscle had very little, if any, inherent tone. For this reason, studies on relaxant drugs were carried out on tissue preparations using agonists which produced contractions that were sustained for at least fifteen minutes. Acetylcholine, histamine, 5-hydroxytryptamine and potassium chloride produced dose-dependent contractions of the tracheal chain preparation. Acetylcholine and 5-hydroxytryptamine were found to be roughly equipotent on molar basis, whereas histamine and potassium were considerably less potent. 5-hydroxytryptamine and histamine were found to be unsuitable agonists because of tachyphylaxis. Similarly, potassium chloride was unsuitable because, in the concentrations used, it was almost impossible to relax such tissues, even with high doses of isoprenaline. In most preparations, contractions produced by carbachol were not reproducible enough to be useful. However, acetylcholine (7.5×10^{-8} - 7.5×10^{-5}M) in the presence of physostigmine (3.0 - 5.0×10^{-8}M) produced well-sustained contractions which were readily relaxed with drugs. Acetylcholine was therefore used in most of the experiments reported here.

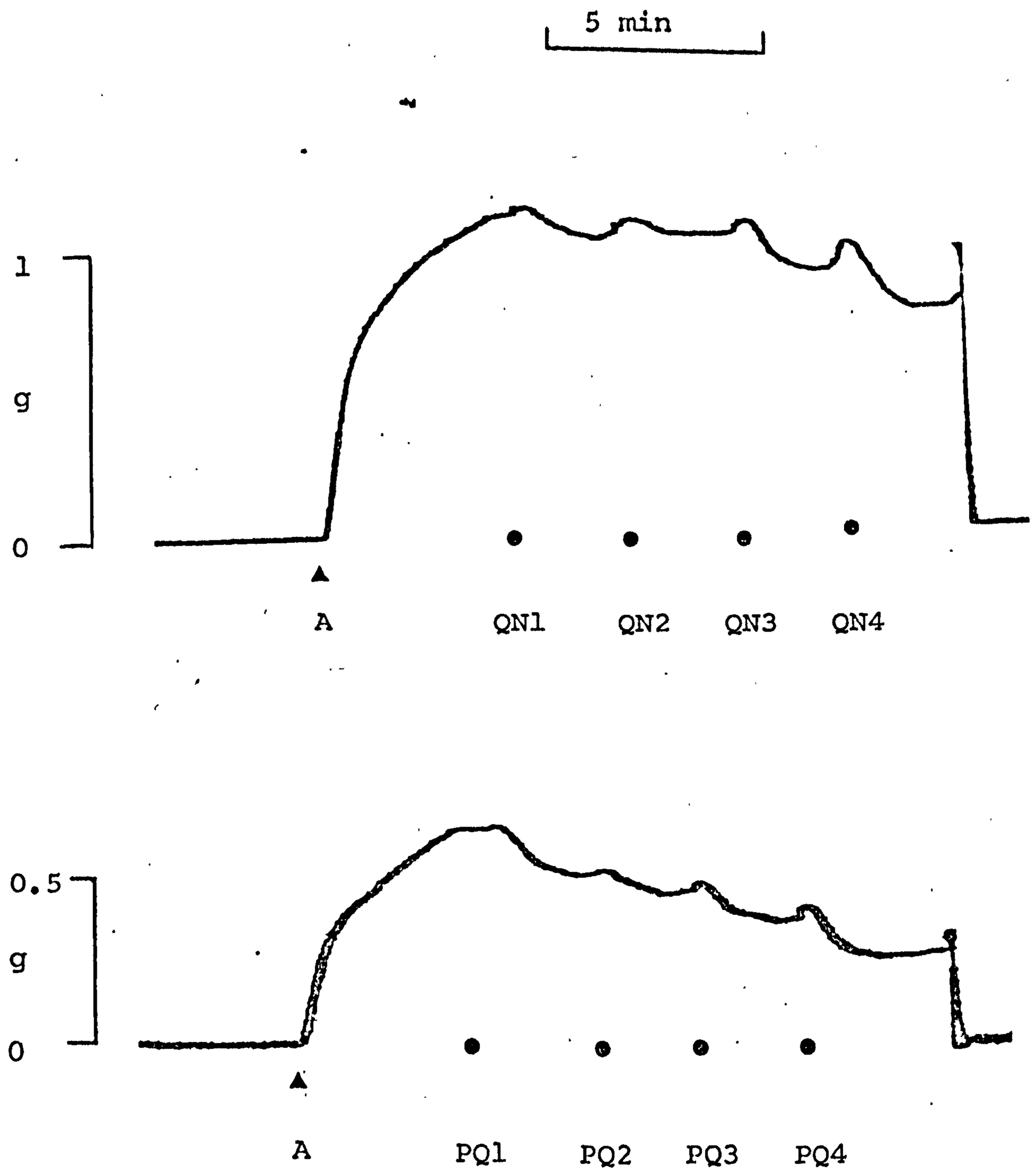


FIGURE 20

Guinea-pig isolated tracheal chain smooth muscle. Effects of quinine (QN) and primaquine (PQ) on tracheal chain smooth muscle contracted with acetylcholine (A, 7.5×10^{-8}M, in presence of physostigmine 5.0×10^{-8}M). QN1, QN2, QN3 and QN4 denote quinine 2.5×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M and 2.5×10^{-4}M respectively, while PQ1, PQ2, PQ3 and PQ4 represent primaquine, 2.5×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M and 2.5×10^{-4}M.

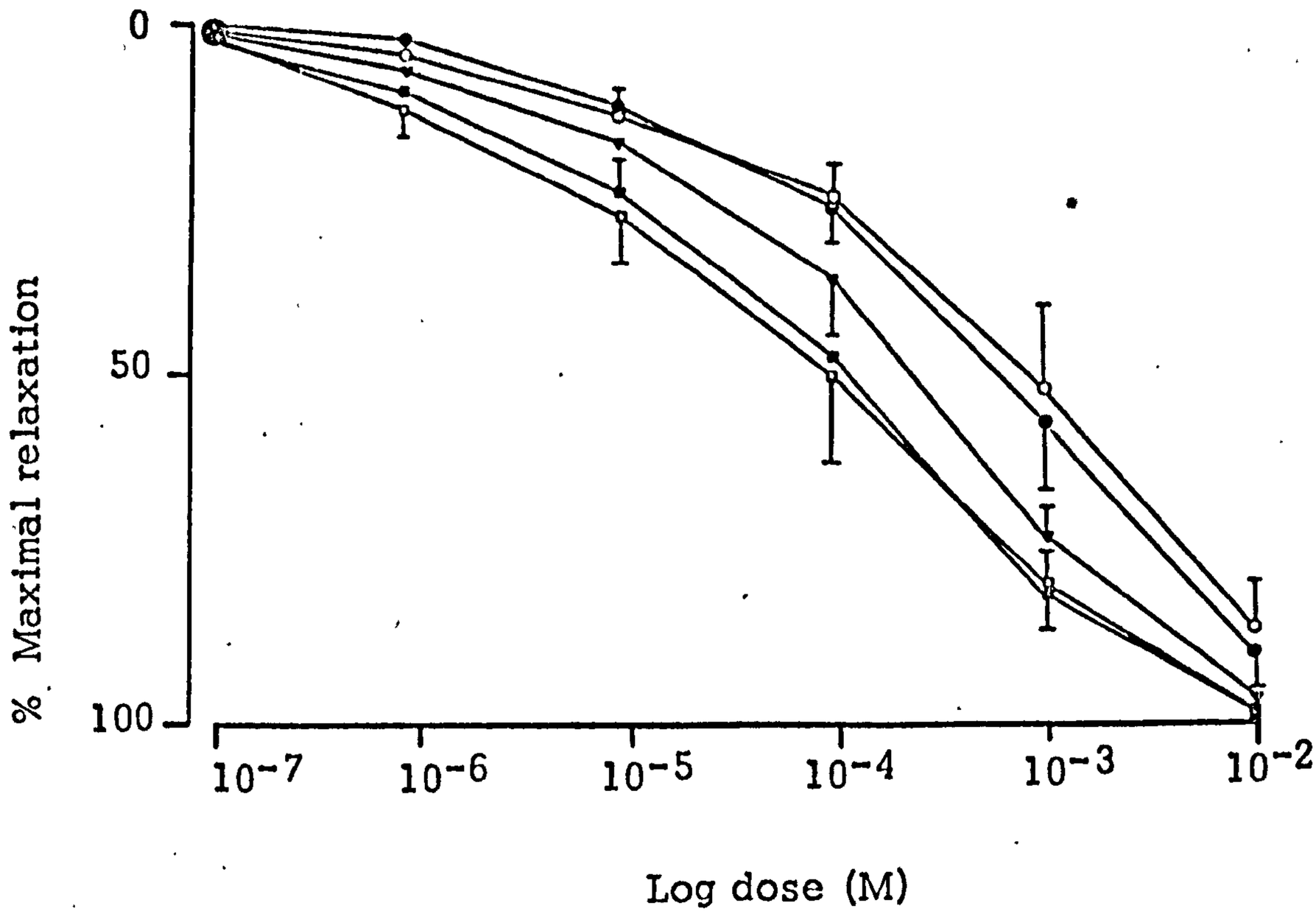


FIGURE 21

Guinea-pig isolated tracheal chain smooth muscle.

Mean cumulative dose/response curves to chloroquine (□), primaquine (■), quinine (▼), proquanil (◎) and pyrimethamine (o) on guinea-pig isolated tracheal chain smooth muscle preparations contracted with acetylcholine (in the presence of physostigmine). Each point is the mean of 6 - 9 observations, and the vertical bars denote s.e. of means.

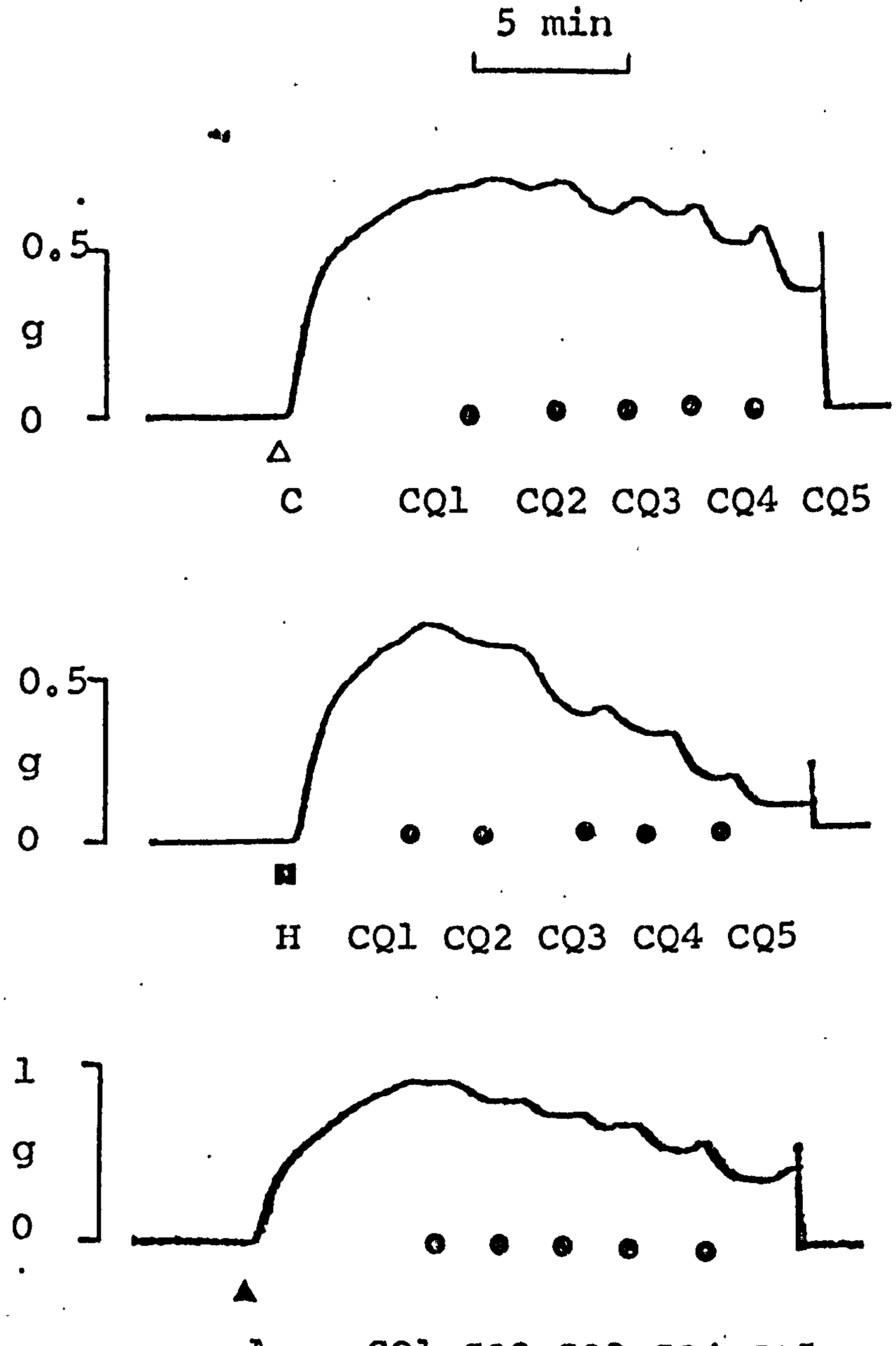


FIGURE 22

Guinea-pig isolated tracheal chain smooth muscle. Effect of chloroquine (CQ) on tracheal chain smooth muscle contracted with carbachol (C, 7.5×10^{-8}M, upper trace), histamine (H, 7.5×10^{-5}M, middle trace), or acetylcholine (A, 2.5×10^{-8}M, lower trace). CQ1, CQ2, CQ3, CQ4 and CQ5 denote chloroquine, 2.5×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 2.0×10^{-4} M and 4.0×10^{-4}M respectively.

TABLE 2

Drug	ED50 (M)
Chloroquine	2.25×10^{-5} (8)
Primaquine	2.80×10^{-5} (6)
Quinine	4.32×10^{-5} (7)
Proguanil	9.64×10^{-5} (4)
Pyrimethamine	1.33×10^{-4} (5)
Isoprenaline	4.56×10^{-8} (7)
Noradrenaline	5.42×10^{-7} (6)
Salbutamol	3.21×10^{-7} (5)
Papaverine	2.70×10^{-6} (4)

Guinea-pig isolated tracheal chain smooth muscle. ED50 values for various drugs on tracheal chain preparations contracted with acetylcholine (in the presence of physostigmine, 3.0×10^{-8}M). The values are the means obtained from 4 to 8 observations (number in parenthesis).

All the antimalarial drugs (5.0×10^{-7} - 1.0×10^{-2}M) produced dose-related relaxations of tracheal smooth muscle contracted with acetylcholine in the presence of physostigmine. Figure 20 shows typical traces whilst Figure 21 illustrates the dose/response curves obtained.

All the antimalarial compounds investigated also produced dose-dependent relaxations of the tracheal smooth muscle preparations contracted with 5-hydroxytryptamine (three preparations for each compound) and carbachol (two preparations for each drug). Histamine-induced contractions of the tracheal chain smooth muscle were readily relaxed by the aminoquinolines but only slightly relaxed by proguanil and pyrimethamine. Figure 22 shows typical traces obtained with three different agonists.

The effects of the antimalarials on tracheal smooth muscle was compared with those of some other drugs. Table 2 shows the ED50 values for the antimalarials and for isoprenaline, noradrenaline, salbutamol and papaverine. On a molar basis, the order of potency of the drugs in relaxing the tracheal chain smooth muscle contracted with acetylcholine in the presence of physostigmine was: isoprenaline $>$ salbutamol $>$ noradrenaline $>$ papaverine $>$ chloroquine $\geq$ primaquine $>$ quinine $\gg$ proguanil $\geq$ pyrimethamine.

The β-adrenoceptor blocking drug, alprenolol (5.0×10^{-8} - 1.0×10^{-6}M) produced a marked shift of the dose/response

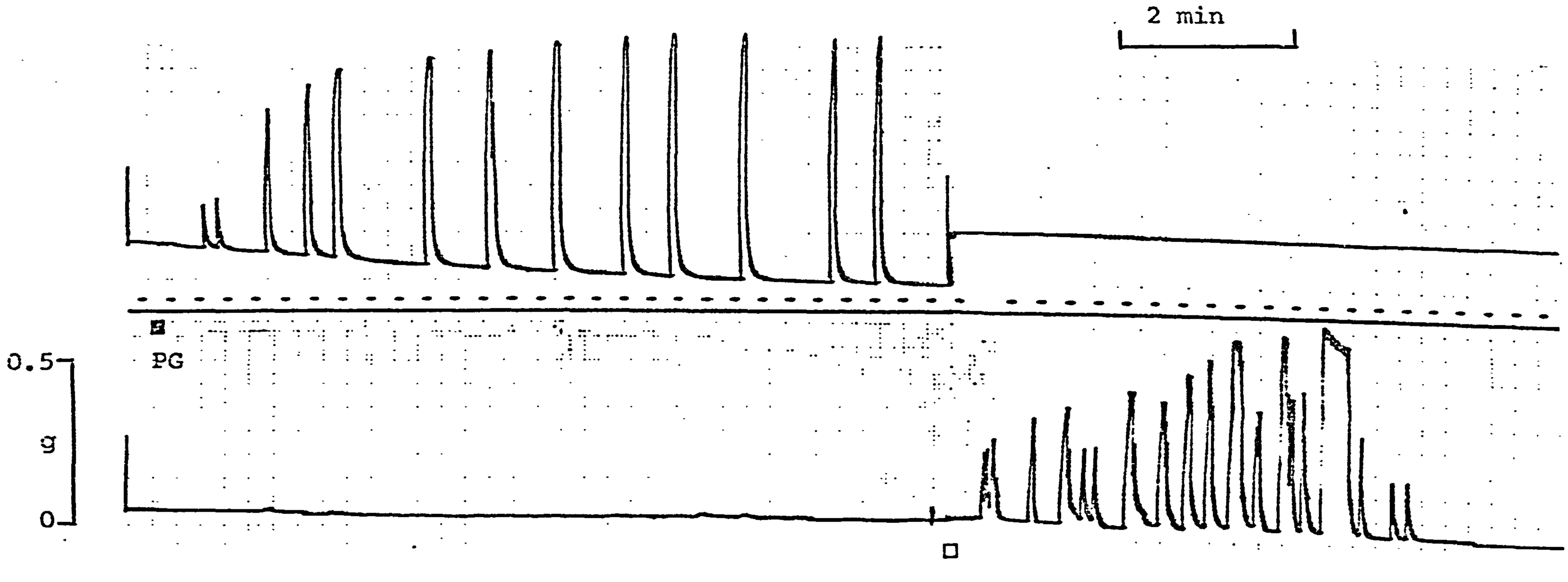


FIGURE 23

Rat isolated oestrogen-dominated uterus. Effects of proguanil (PG, 2.5×10^{-6}M) and quinine (QN, 7.5×10^{-6}M) on the quiescent oestrogen-primed rat isolated uteri. Proguanil- or quinine-induced spontaneous rhythmic contractions started 30 - 60 seconds after the drug administration.

curves to isoprenaline and noradrenaline, and a similar effect was obtained with propranolol (5.0×10^{-7} - 1.75×10^{-6}M) against salbutamol. However, these β-adrenoceptor blocking agents, in the concentration range used, did not modify dose/response curves to the antimalarial compounds.

2.22 Rat and guinea-pig isolated uteri

In the oestrogen-dominated isolated uterine preparations (ie. uterine tissues isolated from oestrogen-treated animals), all the antimalarial compounds produced biphasic effects. Low concentrations (2.5×10^{-8} - 7.5×10^{-6}M) stimulated the isolated uterus whilst higher concentrations (1.0×10^{-4} - 2.0×10^{-3}M) inhibited the tissues in a dose-dependent manner.

The responsiveness of isolated uterine preparations to stimulant doses of the antimalarial drugs varied from one animal species to another. Thus proguanil (1.0 - 4.0×10^{-6}M) caused rhythmic contractions of the oestrogen-dominated quiescent rat uterus (Figure 23) but had a much weaker action on the guinea-pig isolated oestrogen-treated uterus. Similarly, quinine (2.5×10^{-6} - 2.5×10^{-5}M) produced powerful contractions of the rat uterus with a smaller effect on the guinea-pig uterus. In most of the preparations used, pyrimethamine (2.5 - 7.5×10^{-6}M) caused contractions of the rat isolated oestrogen-dominated uterus but not that of the guinea-pig. Pyrimethamine-

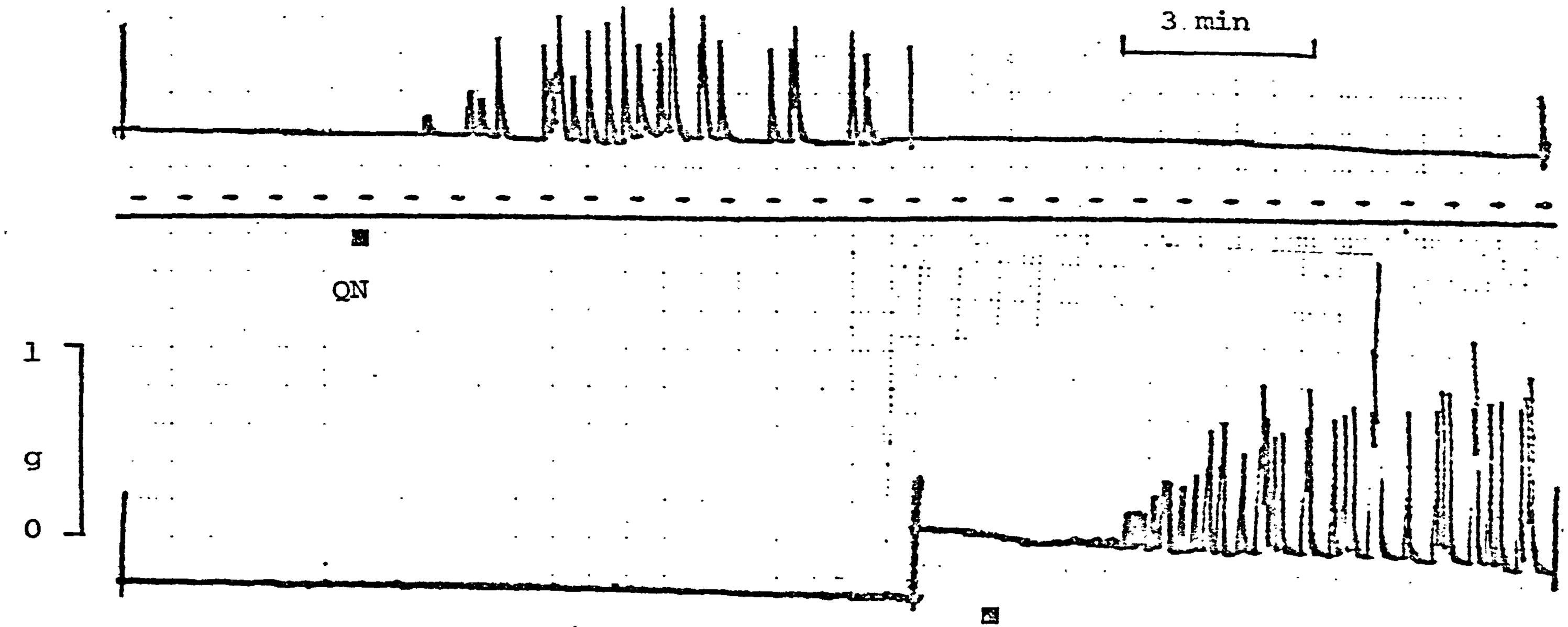


FIGURE 24

Guinea-pig isolated oestrogen-primed uterus. Effect of quinine (QN, 7.5×10^{-6}M on the quiescent oestrogen-dominated isolated uterus. Quinine-induced spontaneous contractions started 30 - 90 seconds after the drug administration.

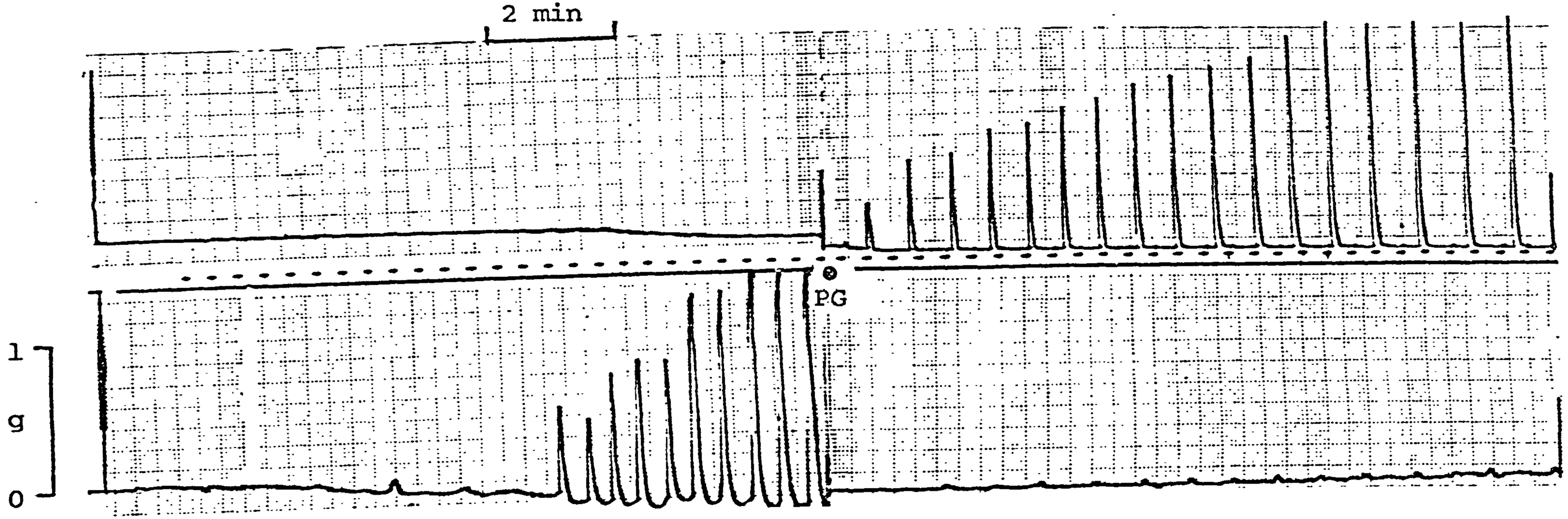


FIGURE 25

Rat isolated oestrogen-primed uterus. Effect of indomethacin (IND, 2.5×10^{-6}M) on spontaneous contractions of the quiescent oestrogen-dominated rat uterus evoked by proguanil (PG, 2.5×10^{-6}M). After indomethacin, the preparation was usually stimulated

induced contractions were at a lower frequency than those produced by proguanil or quinine.

Chloroquine and primaquine ($1.0 \times 10^{-8} - 5.0 \times 10^{-6}$M) produced no appreciable spontaneous contractions of the rat isolated oestrogen-primed uterus. At the same dose levels, however, chloroquine markedly stimulated the guinea-pig isolated oestrogen-dominated uterus. In 40 per cent of the preparations used (ie. four). primaquine ($1.0 \times 10^{-7} - 2.5 \times 10^{-6}$M) also caused spontaneous contractions of the guinea-pig isolated oestrogen-primed uterus. Similarly, quinine ($7.5 \times 10^{-7} - 1.0 \times 10^{-5}$M) always induced spontaneous contractions of the guinea-pig isolated oestrogen-dominated uterus (Figure 24).

The spontaneous contractions of the quiescent, oestrogen-dominated isolated uteri of rats and guinea-pigs induced by the antimalarial compounds were not inhibited by indomethacin ($7.5 \times 10^{-7} - 2.5 \times 10^{-6}$M ; Figure 25). In fact, in most of the preparations, the tissue became more responsive to antimalarials following indomethacin administration (eg. Figure 25 lower trace). In control runs where equal volumes of ethanol (0.01 - 0.05 ml/ml) was administered to the bath, the preparations were not stimulated. The antimalarial-induced spontaneous contractions were also resistant to the action of atropine ($1 - 2.5 \times 10^{-6}$M), mepyramine ($1 - 2.5 \times 10^{-6}$M),

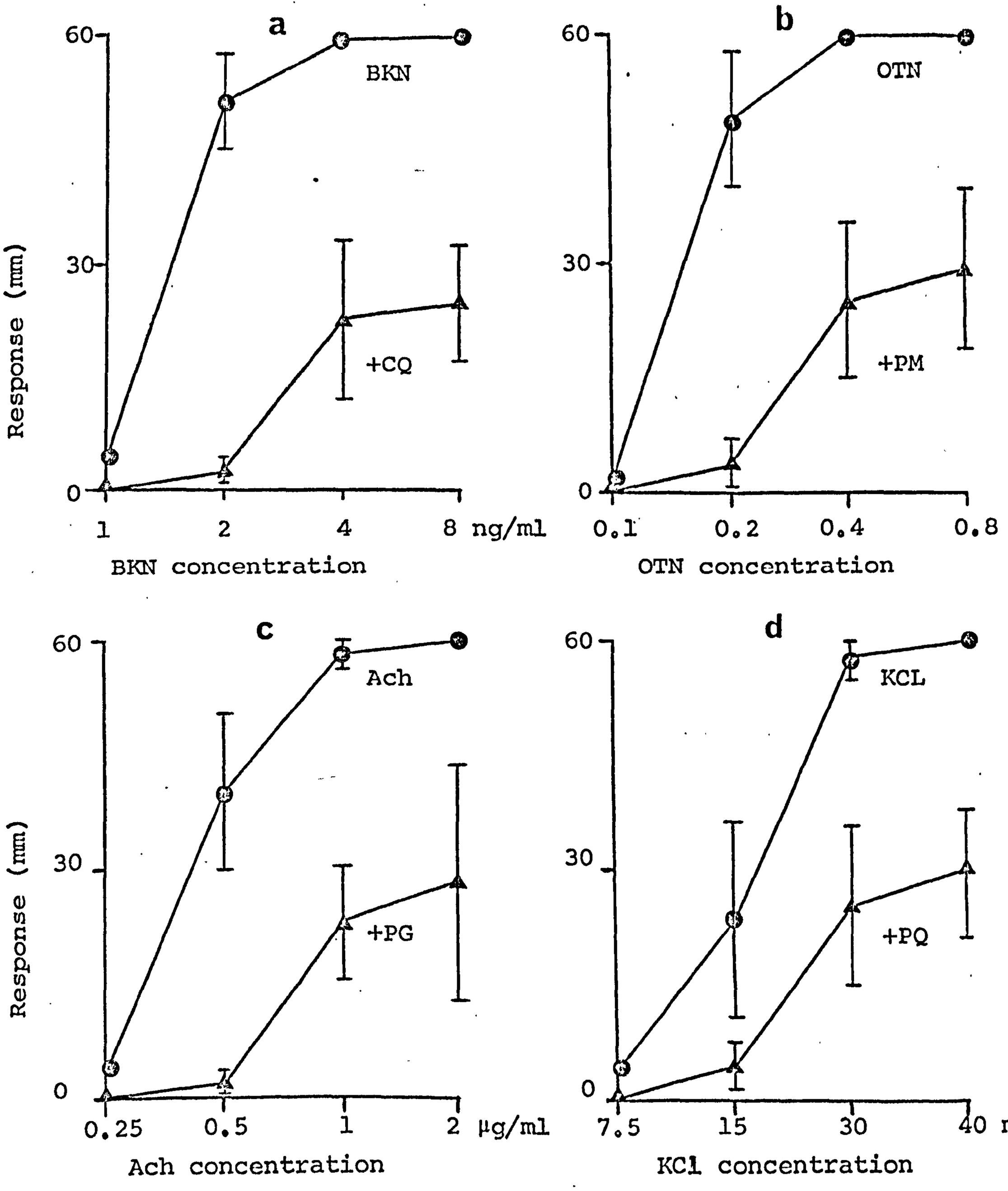


FIGURE 26

Rat isolated oestrogen-dominated uterus. Effects of various concentrations of (a) bradykinin (BKN) in the absence (●) and in the presence (▲) of chloroquine (CQ, 1.0×10^{-4}M); (b) oxytocin (OTN) in the absence (●) and in the presence (▲) of pyrimethamine (PM, 1.0×10^{-4}M); (c) acetylcholine (Ach) in the absence (●) and in the presence (▲) of proguanil (PG, 1.0×10^{-4}M); and potassium chloride (KCl) in the absence (●) and in the presence (▲) of primaquine (PQ, 1.0×10^{-4}M). Each point is the mean of 5 to 7 observations and the vertical bars denote s.e. of means.

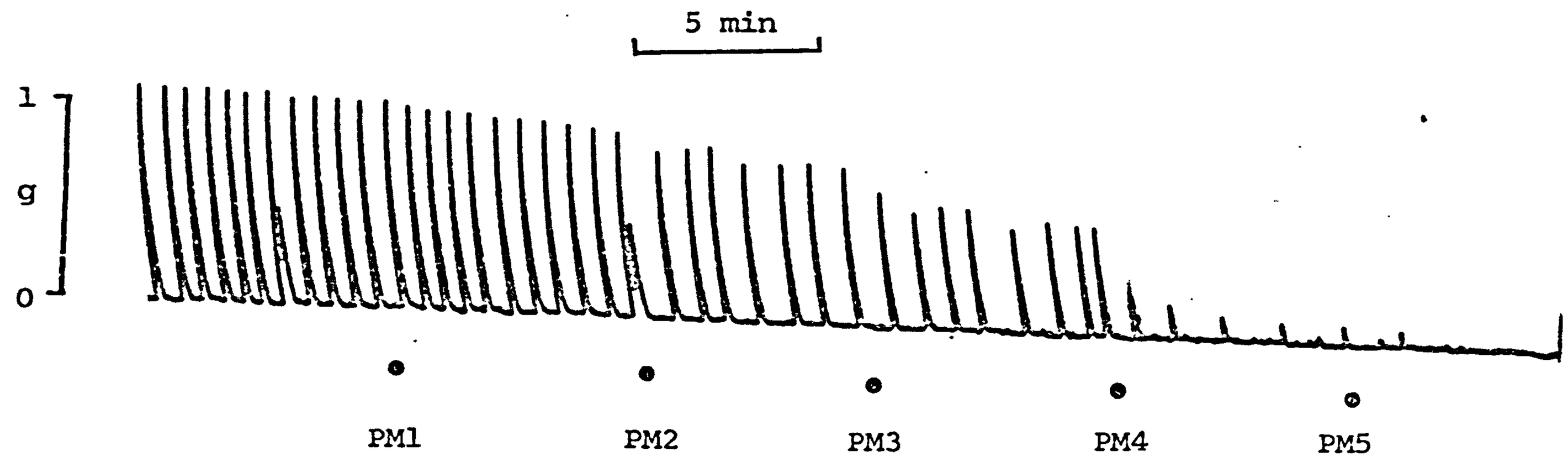


FIGURE 27

Pregnant guinea-pig isolated uterus. Cumulative effect of pyrimethamine (PM) on the spontaneous contractions of an isolated uterus taken from a pregnant guinea-pig. PM1, PM2, PM3, PM4 and PM5 denote pyrimethamine, 5.0×10^{-5}, 1.0×10^{-4}, 5.0×10^{-4}M, 1.0×10^{-3}M and 2.5×10^{-3}M respectively.

hexamethonium ($1 - 2.5 \times 10^{-6}$M), methysergide ($1 - 2.5 \times 10^{-6}$M), or phentolamine ($1 - 2.5 \times 10^{-6}$M).

All the antimalarial drugs studied ($7.5 \times 10^{-8} - 2.5 \times 10^{-3}$M) dose-dependently inhibited, or abolished, the stimulant actions of bradykinin, oxytocin, acetylcholine and potassium chloride on the isolated oestrogen-primed quiescent uteri of rats and guinea-pigs (Figure 26). The antagonism produced (non-specific) was essentially similar to that produced by these agents against the standard spasmogens on the guinea-pig isolated ileum.

The stimulant effects of the compounds observed in the quiescent preparations isolated from non-pregnant, oestrogen-dominated rats and guinea-pigs were not seen in those taken from rats and guinea-pigs in early, middle or late stages of pregnancy. Low to high concentrations of all the antimalarial compounds ($1.0 \times 10^{-6} - 1.0 \times 10^{-2}$M) produced dose-dependent inhibitions of spontaneous contractions of uteri taken from pregnant rats and guinea-pigs. Figure 27 shows a typical trace of all the compounds; primaquine produced the strongest inhibition of these spontaneous contractions. Figure 28 shows dose/response curves obtained for all the antimalarials on pregnant spontaneously contracting guinea-pig uteri. No qualitative or quantitative differences related to the stage of pregnancy were observed, and the inhibitory effects of the antimalarial compounds were resistant to the actions of

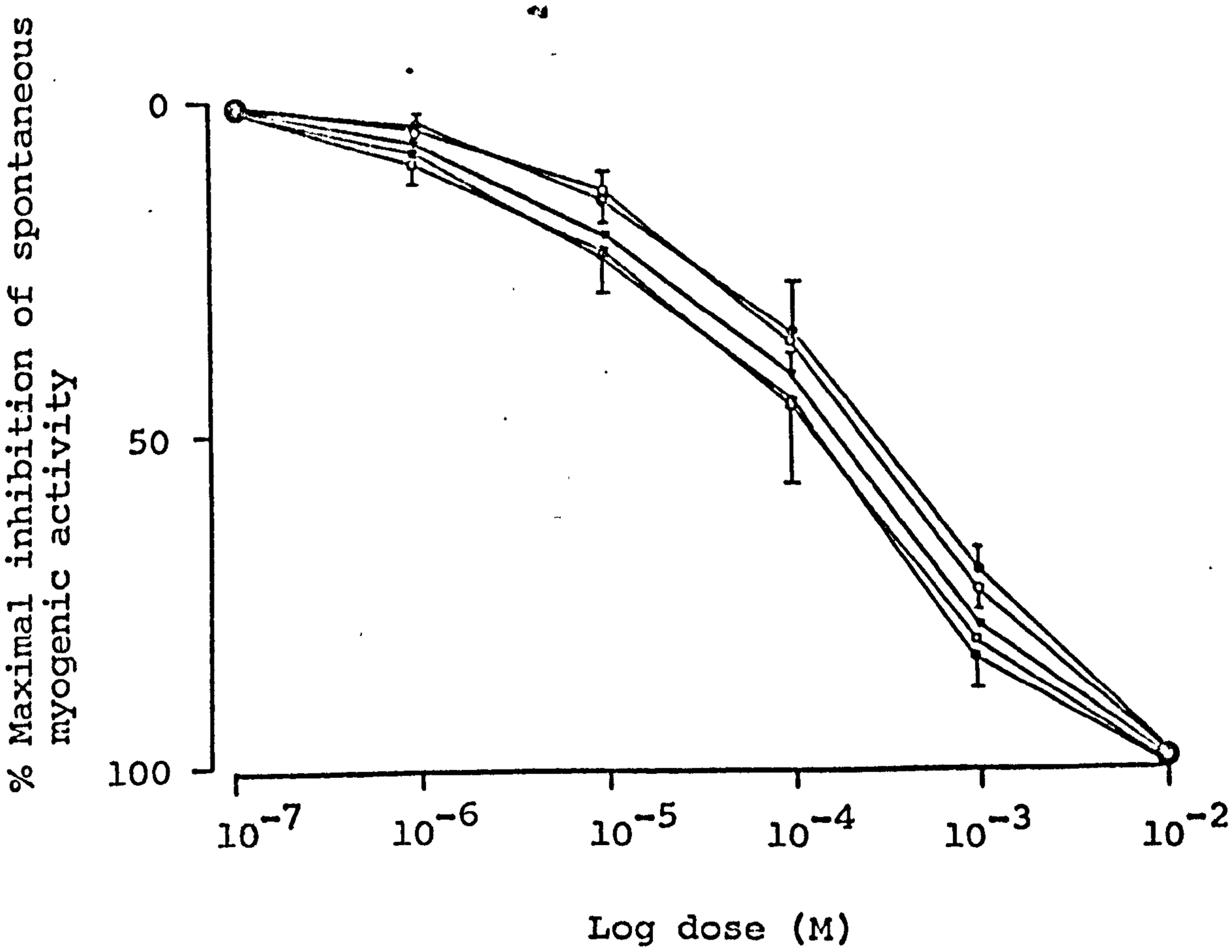


FIGURE 28

Pregnant guinea-pig isolated uterus. Mean cumulative dose/response curves to primaquine (□), chloroquine (■), quinine (▼), proguanil (●) and pyrimethamine (○) on the isolated spontaneously-contracting uterus of pregnant guinea-pig. Each point is the mean of 6 - 8 observations, and the vertical bars denote s.e. of means.

atropine (1 - 2.5 x 10^{-6}M), mepyramine (1 - 2.5 x 10^{-6}M), hexamethonium (1 - 2.5 x 10^{-6}M), methysergide (1 - 2.5 x 10^{-6}M), phentolamine (1 - 2.5 x 10^{-6}M) and propranolol (1 - 2.5 x 10^{-6}M).

Because the antimalarial compound solutions used were acidic, buffered solutions were prepared to the same pH as those of the antimalarial agents. This was done in order to check the possible influence of pH on the stimulant and inhibitory uterine effects of the compounds. Volumes of buffered solution equal to those of the antimalarial solutions used, did not produce any marked effect on either quiescent uterine strips taken from oestrogen-primed rats and guinea-pigs (stimulated by low to medium concentrations of antimalarial drugs) or on spontaneously contracting uteri of pregnant rats and guinea-pigs (inhibited by low to high doses of all the antimalarial agents).

2.23 Guinea-pig and rat isolated vasa deferentia

The response of guinea-pig and rat isolated vasa deferentia to indirect electrical stimulation and to exogenous additions of noradrenaline were found to be similar both quantitatively and qualitatively, hence no distinction has been made between them in describing the results obtained.

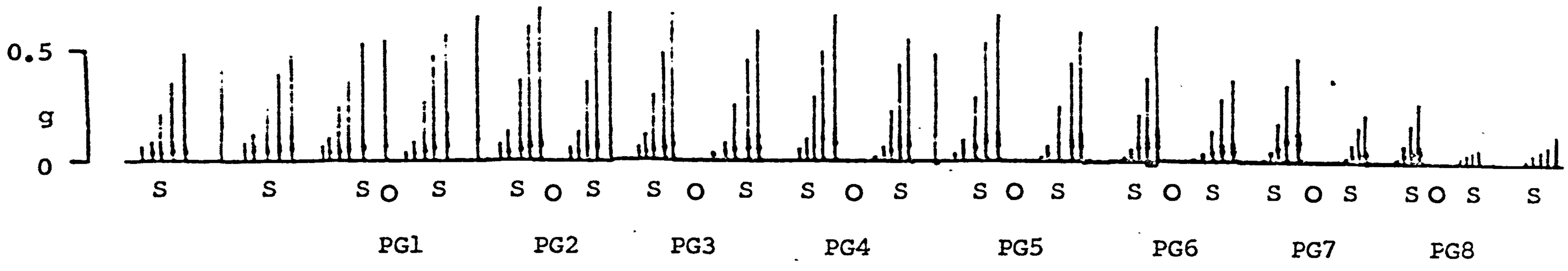


FIGURE 29

Guinea-pig isolated vas deferens. Stimulantory and inhibitory effects of proguanil (PG) on the motor responses of vas deferens to indirect electrical stimulation (S) at various frequencies. PG1, PG2, PG3, PG4, PG5, PG6, PG7 and PG8 represent proguanil 2.5×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 2.5×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M, 2.5×10^{-3}M and 5.0×10^{-3}M respectively. Doses of proguanil were allowed to act on the tissue for 30 seconds before stimulation, and washed out 3 times after the subsequent stimulation.

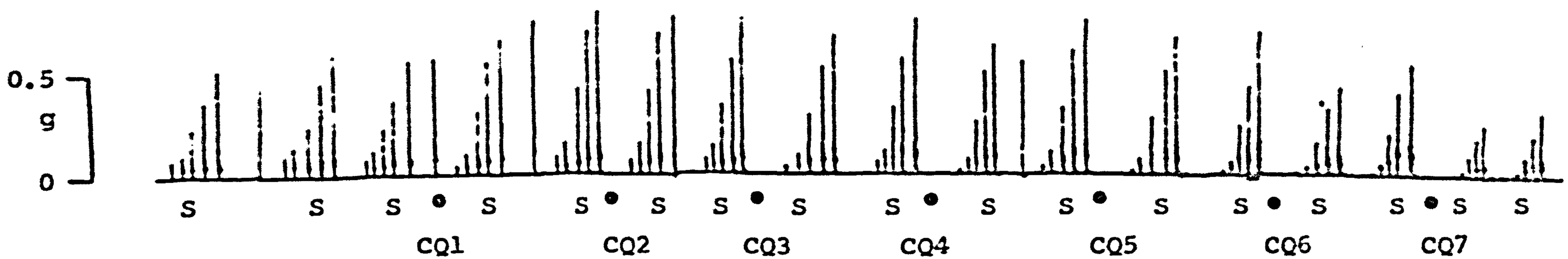


FIGURE 30

Guinea-pig isolated vas deferens. Stimulatory and inhibitory effects of chlorcquine (CQ) on the motor responses of vas deferens to indirect electrical stimulation (S) at various frequencies. CQ1, CQ2, CQ3, CQ4, CQ5, CQ6 and CQ7 represent chloroquine 1.0×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 2.5×10^{-4}M, 5.0×10^{-4}M, 7.5×10^{-4}M and 1.0×10^{-3}M respectively. Different doses of chloroquine were added to the bath 30 seconds before electrical stimulation was started.

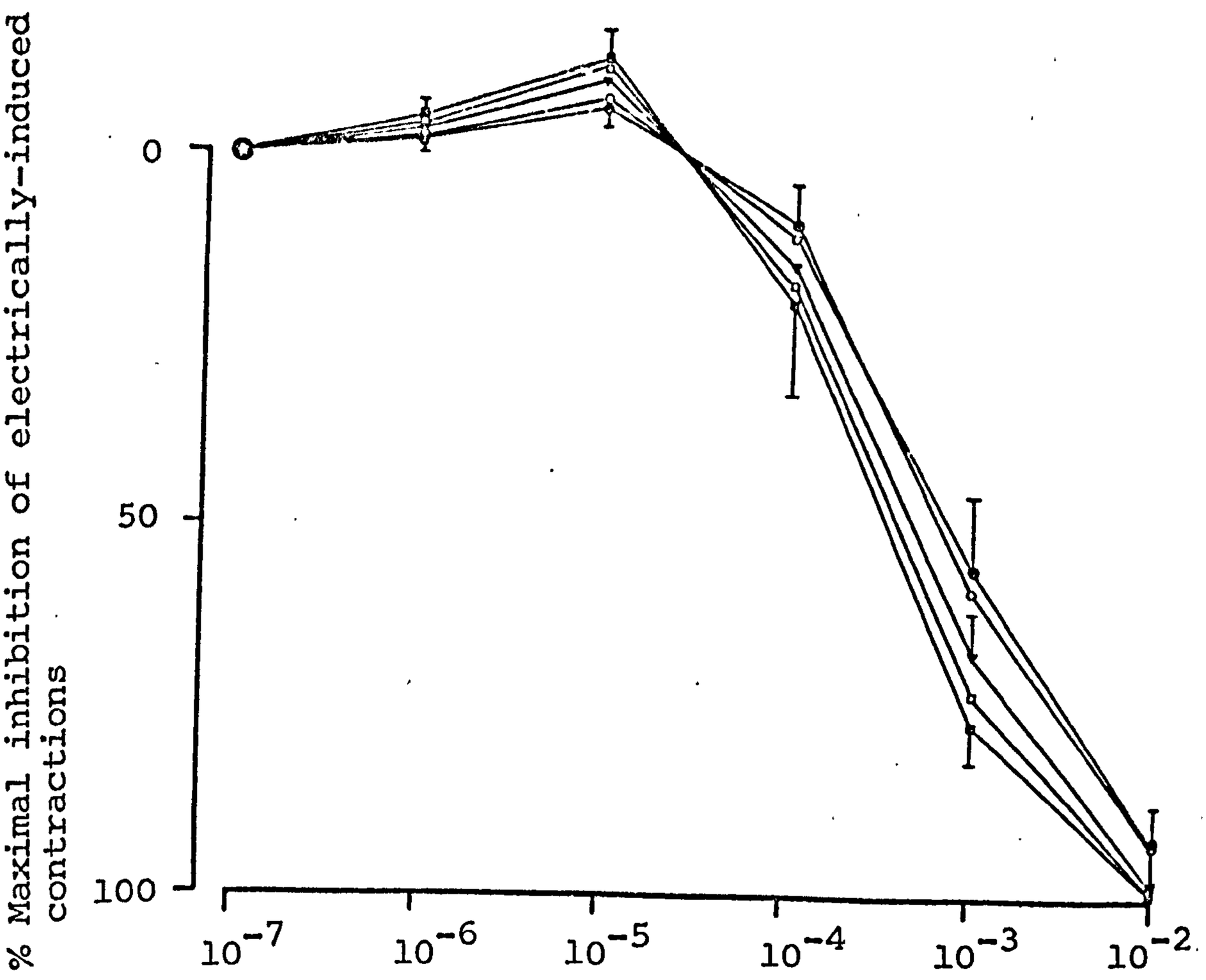


FIGURE 31

Guinea-pig isolated vas deferens. Mean dose/response curves to primaquine (◘), chloroquine (□), quinine (▼), pyrimethamine (O) and proguanil (❍) on guinea-pig isolated vasa deferentia. Each point is the mean of 4 - 8 observations, and the vertical bars represent s.e. of means.

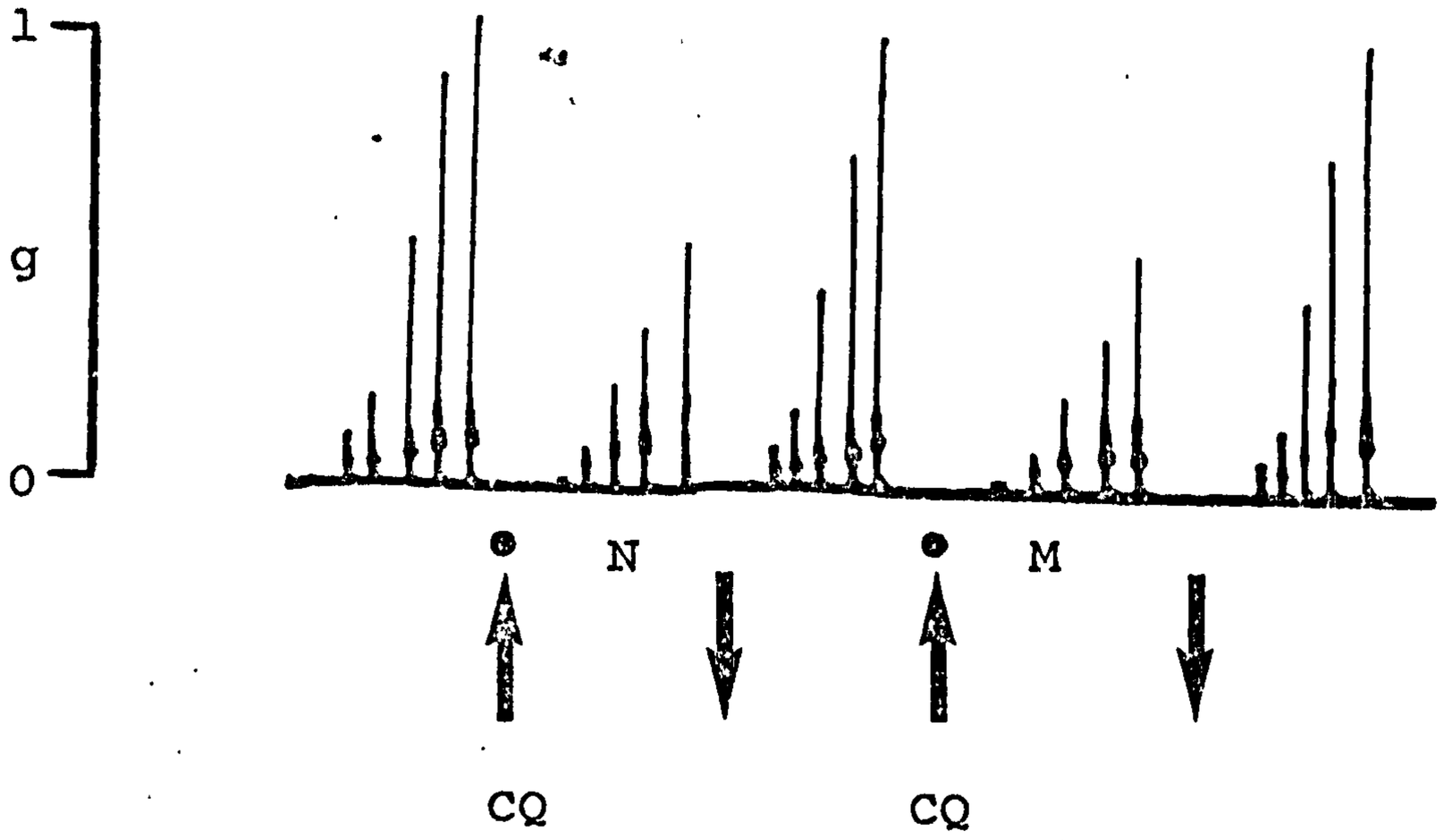


FIGURE 32

Guinea-pig isolated vas deferens. Effects of chloroquine (CQ, 5.0×10^{-4}M) added to the 'nerve' or 'muscle' compartment at the upward arrow. The drug was washed out at the downward arrow. N and M represent nerve and muscle compartments respectively. In both cases, the muscle was indirectly stimulated through the hypogastric nerve. The contractions were inhibited to about the same extent.

In the concentration range 7.5×10^{-7} - 5.0×10^{-5}M, all the antimalarial drugs studied augmented motor responses obtained by hypogastric nerve stimulation, whilst higher doses (1.0×10^{-4} - 1.0×10^{-2}M) depressed or abolished them. This effect depended on the concentrations used. Figures 29 and 30 show traces obtained with proguanil and chloroquine respectively. The stimulant and inhibitory effects of all the compounds (summarized in Figure 31) were readily reversed by washing.

In the experiments carried out with 'two-chambered organ-baths', equal doses of medium to high concentrations of antimalarial drugs (1.0×10^{-4} - 1.0×10^{-3}M) added separately, and in turn, into the 'nerve' and 'muscle' compartments inhibited the motor responses of the tissue induced by nerve stimulation to approximately the same extent (Figure 32). This effect was regarded as an index of membrane-stabilising or local anaesthetic activity. The potency of the antimalarials in eliciting this effect was: primaquine $\geq$ chloroquine $>$ quinine $\gg$ proguanil $\geq$ pyrimethamine.

In some preparations, the inhibitory effects of all the antimalarials on the electrically-evoked contractions of the vas were compared with those produced by the adrenergic neurone blocking agents, guanethidine (2.5×10^{-6}M) and bretylium (5.0×10^{-6}M). Unlike the antimalarial compounds, bretylium selectively inhibited the motor responses of the

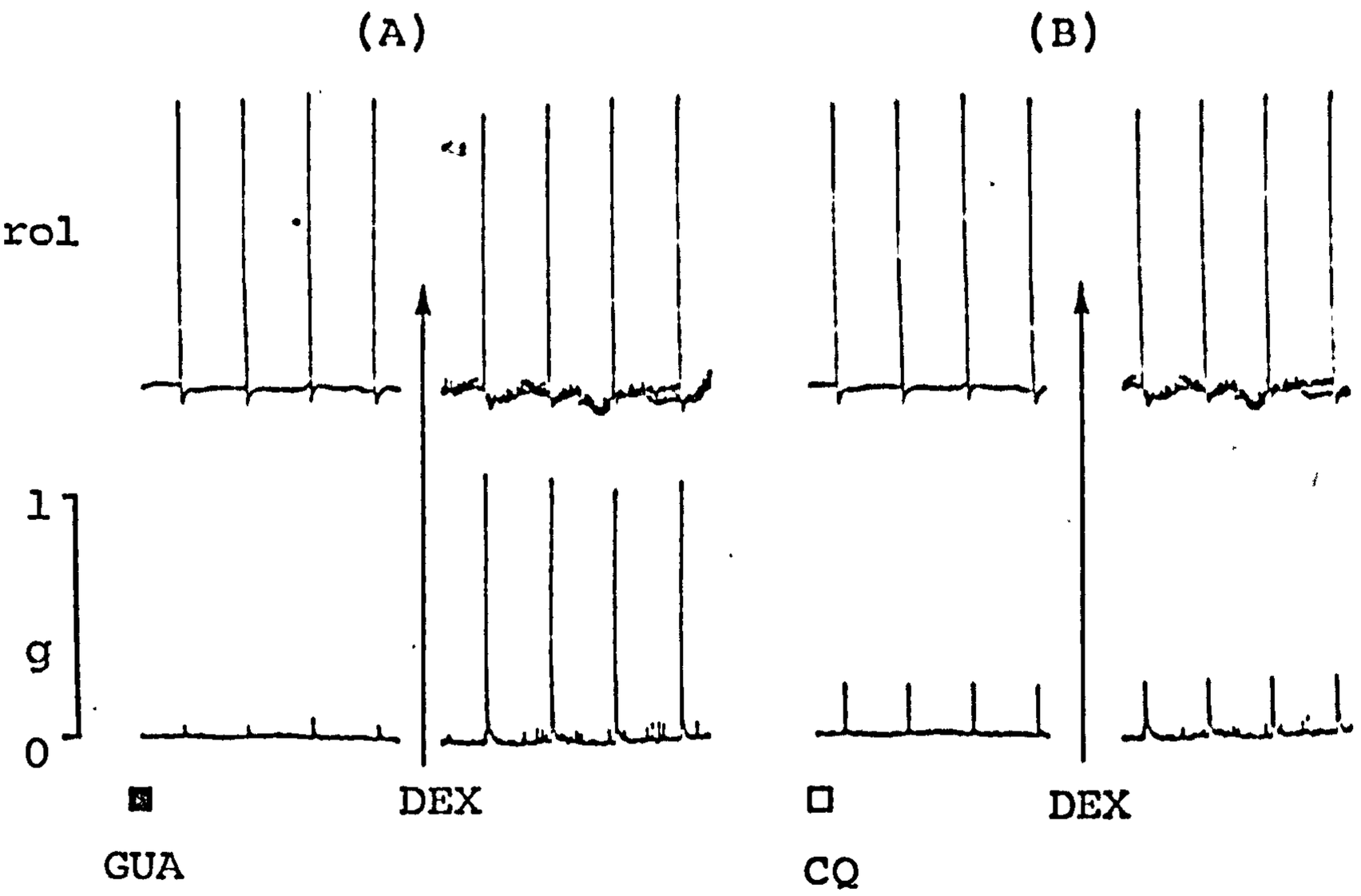


FIGURE 33

Guinea-pig isolated vas deferens. Effects of guanethidine (GUA, 2.5×10^{-6}M), chloroquine (CQ, 7.5×10^{-4}M) and dexamphetamine (DEX, 3.1×10^{-6}M) on the contractions of the vas induced by hypogastric nerve stimulation. In (A), the inhibitory effect of guanethidine was reversed by dexamphetamine whereas the inhibitory effect of chloroquine on the electrically-induced contractions of the muscle was not (B).

isolated vas deferens to high frequency stimulation whereas guanethidine produced a more selective inhibition of low frequency stimulation. The antimalarials inhibited the responses of the tissue induced by both high and low frequency stimulation to about the same extent. Dexamphetamine (7.5×10^{-7} - 5.0×10^{-6}M) reversed the inhibitory effect of guanethidine and bretylium, on the motor responses of the vas deferens to electrical stimulation (Figure 33). However, the same doses of dexamphetamine did not modify the inhibitory effects of antimalarial compounds on this tissue (Figure 33). The inhibitory effects of the antimalarial drugs were also not altered by the action of atropine ($1 - 2.5 \times 10^{-6}$M), mepyramine ($1 - 2.5 \times 10^{-6}$M), hexamethonium ($1 - 2.5 \times 10^{-6}$M), methysergide ($1 - 2.5 \times 10^{-6}$M), propranolol ($1 - 2.5 \times 10^{-6}$M) or phentolamine ($1 - 2.5 \times 10^{-6}$M).

The local anaesthetics, cocaine (1.0×10^{-7} - 1.0×10^{-5}M) and lignocaine (7.5×10^{-7} - 7.5×10^{-5}M) dose-dependently augmented the contractions of the vas deferens evoked by exogenously added noradrenaline, phenylephrine and adrenaline at doses ranging between 5.0×10^{-6} to 2.5×10^{-4}M. A similar effect was seen with all the antimalarial drugs (7.5×10^{-7} - 1.0×10^{-4}M). Higher concentrations of all the antimalarial compounds (2.5×10^{-4} - 1.0×10^{-2}M) dose-dependently inhibited or abolished the catecholamine-induced contractions of the tissue. On molar basis, the order of potency of the antimalarials in producing augmentation and

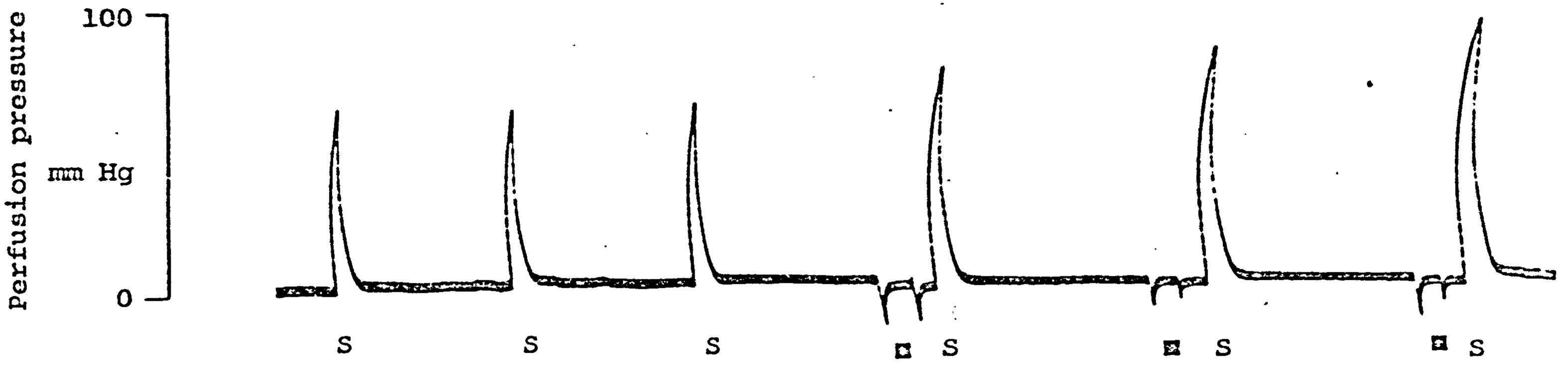


FIGURE 34

Rabbit isolated perfused central ear artery. Effect of primaquine (PM) on the contractions of the isolated perfused ear artery evoked by electrical stimulation (S, at 20 Hz). PQ1, PQ2, and PQ3 denote primaquine 1.0×10^{-5}M, 2.5×10^{-5}M and 5.0×10^{-5}M respectively.

inhibition was: primaquine ≫ chloroquine > quinine > proguanil ≫ pyrimethamine.

2.24 Rabbit isolated perfused ear artery

The isolated perfused central ear artery of the rabbit, unlike the portal vein, doses not show spontaneous myogenic activity or basal tone. In all preparations, the arterial tissue responded to extraluminal periarterial electrical nerve stimulation by producing a frequency-dependent vasoconstriction, indicated by a graded rise in perfusion pressure. Intraluminally added noradrenaline, phenylephrine (5.0×10^{-8} - 5.0×10^{-7}M), and also acetylcholine and nicotine (7.5×10^{-6} - 1.0×10^{-4}M), produced dose-dependent constriction of the arterial preparation.

By themselves, the antimalarial compounds did not elicit vasoconstriction, but rather reduced the perfusion pressure very slightly (ie. they induced a slight vasodilatation). However, low to medium concentrations of the quinoline antimalarials (5.0×10^{-7} - 5.0×10^{-5}M) enhanced the vasoconstrictor effects of periarterial sympathetic nerve stimulation (Figure 34) and intraluminally added noradrenaline or phenylephrine. The augmentation produced by the compounds in this tissue was much less than that produced in the vas deferens or portal vein. Higher concentrations of all five antimalarials (2.5×10^{-4} - 1.0×10^{-2}M) reduced or totally abolished the vasoconstrictor

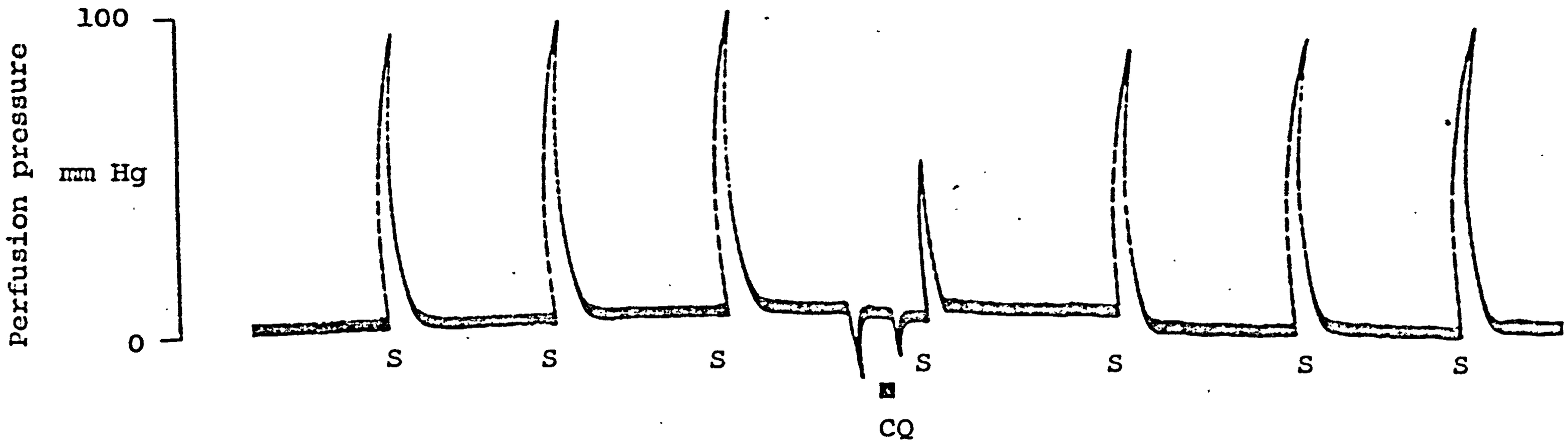
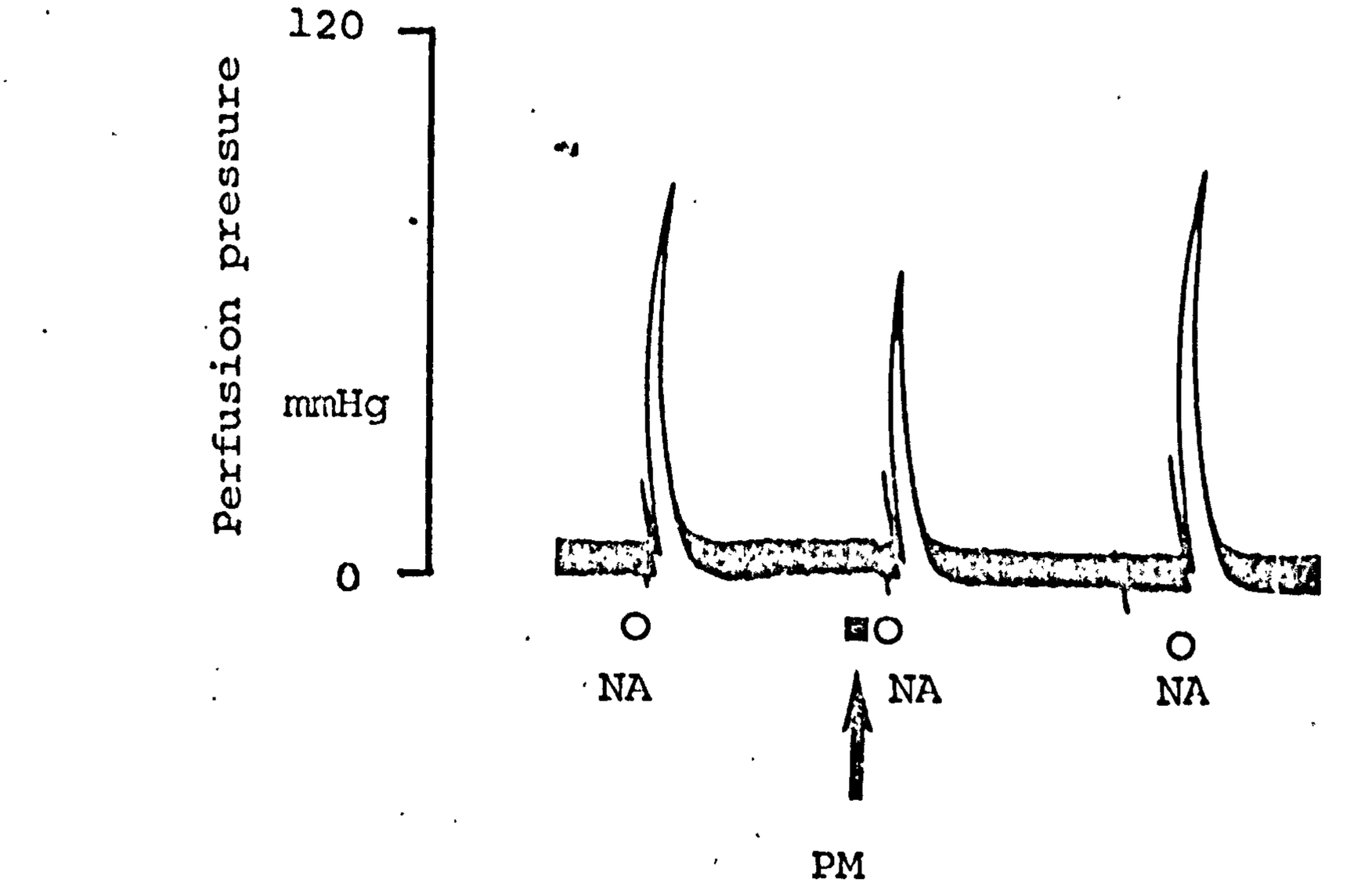


FIGURE 35

Rabbit isolated perfused central ear artery. Effect of chloroquine (CQ, 1.0×10^{-3}M) on electrically-induced rise in perfusion pressure (contraction) of rabbit isolated perfused ear artery.



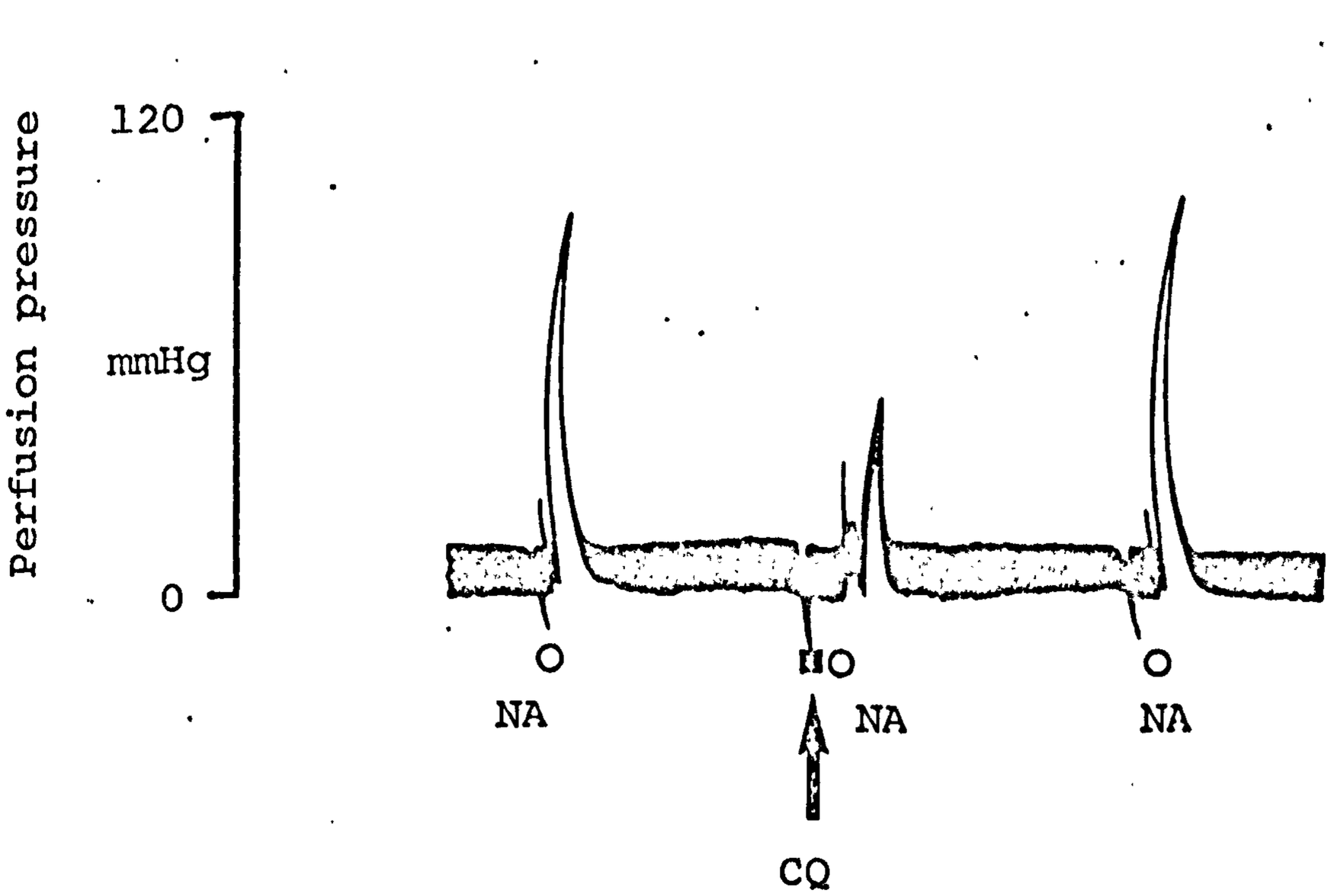


FIGURE 36

Rabbit isolated perfused central ear artery. Effects of pyrimethamine (PM, 7.5×10^{-4}M, upper trace) and chloroquine (CQ, 7.5×10^{-4}M, lower trace) on noradrenaline (NA, 0, 5.0×10^{-8}M)-induced contractions of rabbit isolated ear artery. The inhibitory effect of chloroquine on the rise in perfusion pressure was more marked than that of pyrimethamine.

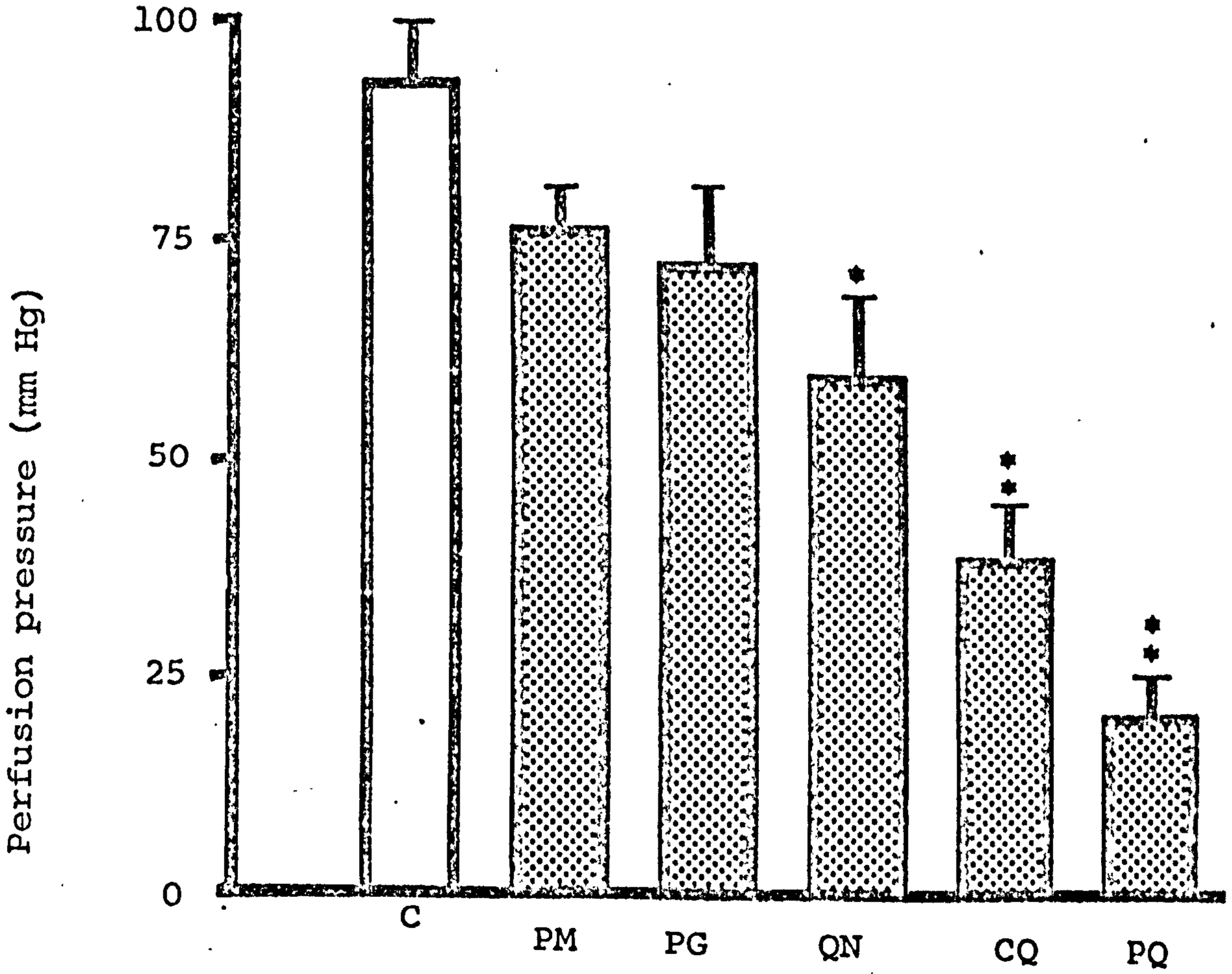


FIGURE 37

Rabbit isolated perfused central ear artery. Effects of pyrimethamine (PM, 1.0×10^{-3}M), proguanil (PG, 1.0×10^{-3}M), quinine (QN, 1.0×10^{-3}M), chloroquine (CQ, 1.0×10^{-3}M), and primaquine (PQ, 1.0×10^{-3}M) on electrically-induced changes in perfusion pressure in the isolated ear artery. The open square is the control (C) whilst close squares represent drug treatment. Vertical bars denote s.e. of means.

* means $P < 0.05$ and ** denotes $P < 0.001$

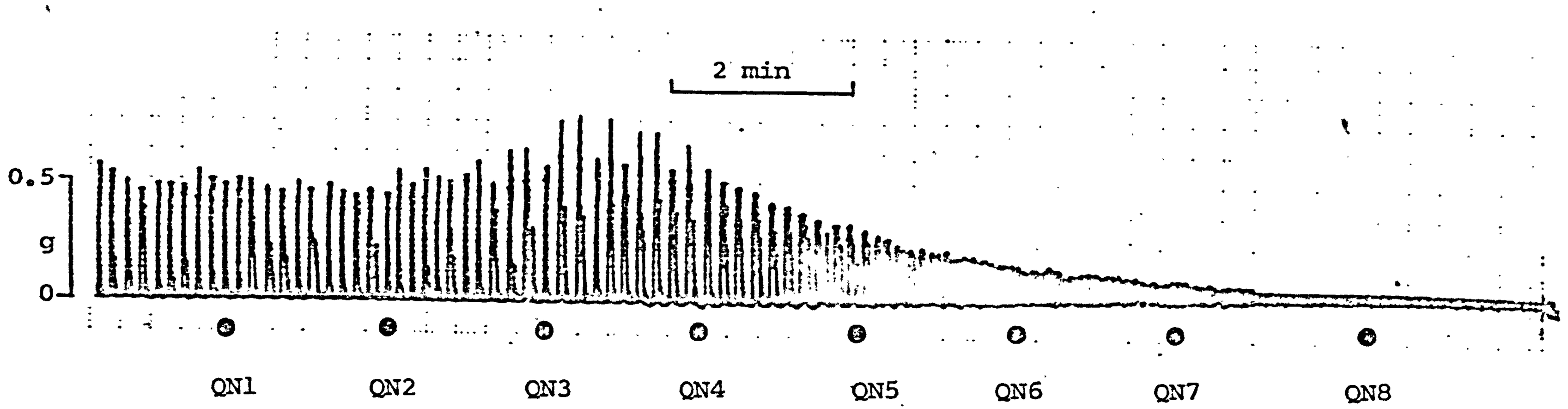


FIGURE 38

Rat isolated portal vein. Effects of quinine (QN) on spontaneous myogenic activity of an isolated portal vein. QN1, QN2, QN3, QN4, QN5, QN6, QN7 and QN8 represent quinine 1.0×10^{-5}M, 2.5×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 2.5×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M and 2.5×10^{-3}M respectively.

responses of the ear artery preparation to extraluminal periarterial nerve stimulation (eg. Figure 35) and to intraluminally added noradrenaline (Figure 36), phenylephrine, acetylcholine and nicotine. Figure 37 summarises the effect of 1.0×10^{-3}M of each antimalarial drug studied on the rise in perfusion pressure evoked by periarterial nerve stimulation at a frequency of 30 Hz.

2.25 Rat isolated portal vein

All the antimalarial drugs studied had biphasic effects on the spontaneous myogenic contractions of the portal vein. In all the preparations (seven for each compound), low to medium doses of each of the antimalarials (7.5×10^{-7} - 1.0×10^{-4}M) dose-dependently enhanced both the amplitude and frequency of the contractions; relatively higher concentrations (2.5×10^{-4} - 1.0×10^{-2}M) depressed (or abolished) both amplitude and frequency in a dose-related manner. Figures 38 and 39 show typical (stimulant and inhibitory) effects of quinine and pyrimethamine, and all the results are summarized in Figure 40. In most of the preparations treated with proguanil or pyrimethamine, the initial stimulatory phase (shown in Figure 38) was absent (Figure 41).

Adrenaline and noradrenaline (2.5×10^{-8} - 7.5×10^{-6}M) produced dose-dependent contractions of the isolated portal vein. Low to medium doses of the antimalarials

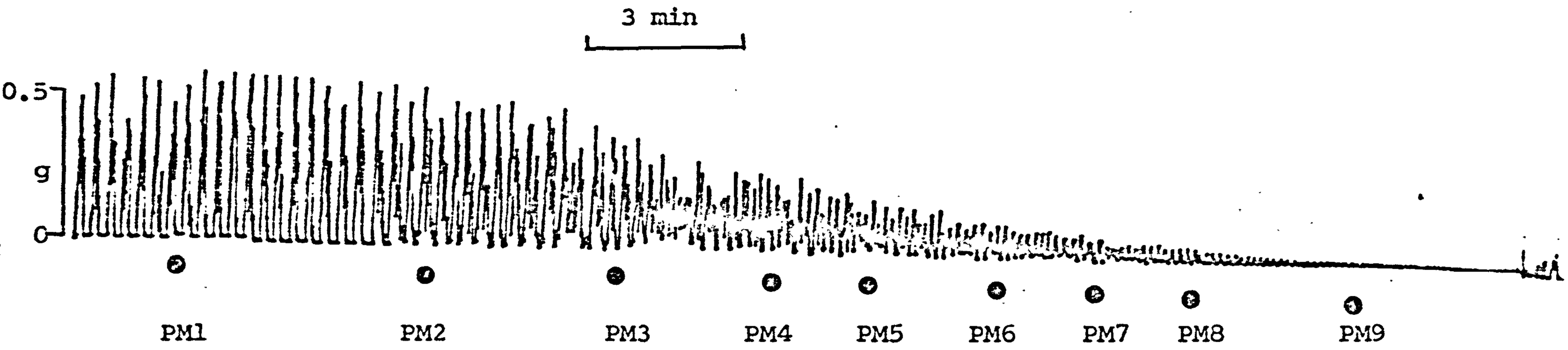


FIGURE 39

Rat isolated portal vein. Effects of pyrimethamine (PM) on spontaneous rhythmic contractions of an isolated portal vein. PM1, PM2, PM3, PM4, PM5, PM6, PM7, PM8 and PM9 denote pyrimethamine 2.5×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 2.5×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M, 2.5×10^{-3}M, 5.0×10^{-3}M and 1.0×10^{-2}M respectively.

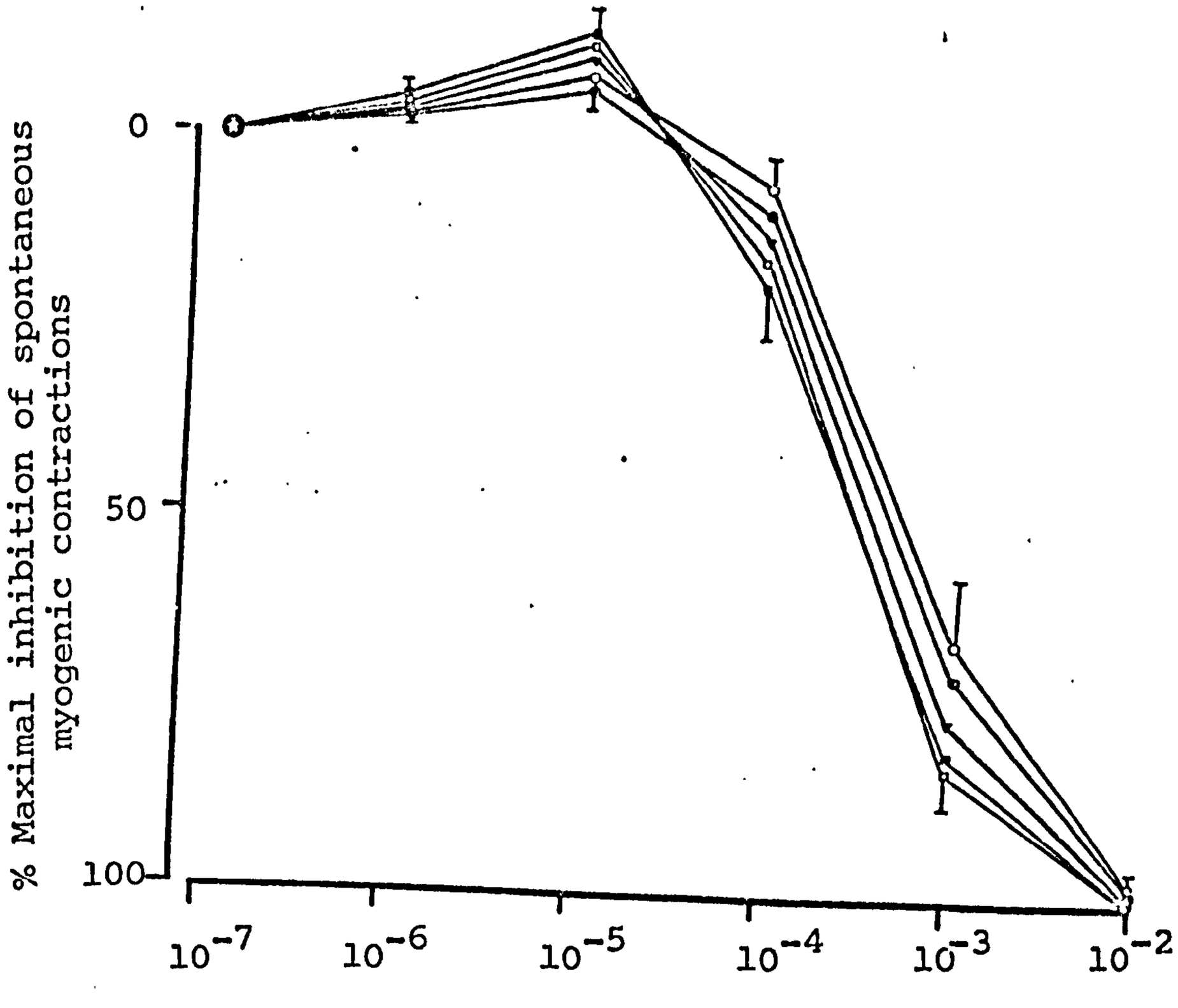


FIGURE 40

Rat isolated portal vein. Mean cumulative dose/response curves to primaquine (■), chloroquine (□), quinine (▼), proguanil (●) and pyrimethamine (O) on rat isolated portal veins. Each point is the mean of 5 - 8 observations, and the vertical bars denote s.e. of means.

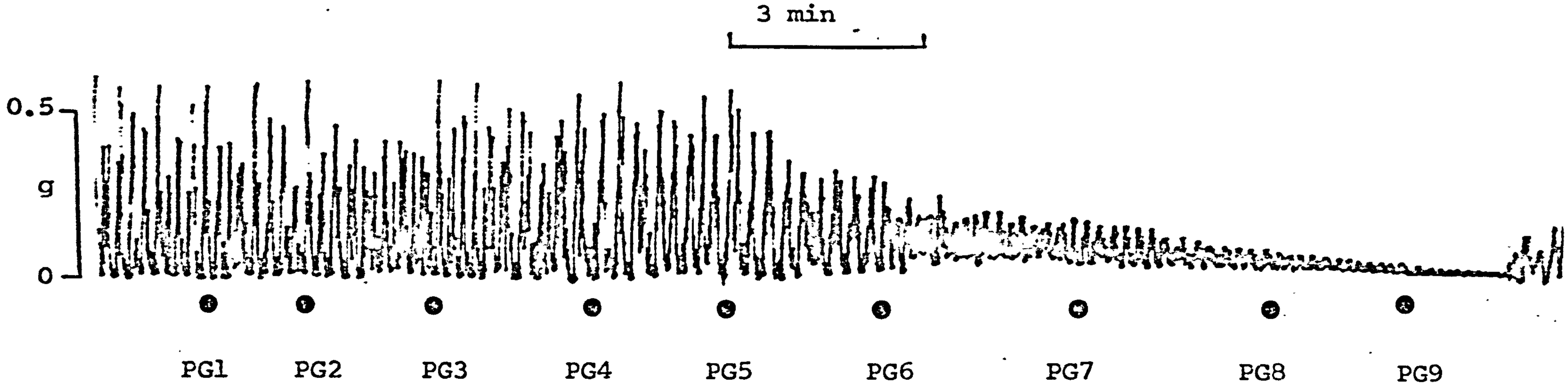


FIGURE 41

Rat isolated portal vein. Effects of proguanil (PG) on spontaneous myogenic activity of an isolated portal vein. PG1, PG2, PG3, PG4, PG5, PG6, PG7, PG8 and PG9 represent proguanil 1.0×10^{-5}M, 2.5×10^{-5}M, 5.0×10^{-5}M, 7.5×10^{-4}M, 1.0×10^{-4}M, 2.5×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M and 2.0×10^{-3}M respectively. The initial stimulant effect often seen with the quinoline compounds (eg. Figure 38) was absent in this preparation.

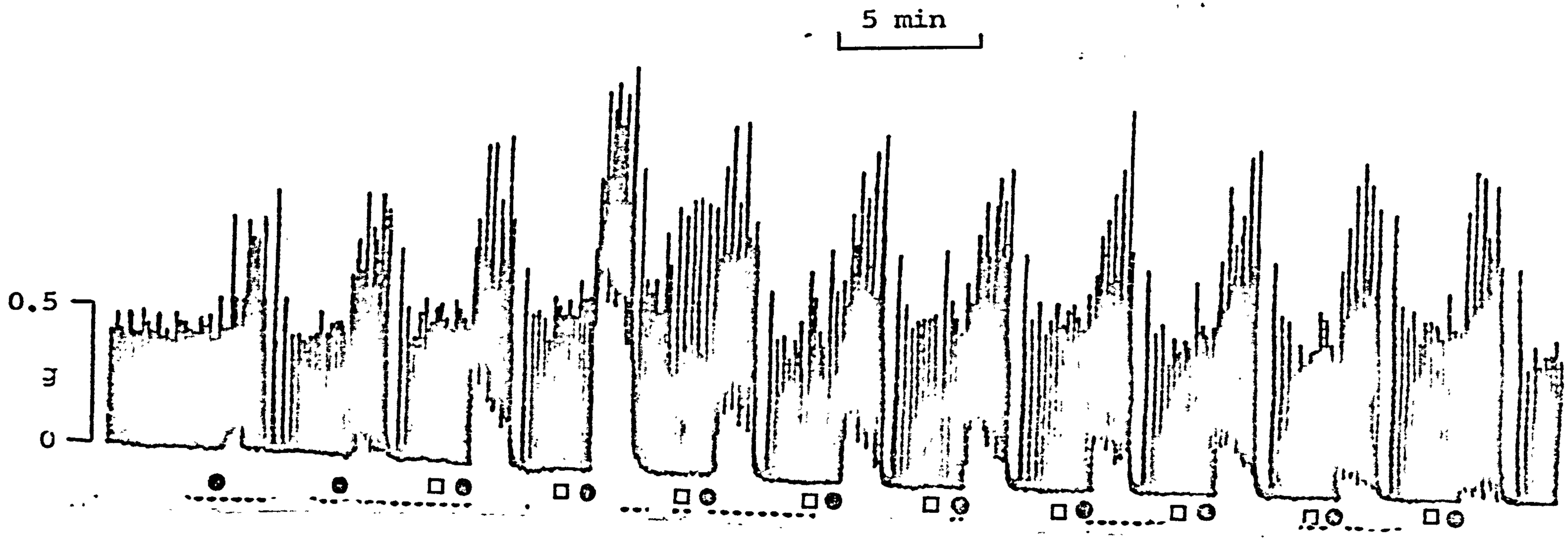


FIGURE 42

Rat isolated portal vein. Effects of various concentrations of chloroquine (CQ, □) on the responses of rat isolated portal vein to exogenously added noradrenaline (NA, ●) 2.5×10^{-8}M. CQ1, CQ2, CQ3, CQ4, CQ5, CQ6, CQ7, CQ8 and CQ9 denote chloroquine 2.5×10^{-5}M, 5.0×10^{-5}, 7.5×10^{-5}M, 1.0×10^{-4}M, 2.0×10^{-4}M, 3.0×10^{-4}M, 4.0×10^{-4}M, 5.0×10^{-4}M and 7.5×10^{-4}M respectively. There was an initial dose-dependent potentiation of noradrenaline effect followed by a gradual inhibition.

(7.5×10^{-7} - 5.0×10^{-5}M) augmented the contractile effects of adrenaline and noradrenaline (Figure 42) on this tissue while higher concentrations (5.0×10^{-4} - 1.0×10^{-2}M) inhibited the actions of the catecholamines.

DISCUSSION

The results reported in this section show that all five antimalarial drugs, despite their different chemical structures and different mechanisms of antimalarial action, have in common, at least superficially, a number of effects on smooth muscle preparations. On most of the preparations used, each drug exerted both inhibitory and excitatory effects depending on the concentration of the drug and the conditions of the experiment.

Relatively low to medium concentrations of the compounds reduced the resting tension of the guinea-pig isolated ileum, the chick oesophagus and the rabbit duodenum, inhibited pendular movements of the last named preparation, and depressed contractions of all three tissues produced by acetylcholine, histamine, nicotine, 5-hydroxytryptamine, potassium chloride or barium chloride. These observations are in general agreement with those of other workers (Vane, 1949; Agarwal and Deshmankar 1963; Deshpande et al., 1963; Olatunde 1970). It seems likely that the non-specific inhibitory effects of the drugs are a consequence of their membrane stabilising or local anaesthetic activity which has been described by others (Arora 1955; Jindal et al., 1958; Chinyanga et al., 1971, 1972).

Larger concentrations of the compounds produced contractions of the smooth muscle in all three isolated tissues. In the guinea-pig ileum, this effect was shown to be produced only

in innervated longitudinal muscle strips; denervation of the strip abolished the effect, although such preparations continued to respond to agonists such as acetylcholine and histamine. The fact that denervation abolished the contractor effect indicates that it is dependent on the nerves. The antimalarials possess weak anticholinesterase activity as shown by Wright and Sabine (1948), Garcia et al., (1968) and Ayitey-Smith and Boye (1975), and confirmed in the present study. It is possible that the concentrations necessary to produce contractions of the smooth muscle were sufficient to produce some enzyme inhibition. However, it is unlikely that the contractions were produced by accumulating endogenous acetylcholine, since they were unaffected by atropine in concentrations greater than those necessary to block responses to exogenous acetylcholine. The contractions were also unaffected by hexamethonium, mepyramine or methysergide. Thus, although an intact innervation was necessary, the contractions were apparently not evoked by the release of the common endogenous mediators or autocoids (acetylcholine, histamine and 5-hydroxytryptamine), nor were the membrane receptors for these agonists involved in the response. The possibility must therefore be considered that the antimalarials are capable of releasing some other endogenous smooth-muscle contracting substance, and that intact neurones, if not the actual site of storage, are at any rate involved in the release mechanism. Possibilities include ATP, bradykinin or prostaglandins of the F series, but further work is necessary to test these possibilities.

ATP has been shown to produce contraction of the guinea-pig ileum (see Burnstock 1972 for review), and it is possible that it is released in this tissue from what Burnstock has called 'purinergic nerves'. However, ATP inhibits the rabbit duodenum (Burnstock 1972) and therefore cannot be responsible for the contractions in this tissue.

Although high concentrations of the antimalarials were required to produce contractions of the smooth muscles, relatively small concentrations were capable of augmenting contractions of the chick oesophagus stimulated through its cholinergic parasympathetic nerves. It is possible that this effect was a consequence of an anticholinesterase activity. However, this is unlikely since other anticholinesterases, such as physostigmine and neostigmine, augment the evoked contractions of this preparation only in concentrations that also increase the resting tension (Everett 1965). There is evidence that the nerves to the chick oesophagus can, under appropriate conditions, release an unknown excitatory transmitter in addition to acetylcholine (Hassan 1969), and it may be that the antimalarials facilitate the effect of nerve impulses in releasing this transmitter.

Larger concentrations of the antimalarials depressed the responses of the chick oesophagus to nerve stimulation. This effect can probably be attributed to the local anaesthetic action impairing nervous conduction. The same action may have been responsible for abolishing the

inhibitory response to adrenergic stimulation in the Finkleman preparation of the rabbit intestine. Other workers have reported a similar inhibitory effect on adrenergically innervated tissues and have attributed it to an adrenergic neurone blocking action (Jindal 1970; Achari, Banerji and Kapoor 1972). However, the present results showed that the effect was not reversed by dexamphetamine, indicating that the adrenergic neurone blockade produced differs from the specific type produced by drugs such as guanethidine.

All five antimalarial drugs inhibited contractions of guinea-pig isolated tracheal chains produced by acetylcholine, 5-hydroxytryptamine or histamine. This effect too can probably be attributed to the membrane stabilising actions of the compounds.

Relatively low to medium concentrations of quinine, proguanil, chloroquine, and, to a lesser extent, pyrimethamine produced contractions of isolated oestrogen-dominated uterine strips and often gave rise to persistent rhythmic oscillations of tension. These findings are in agreement with the reports of Chen and Anderson (1947) and Joseph and Jindal (1957), but at variance with those of Jindal and Joseph (1957) and Abdel-Aziz et al., (1971) which claim that proguanil and chloroguanil (two drugs which have a common metabolite) possess only inhibitory effects in the uterus.

The mechanism of action of the stimulant action of antimalarials on uterine strips taken from oestrogen-primed animals is not fully known. It resembled that on the other smooth muscles studied in that it was not blocked by atropine, methysergide, mepyramine or propranolol. All of the antimalarial drugs antagonized contractions of the oestrogen-dominated uterine strips induced by oxytocin, ergometrine, acetylcholine, 5-hydroxytryptamine, bradykinin, potassium chloride or barium chloride. Similar observations have been reported by Joseph and Jindal (1957), Garcia et al., (1968), Kurantsin-Mills and Chinyanga (1972). This antagonistic action is probably a consequence of the non-specific membrane stabilising action described above for other smooth muscle.

All the antimalarial agents studied always produced dose-related inhibitory effects on isolated uterine strips taken from pregnant rats and guinea-pigs. This finding is in agreement with the work of Goldsmith (1946), Morwin and Winkelmann (1962), Dziubinski et al., (1962), Stone (1962), Klumpp (1965) and Abdel-Aziz et al., (1971). The effect is probably attributable to the local anaesthetic activity of the compounds. No basis for the abortifacient action of the antimalarial drugs was revealed in the present study. It is possible that this involves release of prostaglandins and/or some other mediators. The failure of indomethacin (an inhibitor of prostaglandin synthesis) to antagonise the stimulant effects of the compounds on the isolated uterine

preparations used does not preclude a possible involvement of prostaglandins in the 'oxytocic' action of the compounds.

The results obtained from the 'two-chambered organ bath experiments' with the vas deferens indicate that all the five antimalarial drugs examined possess local anaesthetic activity. Like the standard local anaesthetics, cocaine and lignocaine, low to medium concentrations of all the antimalarial drugs augmented the contractions of the vasa deferentia evoked by indirect electrical stimulation and by exogenous noradrenaline, adrenaline or phenylephrine; whilst high doses of the compounds depressed them. The observation that the stimulant effects induced by low to medium concentrations of the aminoquinoline compounds on electrically-evoked contractions of the tissue were markedly reduced by reserpinization of the animals suggests that the amino quinoline group of antimalarials release catecholamines from their tissue stores. The results obtained on other mainly adrenergically-innervated muscle preparations (eg. the isolated central ear artery of the rabbit, rat portal vein and cardiac muscle preparations) strenghten this view. Nevertheless, the inhibitory effects of all the compounds on the electrically-induced contractions of this muscle preparation were not reversed by dexamphetamine. This result further strengthens the suggestion made earlier that the antimalarial compounds are not likely to be adrenergic neurone blocking agents like guanethidine and bretylium (whose inhibitory effects on the vas were reversed by dexamphetamine).

The effects of the antimalarial compounds on the blood vessels studied were biphasic and quantitatively similar to those produced on the vas deferens. Relatively low to medium doses of the agents augmented the electrically-induced, or exogenous catecholamine evoked contractions of the isolated perfused central ear artery of the rabbit, and the spontaneous myogenic contractions of the rat portal vein; whilst high concentrations of the agents inhibited these contractions. As in the vas deferens, the excitatory and inhibitory actions of the compounds on the vascular tissues are thought to be mediated by the membrane stabilising activity of the agents. On their own accord, the antimalarial compounds dilated the ear artery preparations. This vasodilator action probably suggests a direct relaxant effect (possibly similar to that observed on other smooth muscles studied) either mediated through the vasodilator innervation (Hughes and Vane 1967; Kalsner 1974), or more likely coupled with their local anaesthetic activity. As in the other smooth muscles used, the lack of sensitivity of the compounds to any of the standard antagonists employed in the vascular preparations (arterial and venous) also probably lends further support to the earlier suggestion that the antimalarial drugs probably do not exert their actions through specific receptors. The results obtained in Sections 3 and 4 of this thesis (see later) strengthen the observations discussed in the present section.

SECTION 3

SKELETAL MUSCLE PHARMACOLOGY OF THE ANTIMALARIAL DRUGS

INTRODUCTION

3.1 Actions of quinine and quinidine on skeletal muscle

Several investigators have reported the actions of quinine and its dextro-rotatory isomer, quinidine, on skeletal muscles. Santesson (1892) found that the response of frog skeletal muscle to a single stimulus was increased after an injection of small doses of a quinine salt. Because he also obtained this 'quinine action' in curarized frogs, he suggested that the action was not on the motor nerves but on the skeletal muscle fibre itself. With tetanizing stimuli, he further found that the heights of contractions were always below normal after the administration of quinine, and that the muscle fatigued much earlier and soon passed into rigor. He concluded that quinine possessed a local action on skeletal muscle, causing fatigue with depression of contractility and irritability. Furth and Schwartz (1909) observed that after curarization, small doses of quinine increased the responses of cat skeletal muscle to electrical stimulation with single shocks. In 1915, Secher confirmed that quinine and related cinchona alkaloids depress tetanic contraction and cause early fatigue. Smith and Fantus (1916) working with frogs also reported that the tetanic response to nerve faradization was changed to a single twitch response after quinine, although the muscle still responded fully to direct stimulation. Piccinini (1920, 1922) reported that small doses of quinine increased the muscular response of the

frog to electrical stimulation. In 1923, Brody and Sollmann studied the effects of quinidine and other cinchona alkaloids on some skeletal muscle preparations and observed that quinine and quinidine had strikingly similar effects. They concluded that quinine and quinidine caused a depression of the response of frog muscle, this being manifested by lowered contraction, more rapid fatigue and prolongation of refractory period. Weiss (1926) found that quinine and quinidine prevented the fibrillation of skeletal muscle after physostigmine in the dog, and in 1930, Buchbinder showed that quinidine had an inhibitory influence on the fibrillation of the dog's tongue following hypoglossotomy. After tests on human subjects (soldiers) Vondracek (1932) suggested that quinine increased the muscle work not so much through a direct muscle action but through an action on the central nervous system. Wolf (1936) made the observation that myotonic dystrophy could be symptomatically relieved by quinine in human beings. Kennedy and Wolf (1937) reported four cases of myotonic atrophy favourably influenced by quinine, and pointed out that quinine increased the muscular weakness in cases of myasthenia gravis. These workers further showed, that neostigmine had an unfavourable effect upon myotonia. Smith (1937) reported three other cases of congenital myotonia and confirmed the value of quinine in the treatment of this disease. Kolb, Harvey and Whitehill (1938) studied eight cases of myotonic atrophy and one case of congenital myotonia in patients undergoing quinine

sulphate treatment and found that quinine abolished the spasticity but had no effect on muscular strength. The investigators further demonstrated that quinine counteracted the effect of neostigmine in two cases of myasthenia gravis and increased the symptoms and signs of the disease. This team of workers also showed that neostigmine increased spasticity in myotonic conditions and directly antagonized the full therapeutic effect of quinine sulphate.

Later experimental studies have lent more weight to Santesson's earlier work and shed more light on the pharmacology of quinine and quinidine on skeletal muscles. For example, Harvey (1939) studied the actions of quinine on mammalian, avian and amphibian skeletal muscles and found that it has a number of actions on the skeletal muscle itself, on the motor end-plate, and on the responses of the muscle to various other drugs. He found that quinine caused an increase in the tension response to a single maximal stimulus in normal, curarized and denervated muscles. This mechanical potentiation was accompanied by an increase in the amplitude and duration of the muscle action potential. The ability of the muscle to respond to a tetanus was diminished on account of an increase in refractory period. The excitability at the motor end-plate was lowered by quinine, and the ability of a tetanus to facilitate conduction at the end-plate was reduced or abolished. Harvey also found that physostigmine and veratrine were no longer able to evoke a repetitive

response to a single stimulus in a muscle which had been treated with quinine; and he went on to show that the response of the normal mammalian muscle to injected acetylcholine was abolished by quinine. He attributed the latter phenomenon to a curariform action of the drug since the otherwise very similar response to an injection of potassium was only very slightly affected. Oester and Maaske (1939) showed that quinine potentiated skeletal muscle twitches in low doses, depressed muscle tetanus in larger doses, and prolonged the least effective interval for the summation of two successive stimuli when administered to dogs. They also found that quinine blocked potentiation by physostigmine, and also blocked the post-physostigmine acetylcholine muscle effects. It produced additional depression of muscle twitches in a partially curarized animal, blocked the acetylcholine decurarization and potentiated denervated muscle twitch though it blocked the effects of acetylcholine on denervated muscle. Quinine has a strong curare-like action in cats, blocking transmission at the neuromuscular junction and at the superior cervical ganglion without interfering with the normal liberation of acetylcholine from the nerve endings (Harvey 1940). Ravin (1940) also found, in the same species, that in small doses, quinine increased the response of skeletal muscle to slow rates of direct or indirect stimulation, while depressing the response to high rates. With increasing doses of quinine, the rate of stimulation required to show depression decreased and finally,

depression was evident at all rates of stimulation. Even larger doses of quinine were required to produce depression in directly stimulated muscle. Quinine antagonised the action of physostigmine on skeletal muscles as effectively as, curare. Physostigmine was, however, not as effective in antagonising the actions of quinine as it was in antagonising the actions of curare. Ravin confirmed that quinine inhibited the fibrillation of denervated muscle. In isolated phrenic nerve-diaphragm preparations, low doses of quinidine have been shown initially to increase the effect of both nerve and muscle stimulation; in high doses quinidine depressed the twitches (Stephenson 1948; Dutta 1949). Rummel and Schulz (1954) found that after quinidine administration, the response to direct stimulation was almost entirely abolished while shocks administered through the nerve were still effective. Lammers and Ritchie (1955) demonstrated that quinine increased the peak twitch tension and contraction time of the cat soleus and tibialis anterior muscles and of the frog sartorius muscle. Quinine also decreased the fusion frequency of cat soleus and tibialis anterior muscles. These workers showed that quinine had no effect on the maximum tetanic tension of the cat soleus and tibialis anterior muscles, but increased the peak tension developed during an unfused tetanus. They found that quinine decreased the maintained tetanic tension of these muscles evoked by high frequencies of stimulation, but increased tension when evoked by low frequencies of stimulation.

Quinine increased the duration of the active state of frog muscle and Lammers and Ritchie assumed that this change accounted for the observed increase in twitch tension and contraction time. Tubocurarine and physostigmine neither affected the active state of frog muscle nor affected the action of quinine on the active state. Quinidine had similar effects as quinine on muscular contraction. The prolongation of the duration of the active state thus appears to be the cause of the various effects of quinine and quinidine on cat skeletal muscles.

There is well-documented evidence that indicates curare-like actions of cinchona alkaloids (for references, see Goodman and Gilman, 1975). Although quinine has long been known to possess neuromuscular blocking properties (Harvey 1940; Oester and Maaske 1939; and Matthes 1939) the actual mechanism of action is obscure. The most favoured mechanism claims that the drug probably increases the threshold of excitability of the motor end-plate through a curare-like action. Recently however, quinidine has been reported to interact with muscle relaxants to produce prolonged neuromuscular blockade. Schmidt, Vick and Sadove (1962, and 1963), noted that the administration of quinidine to a patient just after emergence from anaesthesia, in which dimethyl tubocurarine had been used as an adjuvant to anaesthesia caused "recurarization" of the muscles and signs of respiratory paralysis to reappear. Those workers found that quinidine had no effect

on the amount of muscle relaxant required to produce a flaccid paralysis of the neck muscles ('head drop test') in rabbits, but when quinidine was given <u>after</u> recovery from tubocurarine, decamethonium, or suxamethonium, paralysis re-occured. Grogono (1963) also reported the return of respiratory paralysis in two patients when quinidine was injected intravenously during recovery from suxamethonium, and the initial recurarization was also observed. Boere (1964); Katzung and Way (1966); Cuthbert (1966); Way, Katzung and Larson (1967); Miller, Way and Katzung (1967) and Usubiaga (1968) have also demonstrated both the recurarizing and potentiating actions of quinidine on neuromuscular blocking agents.

Isaacson, Yamaji, and Sandow (1970) found that raising the pH of the experimental medium (from 6.2 to 8.2) enhanced the capability of quinine (0.5 - 5 mM) to cause contracture in frog sartorius muscles, and to increase the rate of release of ^{45}Ca during the efflux of the ^{45}Ca slow component of these muscles, and they inferred that the observed increase in ^{45}Ca efflux represented release from the sarcoplasmic reticulum and that the contracture resulted from this release. Suarez-Kurtz and Paumgartten (1973) demonstrated that procaine and tetracaine reversibly block contractures and the attendant increase in ^{45}Ca efflux induced by quinine (0.15 - 20 mM) in the sartorius muscle of the frog. They further observed that blockade of the contractures was obtained in normally polarized and in

depolarized muscles whether the local anaesthetics were applied prior to, or, after the elicitation of the contracture. These authors concluded that procaine and tetracaine act as competitive inhibitors of quinine in frog sartorius muscle.

Several workers have reported the alkaloids quinidine and caffeine to have rather similar effects on tension development. In particular, low concentrations of these drugs have been reported to cause potentiation of twitch tension whereas higher concentrations cause contractures (Axelsson and Thesleff 1958; Sandow and Brust 1966; Isaacson and Sandow 1967; and Luttgau 1970). It is generally accepted that in skeletal muscle, contraction is coupled to excitation through the release of Ca^{++} from the sarcoplasmic reticulum (Hasselbach 1964; Weber 1966; Sandow 1965; Ebashi and Endo 1968). There is a growing body of evidence suggesting that agents that modify the contractile states of muscle, and that have no relevant effects on the cell membrane, do so through their ability to alter the calcium binding properties of intracellular stores, in particular those of the sarcoplasmic reticulum (Sandow 1965; Ebashi and Endo 1968; Bianchi 1961; Isaacson and Sandow 1967; Weber and Hertz 1968; Isaacson, Yamaji and Sandow 1970). According to Sandow (1965), Isaacson and Sandow (1967), and Isaacson et al. (1970), both quinidine and caffeine increase the flux of ^{45}Ca in living frog muscles in association with their effects on contraction. Both

those drugs can induce contracture of frog skeletal muscle in the absence of extracellular calcium (Andersson 1972; and Frank 1962). It has been suggested that drugs that potentiate twitches and also induce contractures act directly on the sarcoplasmic reticulum to cause a release of calcium or cause an inhibition of uptake, thereby increasing the free myoplasmic calcium concentration. In support of this hypothesis, reports showing that caffeine (Weber and Herz 1968; and Ogawa 1970) and quinidine (Carvalho 1968; Fuchs, Gertz and Briggs 1968; and Balzer 1972) can inhibit the binding of calcium by the isolated sarcoplasmic reticulum have recently appeared in the literature. In order to ascertain a plausible mechanism for some of the actions of quinine, quinidine and caffeine on skeletal muscles, Batra (1974) studied the effects of quinidine and caffeine on calcium uptake and release by mitochondria and fragmented sarcoplasmic reticulum of frog muscle. He found that quinidine (1 - 2 mM) released considerable calcium from preloaded mitochondria but had little effect on preloaded fragmented sarcoplasmic reticulum. Calcium uptake by both mitochondria and sarcoplasmic reticulum was inhibited by higher concentrations (2 or 1 mM) of quinidine but the inhibition of mitochondrial calcium uptake was much greater. With lower concentrations of quinidine (0.4 mM), there was no significant effect on calcium uptake by fragmented sarcoplasmic reticulum, but a 48 per cent inhibition of mitochondrial calcium uptake. Caffeine (2 - 10 mM) inhibited calcium uptake both by

mitochondria and fragmented sarcoplasmic reticulum, and here again, inhibition of uptake by mitochondria was greater than by fragmented sarcoplasmic reticulum. In contrast to quinidine, caffeine (10 mM) released calcium from sarcoplasmic reticulum and not from mitochondria. This calcium releasing effect of both caffeine and quinidine increased when the ratio of the drug and fragmented sarcoplasmic reticulum protein increased. The calcium releasing concentrations of these drugs were comparable to those reported to elicit contractures of living muscle and the lower concentrations, which inhibited calcium uptake, were comparable to those which potentiated twitches.

3.2 Actions of synthetic antimalarials on skeletal muscle

The availability of new synthetic antimalarial agents, from about the middle of this century, led to the study of their pharmacological actions on various organ systems of experimental animals and man. Although the compounds differ widely in their chemical structures, some of them mimic quinine not only in its action as an antimalarial but also in most other pharmacological properties (for instance, antifibrillatory (Burn and Vane 1949; Arora, Sharma and Madan 1955), antiveratrinic (Arora 1955), and antihexokinase agent (Armitage 1957)).

Until fairly recently however, not much attention was paid to the neuromuscular actions of the synthetic antimalarials.

Nevertheless, on the isolated rectus abdominis muscle of the frog, Vane (1949) found that proguanil had a biphasic action on contractions of the muscle evoked by acetylcholine; in small doses it augmented the contractions and in higher concentrations it depressed them. Larger doses of proguanil caused the muscle to contract in a dose-related manner.

Vane also found that the twitch evoked by single maximal nerve volleys in the cat sciatic-gastrocnemius preparation was depressed by arterial injections of proguanil; this curariform action of the drug was also observed on the isolated phrenic nerve-diaphragm preparation of the rat. Dallemagne and Philippot (1955) also found that intravenous injections of proguanil inhibited neuromuscular transmission in the cat. The inhibitions were found to be more marked in the soleus than in the tibialis anterior muscle. Similar effects were observed in the dog and rat. Recovery from the block was delayed by decamethonium and by physostigmine. Where decamethonium administration preceded proguanil, they found that proguanil first increased the block but thereafter, there was a rapid restoration of transmission. If the block was first developed under curare, proguanil intensified it and there was no such rapid recovery of transmission. A recurarization effect of proguanil was also observed where transmission had just recovered from block by either decamethonium or tubocurarine.

Grewal and Sharma (1960) showed that quinine, quinidine, mepacrine and chloroquine produced the same type of depression of twitch tension, tetanus and the acetylcholine response of the skeletal muscle. They found that quinine was the most potent on the frog rectus abdominis muscle, quinidine on the frog sciatic nerve-gastrocnemius preparation, and chloroquine on the sciatic nerve-gastrocnemius preparation of the dog. These workers concluded that chloroquine and mepacrine resembled quinine and quinidine in their actions on skeletal muscle and suggested that chloroquine and mepacrine might prove superior to quinine in the treatment of congenital myotonia. Wislicki (1960) injected quinidine intra-arterially into cats and found that the drug transiently blocked neuromuscular transmission and reduced gradually (and for more prolonged periods of time) peak twitch tension. At low rates of stimulation the reduction was preceded by a slight increase in twitch height. Wislicki concluded that the effects of quinine on striated muscle were more cumulative as additional injections of the same dose depressed to a higher degree both neuromuscular transmission and contraction after direct stimulation.

Jui-Yen (1971) studied the action of chloroquine both clinically and experimentally on striated muscles and found that chloroquine had a direct blocking action on the neuromuscular junction. This 'chloroquine effect' was antagonized by neostigmine but not by calcium. He there-

fore postulated that chloroquine had a non-depolarising blocking action at the neuromuscular junction and further showed using microelectrode techniques, that the action potential evoked by direct stimulation disappeared within three minutes of chloroquine administration. The blocking action of chloroquine appeared to take place neither in the nerve nor muscle fibre but at the neuromuscular junction. Chinyanga, Greenberger and Vartanian (1971); Chinyanga, Vartanian, Okai and Greenberger (1972), Vartanian and Chinyanga (1972) also studied the action of chloroquine on the neuromuscular junctions of frogs and cats and found that chloroquine depressed and finally blocked neuromuscular transmission in doses as low as those used in clinical practice. Studies on end-plate and action potentials in muscle fibres and in single nerve fibres indicated that the chloroquine-induced depression of neuromuscular transmission resulted from depression of the excitability of the electrically excitable membranes of the axon and muscle fibre. This effect led to a decrease in the height of the nerve action potential and a reduction of transmitter output at the end-plate. The mechanism of action of chloroquine was thus similar to that of local anaesthetics. Ayitey-Smith and Vartanian (1975) studied the effect of chloroquine on frog skeletal muscle and found that it had a dual action on the muscle. Relatively low doses of the drug inhibited cholinesterase, potentiated acetylcholine-induced contractions of the muscle and antagonised carbachol and caffeine contractions, as well as acetylcholine-induced

contractions of oserinized muscle. High concentrations of the drug antagonized, in a non-competitive manner, contractions evoked by acetylcholine, carbachol, caffeine and potassium. The blocking action of chloroquine thus, appeared to be due to its local anaesthetic property and/or with interference with intra-cellular calcium movements.

3.3 Aims of this study

The present study was carried out in order to systematically study the effects of some newer synthetic antimalarial compounds on amphibian, avian and mammalian skeletal muscle preparations, to compare their actions with those of quinine and quinidine and to examine possible mechanisms of action of these drugs.

METHODS

A: 'IN VITRO' EXPERIMENTS

3.4 Frog isolated rectus abdominis muscle

Frogs (Rana temporaria) of both sexes, weighing between 35 and 80 g. were stunned and decapitated, and the spinal cord destroyed. The rectus abdominis muscles were isolated, divided at the mid-line, and the pair suspended separately in 10 ml organ baths containing frog ringer solution (composition g/l: NaCl 6.5; KCl 0.14; $CaCl_2$ 0.12; $NaHCO_3$ 0.20; NaH_2PO_4 0.01; and glucose 2.0), maintained at room temperature and continuously aerated with a mixture of 5% carbon dioxide in oxygen. The muscles were subjected to a resting tension of 1 g. and were allowed to equilibrate for a period of 45 - 60 minutes before they were exposed to drugs. Doses of the drugs were administered sequentially (non-cumulatively); contractures of the tissue evoked by drug additions were recorded isometrically by means of Devices force-displacement transducers (type UF1), pre-amplifiers (model M2P) and a two-channel pen recorder (model M2R). After washing out each drug by three additions of solution, relaxation of the tissue was aided by gently pulling on the thread which attached the tissue to the transducer. Doses were repeated at regular intervals of 10 or 15 minutes.

3.5 Chick isolated biventer-cervicis nerve-muscle preparation

Young chicks (aged between 3 and 15 days after hatching) were killed by deep ether anaesthesia. Paired biventer cervicis muscles were isolated according to the method of Ginsborg and Warriner (1960). The preparations were separately set up in 10 ml organ baths containing Krebs-Henseleit solution (composition g/l: NaCl 6.92; KCl 0.34; NaH_2PO_4 0.15; $NaHCO_3$ 2.1; $MgCl_2$ 0.11; $CaCl_2$ 0.26; and glucose 1.0), maintained at 32°C and continuously gassed with a mixture of 5% carbon dioxide in oxygen. The muscles were electrically stimulated indirectly, or directly, with bipolar platinum ring electrodes (similar to those described by Burn and Rand; 1960). The preparations were subjected to a resting tension of 1.0 g and allowed to equilibrate until the force of contraction was stable (usually 30 - 60 minutes). Indirect stimulation was effected by placing the electrode around the muscle tendon and stimulating with rectangular wave pulses of 0.1 - 0.2 msec duration at a frequency of 0.1 Hz and supramaximal voltage of 5 - 10 volts delivered from an SRI stimulator. Direct stimulation was applied with the same electrode positioned on the muscle using square wave pulses of 0.1 msec duration at a frequency of 0.1 Hz and at supramaximal voltage sufficient to elicit contractions equal in amplitude to the indirectly-evoked maximal twitches. Antimalarial drugs were added cumulatively to both directly and indirectly stimulated muscle preparations. In some experiments, the muscles were fully or partially

curarized (by administering d-tubocurarine at a dose level of 2.5 - 8.0 x 10^{-6}M). "Acetylcholine was applied between electrical stimulation in some cases. Some preparations were not electrically stimulated, and those were used to investigate the direct effects of the drugs under study. Both the electrically-induced and drug-evoked contractions and contractures of the muscle were recorded isometrically with Devices force-displacement transducers (type UF1), pre-amplifiers (model M2P) and a two-channel pen recorder (model M2R). Doses were repeated where possible at regular intervals of 10 or 15 minutes after 2 or 3 washings.

3.6 Rat isolated phrenic nerve-hemidiaphragm muscle preparation

Adult albino rats of either sex weighing between 300 and 800 g were killed by a blow on the head and bled out. The phrenic nerve-hemidiaphragm muscle preparations were carefully isolated according to the method of Bulbring (1946) and were set up in 100 ml organ baths containing Krebs-Henseleit solution, maintained at a temperature of 37°C and continuously aerated with a mixture of 5% carbon-dioxide in oxygen. The muscles were electrically stimulated either indirectly, or directly, using platinum wire perspex electrodes. Each preparation was allowed to equilibrate until the force of contraction was stable (usually 30 - 45 minutes after setting up) under an applied tension of 1 g. Indirect stimulation was effected via the phrenic nerve using rectangular wave pulses of 0.2 msec duration, at a

frequency of 0.1 Hz and a supramaximal voltage delivered by a SRI stimulator. Direct stimulation was carried out between the two points on the perspex platinum wire electrode to which the diaphragm muscle was secured using rectangular wave pulses of 0.1 msec duration at a frequency of 0.1 Hz and supramaximal voltages. Antimalarial drugs were added cumulatively. Some of the preparations were either partially or fully curarized (muscles treated with 2.5×10^{-6} - 1.0×10^{-5}M d-tubocurarine). Single doses of antimalarial drugs were administered to curare-treated muscle preparations. Doses of drugs were repeated where possible at regular intervals of 10 or 15 minutes and the muscle washed three times after each drug addition. Electrically-induced contractions of the muscle (twitches) were recorded isometrically by means of Devices force-displacement transducers (type UF1), pre-amplifiers (model M2P) and a two-channel pen recorder (model M2R).

3.7 Determination of anticholinesterase activity

The anticholinesterase activities of chloroquine, primaquine, quinine, proguanil and pyrimethamine were determined and compared with that of physostigmine by measuring the cholinesterase activity of chick biventer cervicis muscle homogenates in the presence of the drugs, using the colorimetric method of Ellman, Courtney, Andres and Featherstone (1961).

Chicks aged between 3 and 10 days were killed by deep ether inhalation. Their biventer cervicis muscles were removed and homogenized with an Ultra Turrax homogenizer (type TP18/2) for 1 minute, using 20 mg of tissue per millilitre of 0.1 M phosphate buffer of pH 8.0. The homogenate was filtered through fine gauze into a flask immersed in ice; 0.5 ml of the homogenate was added to 5.1 ml of phosphate buffer, pH 8.0, in a flask and incubated at 37°C for 5 minutes, shaking the solution continuously by means of a mechanical shaker. This was continued throughout the experiment. After 5 minutes 0.2 ml of each of the drugs under study (antimalarial drug or physostigmine) was then added and the solution further incubated for another 15 minutes. Acetylthiocholine (0.2 ml; 1.0 mM) was added to serve as the substrate and samples were taken at 15 minute intervals for the estimation of anticholinesterase activity. Five minutes before sampling, 0.1 ml of 5.5-dithiobis-2-nitrobenzoic acid reagent was added to 2.9 ml of phosphate buffer, pH 8.0, in a photocell, mixed gently, and then 0.2 ml of the incubated solution was added.

The absorbance of the sample was read against a blank at wavelength 412 mμ using a Unicam SP600 spectrophotometer. The blank solution was treated in the same way as the solution used for the experiment except that the flask for the blank contained 5.5 ml phosphate buffer, pH 8.0, and 0.5 ml homogenate but no anticholinesterase agent (anti-malarial drug or physostigmine) or acetylthiocholine.

The determination is based on the following reactions:

1. Acetylthiocholine $\xrightarrow{\text{(enzyme)}}$ thiocholine + acetate

2. Thiocholine + dithiobisnitrobenzoate ⟶ yellow colour

METHODS

B: 'IN VIVO' EXPERIMENTS

3.8 Cat experiments

Adult cats of either sex weighing between 1.8 and 3.5 kg were anaesthetized with a mixture of chloralose (80 mg/kg) and sodium pentobarbitone (6 mg/kg) injected intraperitoneally. Each cat was laid on its back on the operating table. The trachea was cannulated but the animal was still allowed to breathe spontaneously (until the need arose for artificial respiration). Blood pressure was recorded from a common carotid artery by means of a Statham pressure transducer (model P23AC) coupled to a Grass six-channel curvilinear recorder (model 7). The heart rate, and in some experiments, the response of the nictitating membrane to sympathetic nerve electrical stimulation were also recorded. Drugs were injected intravenously through a polythene cannula in the right brachial vein in volumes not exceeding 0.5 ml and washed in with 0.2 ml of 0.9% $^{w}/_{v}$ NaCl solution (normal saline). In some experiments, drugs were injected intra-arterially into the femoral artery. In all experiments, injections of normal saline were used as controls. However, in cases where a drug was dissolved in a vehicle other than normal saline, injections of that vehicle were used as controls.

3.9 Soleus muscle

A skin incision was made from the level of the Achilles tendon to the popliteal space. The soleus was separated from the neighbouring muscles and its tendon of insertion cut. The tendon was attached to a Grass (model FT03) strain gauge coupled to the Grass six-channel recorder. The sciatic nerve was exposed, ligated and cut high in the thigh. Small shielded bipolar platinum stimulating electrodes were placed on the peripheral portion of the sciatic nerve. The soleus muscle and all other exposed tissues were lubricated with heavy liquid paraffin (B.P). With the cat supine on the operating table, the hind limbs were clamped in a horizontal position by means of drills through their femur, tibia and fibula. Maximal muscle twitches were elicited indirectly by stimulating the sciatic nerve at a frequency of 0.1 Hz with rectangular wave pulses of 100 μsec duration delivered from a Grass (model S88) stimulator and about twice the strength necessary to evoke a maximal twitch. In experiments in which incomplete tetanic contractions were recorded, trains of impulses, each of 100 μsec duration, were delivered from a Grass (model S88) stimulator at a frequency of 6 to 7 Hz for 1 second and at regular intervals of 10 seconds. In some experiments, the muscle twitch record was integrated by means of a Grass (model 7P10A) integrating circuit to give a record of the area under the tension curve. The resting tension on the muscle (60 to 100 g) was adjusted

to that which gave the greatest tension on stimulation.

3.10 Tibialis anterior muscle

The tendon of insertion of the right tibialis anterior muscle was exposed and cut. The muscle tendon was attached to a Grass (model FT10C) strain gauge coupled to a Grass (model 7) six-channel recorder. The sciatic nerve was ligated and cut high in the thigh and shielded bipolar platinum electrodes were placed on the peripheral portion of the nerve. The hind limb was clamped as described for the soleus muscle preparation. Maximal twitches were evoked indirectly by stimulating the sciatic nerve at a frequency of 0.1 Hz with rectangular pulses of 100 μsec duration from a Grass (model S88) stimulator. These were about twice the strength necessary to evoke maximal twitches. In experiments in which tetanic contractions were recorded, impulses at a frequency of 100 Hz delivered from a Grass (model S88) stimulator for approximately 3 seconds were used.

In some experiments; drugs were injected intra-arterially into the tibialis artery. Close-arterial injections into the tibialis anterior muscle were made by injecting retrogradely through a polythene cannula tied into the cut central end of the tibial artery at a point immediately distal to the vessels supplying the tibialis anterior muscle. The volume of drug solution administered intra-

arterially did not exceed 0.2 ml and was washed in with 0.1 ml of normal saline. Injections of normal saline were used as controls.

3.11 Nictitating membrane

In experiments in which the response of the nictitating membrane to pre-ganglionic sympathetic nerve stimulation were studied, the head of the cat was immobilized by placing a wooden support under its neck and securing the jaw to a transverse rod. A piece of fine thread was tied to the cartilagenous portion of the nictitating membrane and the membrane was then attached to a Grass force-displacement transducer (model FT03C) coupled to a Grass (model 7) six-channel curvilinear pen recorder. The vago-sympathetic trunk was carefully exposed, isolated from the carotid artery and the cervical (sympathetic) nerve was then separated from the vagus nerve. The cervical nerve was ligated and electrically stimulated through bipolar platinum wire electrodes. The sympathetic nerve was immersed in liquid paraffin and stimulated pre-ganglionically through a 'stimulus isolation unit' by trains of rectangular pulses of 0.5 msec duration at a frequency of 10 Hz for 10 seconds, and with supramaximal strength sufficient to elicit a maximal contraction of the membrane delivered from a Grass (model S88) stimulator. The membrane was repeatedly stimulated at regular intervals of 75 seconds and the contractions were recorded isometrically. Drugs were

administered intravenously via a brachial vein in volumes not greater than 0.5 ml and washed in with 0.9% $^{w}/_{v}$ NaCl solution (0.5 ml). The dose interval was chosen such that the contractions of the nictitating membrane had been constant in amplitude for 10 - 20 minutes before the next injection.

RESULTS

The actions of the five chemically different antimalarial compounds studied (quinine, chloroquine, primaquine, proguanil and pyrimethamine) were found to be strikingly similar on all the skeletal muscle preparations used. Hence, in most of the figures shown in this section, representative typical examples were merely chosen to illustrate the general pattern of behaviour of the anti-malarial drugs.

3.12 Effects on frog rectus abdominis muscle

Chloroquine, primaquine, quinine, proguanil and pyrimethamine all exhibited a dual action on acetylcholine (Ach)-induced contractures of the frog rectus abdominis muscle preparation. At relatively low concentrations (7.5×10^{-7} - 1.5×10^{-4}M), the compounds augmented Ach-induced contractures of the muscle, while at higher concentrations (2.5×10^{-4} - 2.0×10^{-3}M) they depressed the contractures in a dose-related fashion. When the drugs were washed out of the bath, their depressant effect on Ach-evoked contractures was gradually reversed over a period of 10 - 30 minutes. Low doses of the agents which potentiated the action of Ach reduced carbachol-induced contractures of the muscle, whilst moderate to high concentrations of the compounds markedly inhibited carbachol-evoked contractures in a dose-related manner. Figures 43 and 44 summarise the results obtained with chloroquine.

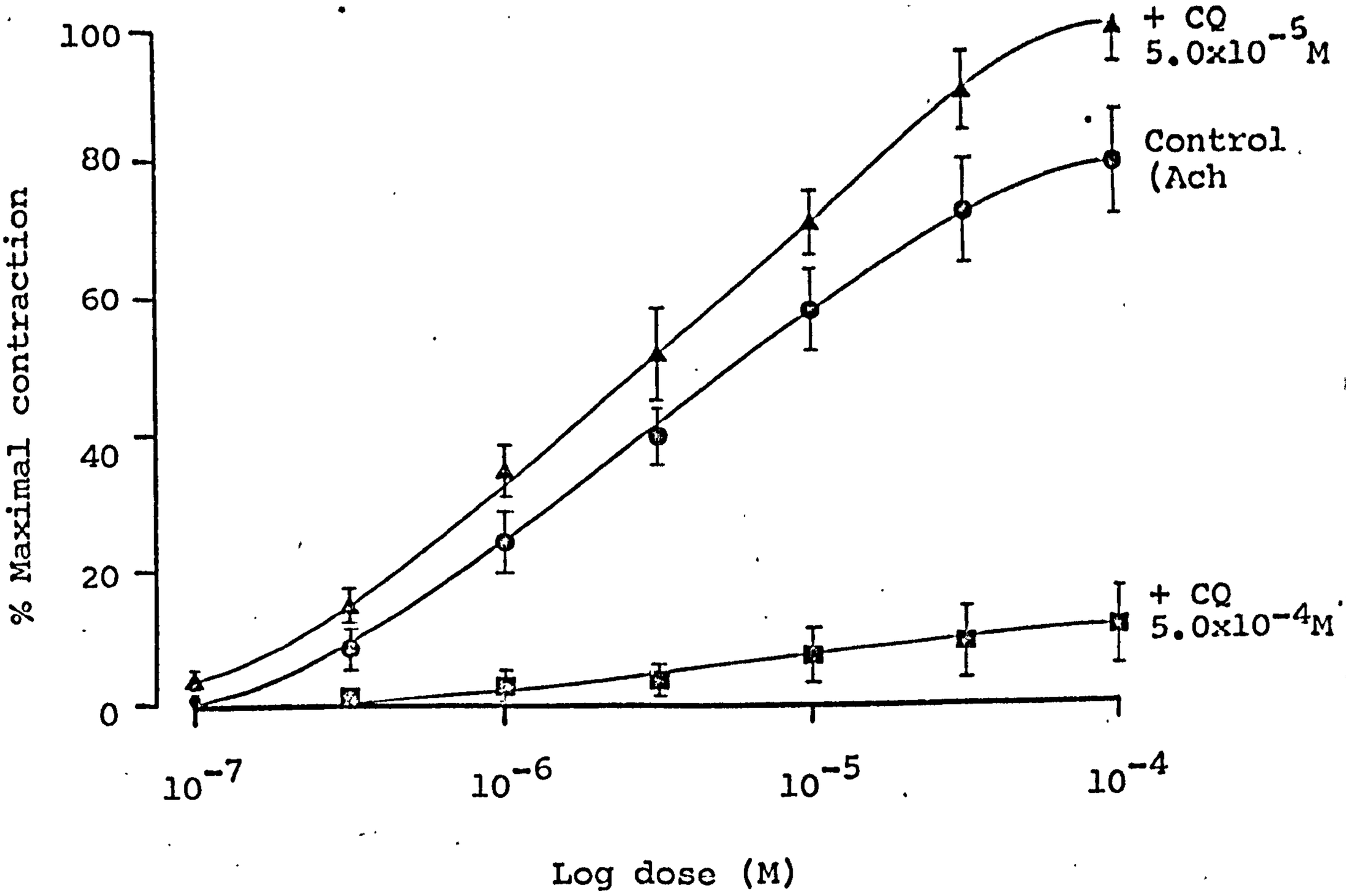


FIGURE 43

Frog rectus abdominis muscle. Effects of chloroquine (CQ, 5.0×10^{-5}M and 5.0×10^{-4}M) on Acetylcholine (Ach)-induced contractions of frog rectus abdominis muscle preparations. The different concentrations of chloroquine (CQ) were added to the bath 10 min before the addition of acetylcholine (Ach). Each point is the mean of 6 - 10 observations. The vertical bars represent s.e. of means.

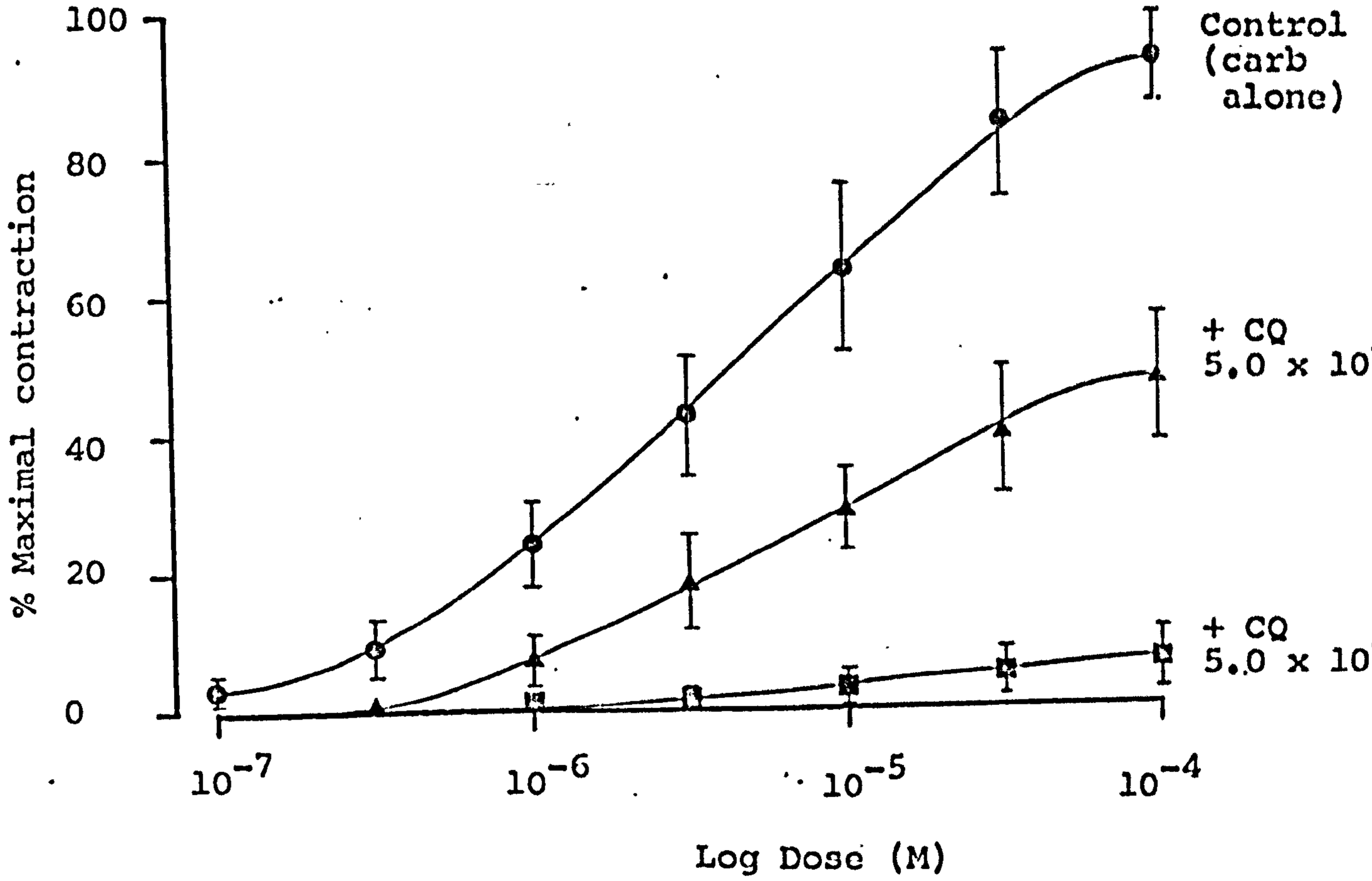


FIGURE 44

Frog rectus abdominis muscle. Effect of chloroquine (CQ, 5.0×10^{-5}M and 5.0×10^{-4}M) on Carbachol-induced contractions of frog rectus abdominis muscle preparations. The different concentrations of chloroquine (CQ) were added to the bath 10 min before the addition of carbachol (Carb). Each point is the mean of 5 - 8 observations, and the vertical bars denote s.e. of means.

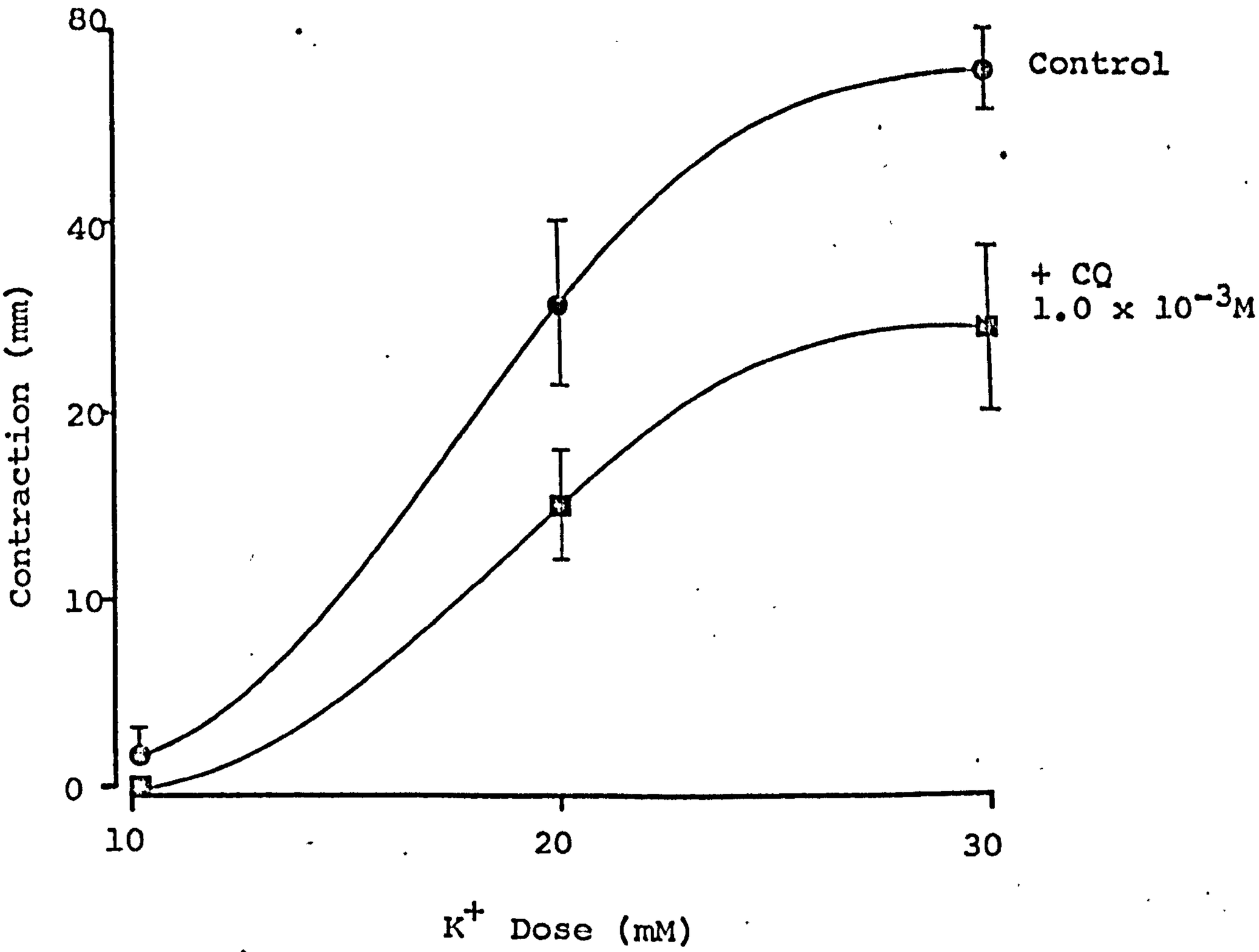


FIGURE 45

Frog rectus abdominis muscle. Effect of chloroquine (CQ, 1.0×10^{-3}M) on potassium-induced contractions of frog rectus abdominis muscles. Each point is the mean of 4 - 7 observations. The vertical bars represent s.e. of means.

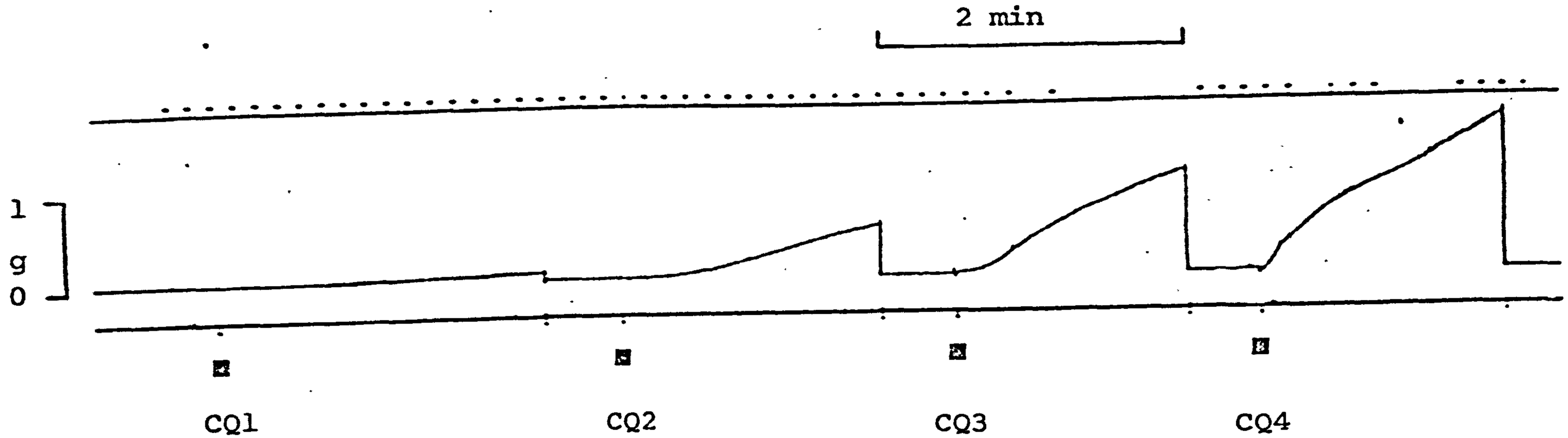


FIGURE 46

Frog rectus abdominis muscle. Effect of chloroquine (CQ) on frog isolated rectus abdominis muscle preparation. CQ1, CQ2, CQ3 and CQ4 denote chloroquine 2.5×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M and 2.5×10^{-3}M respectively.

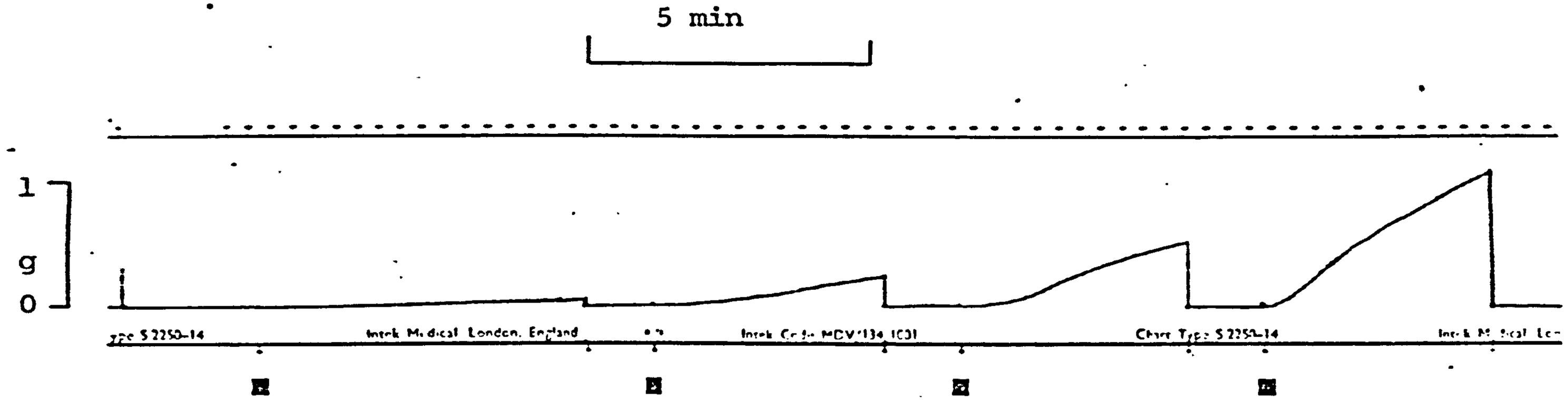


FIGURE 47

Frog rectus abdominis muscle. Effect of pyrimethamine (PM) on frog isolated rectus abdominis muscle preparation. PM1, PM2, PM3 and PM4 represent pyrimethamine 5.0×10^{-4}M, 1.0×10^{-3}M, 2.5×10^{-3}M and 5.0×10^{-3}M respectively.

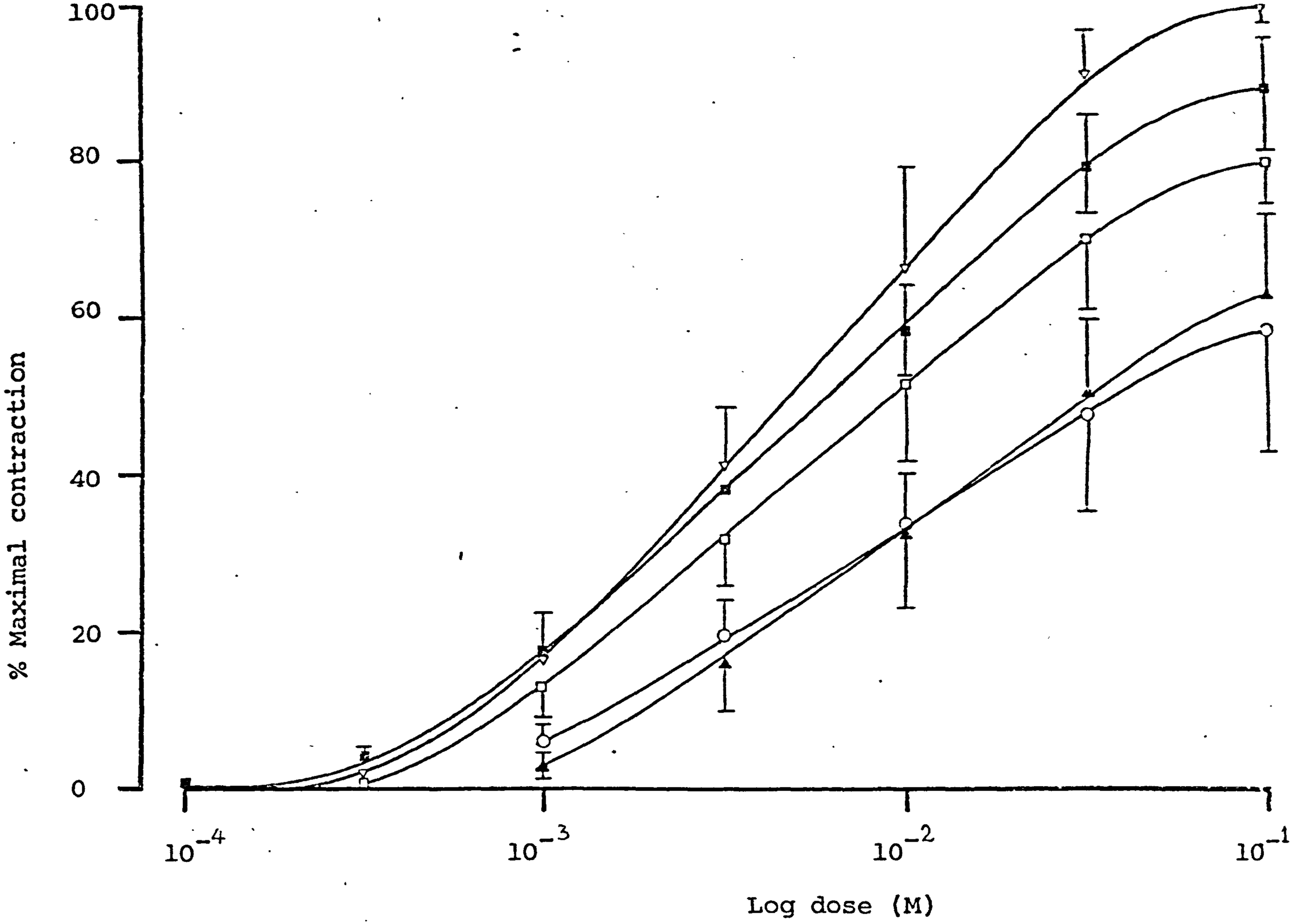


FIGURE 48

Frog rectus abdominis muscle. Mean dose/response curves to chloroquine (▽), primaquine (■), quinine (□), proguanil (▲) and pyrimethamine (○) on frog isolated rectus abdominis muscle. Each point is the mean of 5 - 8 observations. The vertical bars represent s.e. of means.

All doses of the antimalarial drugs used (5.0×10^{-7} - 1.0×10^{-2}M) also antagonized the contractures evoked by acetylcholine in eserinized muscle in a non-competitive manner. The drugs similarly inhibited potassium-induced contractures of the muscle (4 - 7 preparations for each drug) non-competitively. Figure 45 shows the effect of chloroquine on potassium-induced contracture of the muscle.

On their own accord, moderate to high doses of the antimalarial drugs (2.5×10^{-4} - 1.0×10^{-2}M) produced dose-related, sustained contractures of the muscle. Figures 46 and 47 show typical traces, and Figure 48 summarises the results obtained. The relative potency of each antimalarial drug in causing a sustained contracture of the rectus abdominis muscle was compared with those of acetylcholine, carbachol and nicotine and the results are summarized in Figure 49.

3.13 Effects on chick biventer cervicis nervo-muscle preparation

The results obtained with the chick biventer muscle were similar to those obtained using the frog rectus abdominis muscle preparation. Relatively low concentrations of the antimalarial compounds (7.5×10^{-7} - 1.0×10^{-4}M) augmented acetylcholine (Ach)-induced contractions of the biventer muscle. Physostigmine produced a similar effect. Figures 50 and 51 show typical traces obtained. However, these low doses of the antimalarial agents antagonized, in a non-competitive manner, muscle contractions evoked by carbachol,

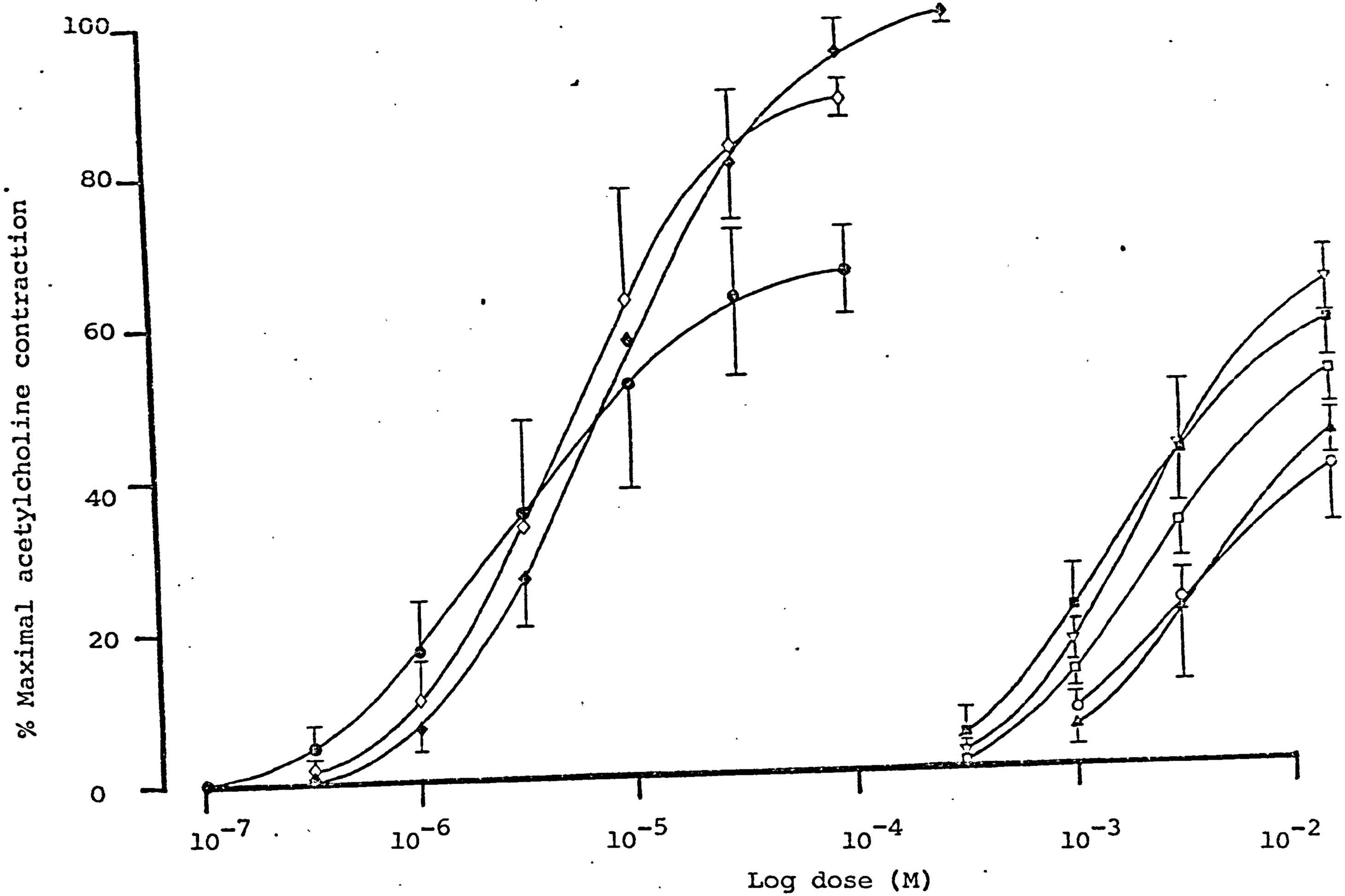


FIGURE 49

Frog rectus abdominis muscle. Mean dose/response curves to acetylcholine (◆), carbachol (◇), nicotine (●), chloroquine (▽), primaquine (▣), quinine (□), proguanil (▲) and pyrimethamine (○) on frog isolated rectus abdominis muscle. Each point is the mean of 6 - 10 observations. The vertical bars denote s.e. of means.

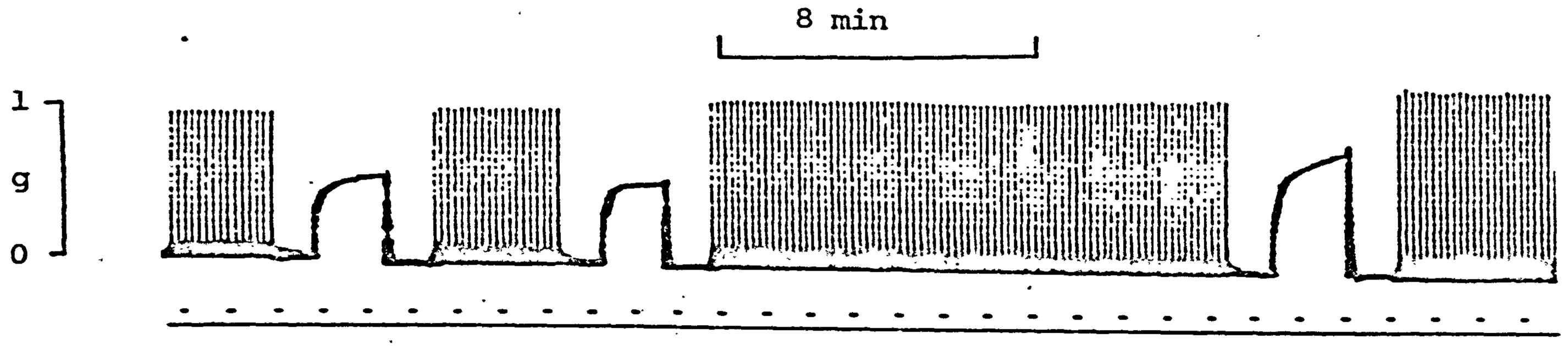


FIGURE 50

Chick biventer cervicis nerve-muscle preparation. Effect of proguanil (PG, 2.5×10^{-6}M, added at the upward arrow) on acetylcholine (A)-induced contractions of chick isolated biventer cervicis nerve-muscle preparations stimulated indirectly at a frequency of 0.1 Hz. At A, electrical stimulation was temporarily stopped and 2.5×10^{-6}M acetylcholine was added to the bath..

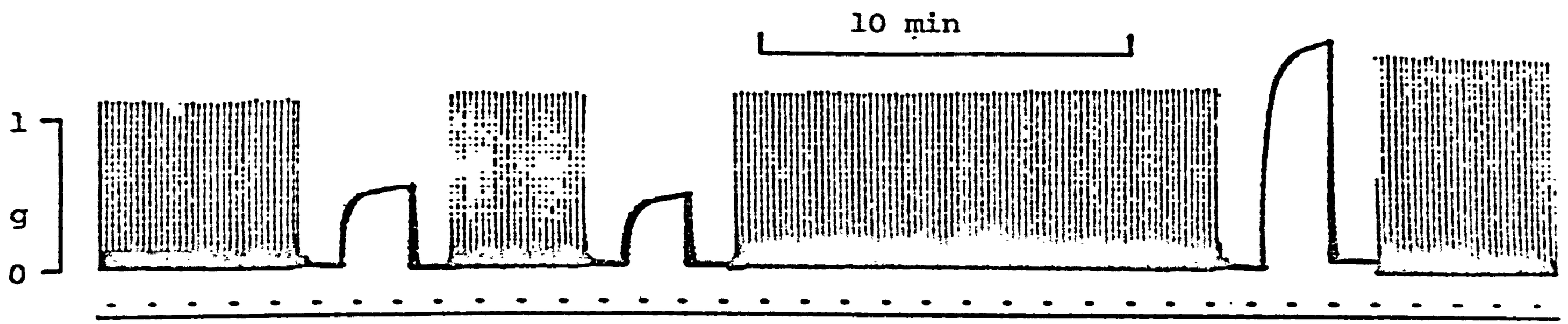


FIGURE 51

Chick biventer cervicis nerve-muscle preparation. Effect of physostigmine (PHY, 2.5×10^{-7}M, added at the upward arrow) on acetylcholine (A)-induced contractions of chick isolated biventer cervicis nerve muscle preparations stimulated indirectly at a frequency of 0.1 Hz. At A, electrical stimulation was temporarily stopped and 2.5×10^{-6}M acetylcholine was added to the bath.

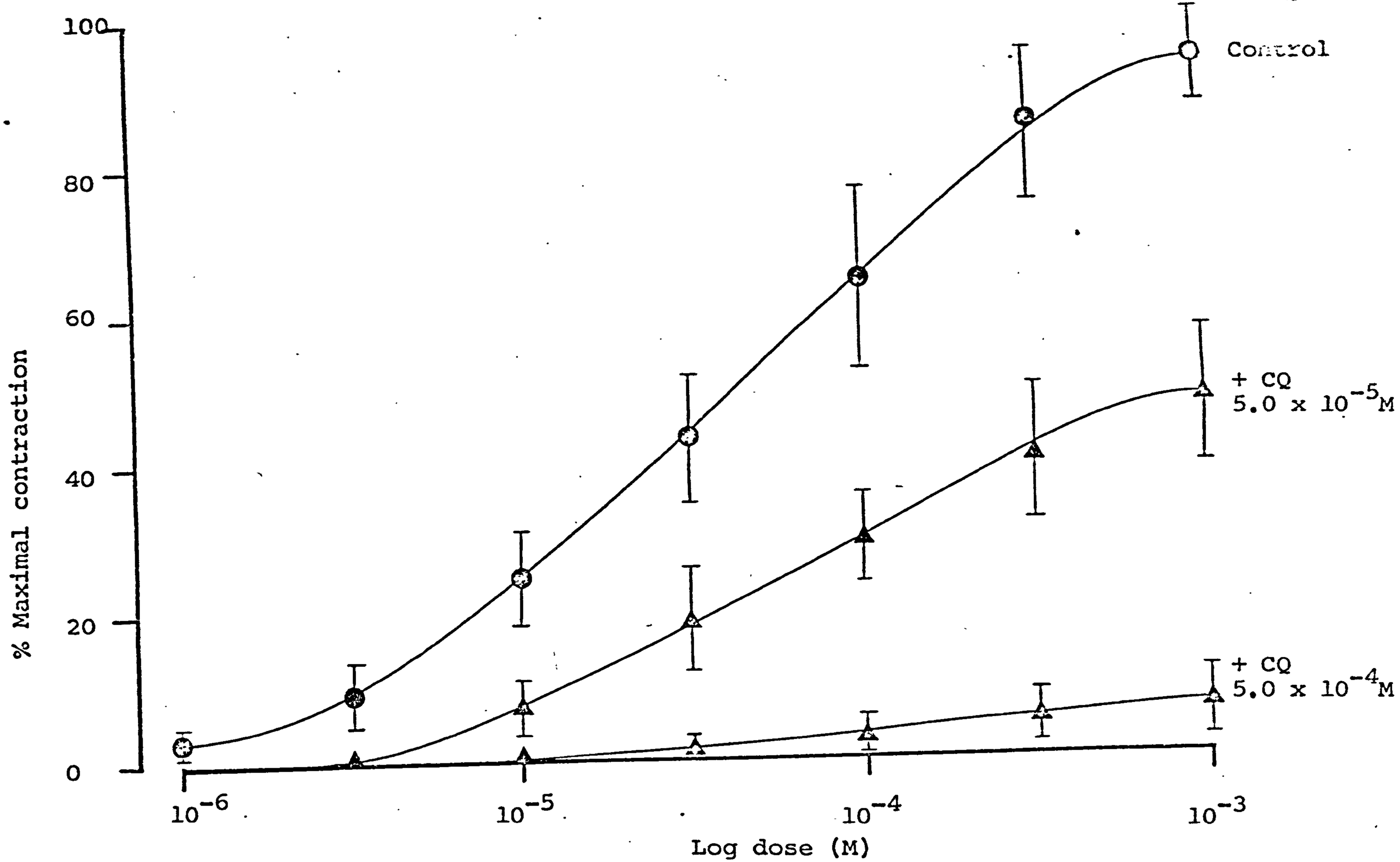


FIGURE 52

Chick biventer cervicis nerve-muscle preparation. Effect of chloroquine (CQ, 5.0×10^{-5}M and 5.0×10^{-4}M) on carbachol-evoked contractions of chick isolated biventer cervicis muscle preparations. The different concentrations of chloroquine (CQ) were added to the bath 10 minutes before the addition of carbachol. Each point is the mean of 4 - 10 observations. The vertical bars represent s.e. of means.

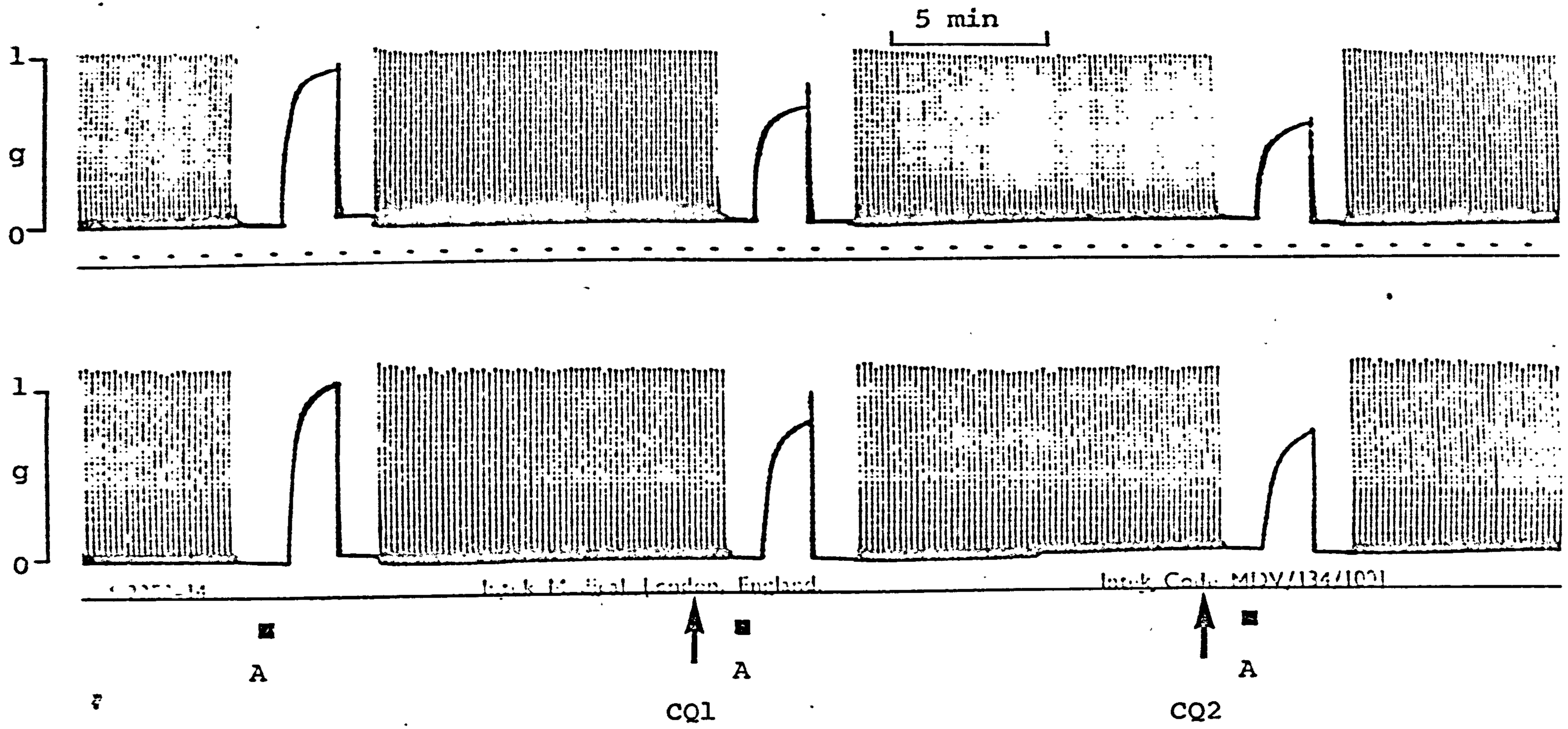


FIGURE 53

Chick biventer cervicis nerve-muscle preparation. Effect of chloroquine (CQ, added at the upward arrows) on acetylcholine (A)-induced contractions of chick isolated biventer cervicis nerve-muscle preparations stimulated indirectly at a frequency of 0.1 Hz. CQ1 and CQ2 denote chloroquine 2.5×10^{-4}M and 5.0×10^{-4}M respectively. At A, electrical stimulation was temporarily stopped and 3.0×10^{-6}M acetylcholine was added to the bath.

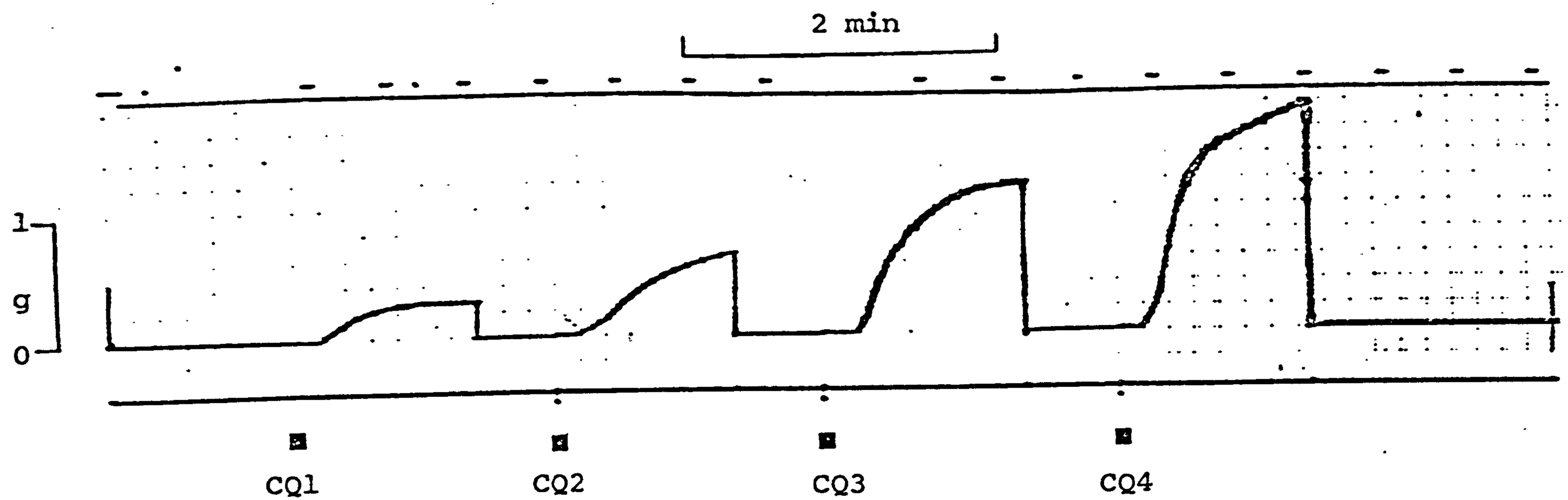


FIGURE 54

Chick biventer cervicis nerve-muscle preparation. Effect of chloroquine (CQ) on chick biventer cervicis muscle preparation. CQ1, CQ2, CQ3 and CQ4 represent chloroquine 5.0×10^{-4}M, 1.0×10^{-3}M, 2.5×10^{-3}M and 5.0×10^{-3}M respectively.

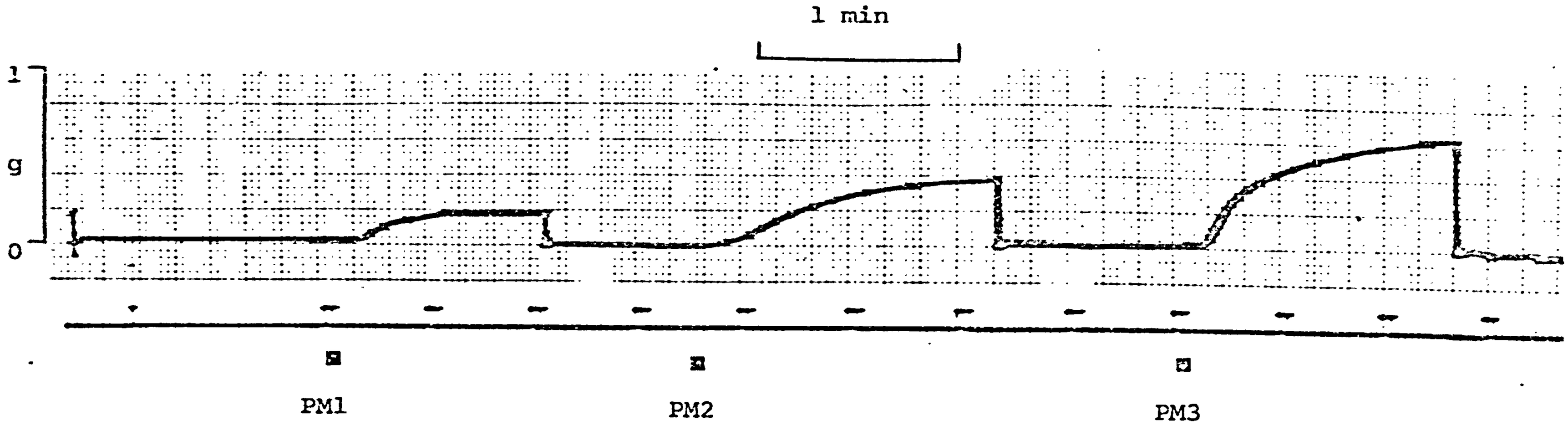


FIGURE 55

Chick biventer cervicis nerve-muscle preparation. Effect of pyrimethamine (PM) on chick biventer cervicis muscle preparation. PM1, PM2 and PM3 denote pyrimethamine 5.0×10^{-4}M, 1.0×10^{-3}M and 5.0×10^{-3}M respectively.

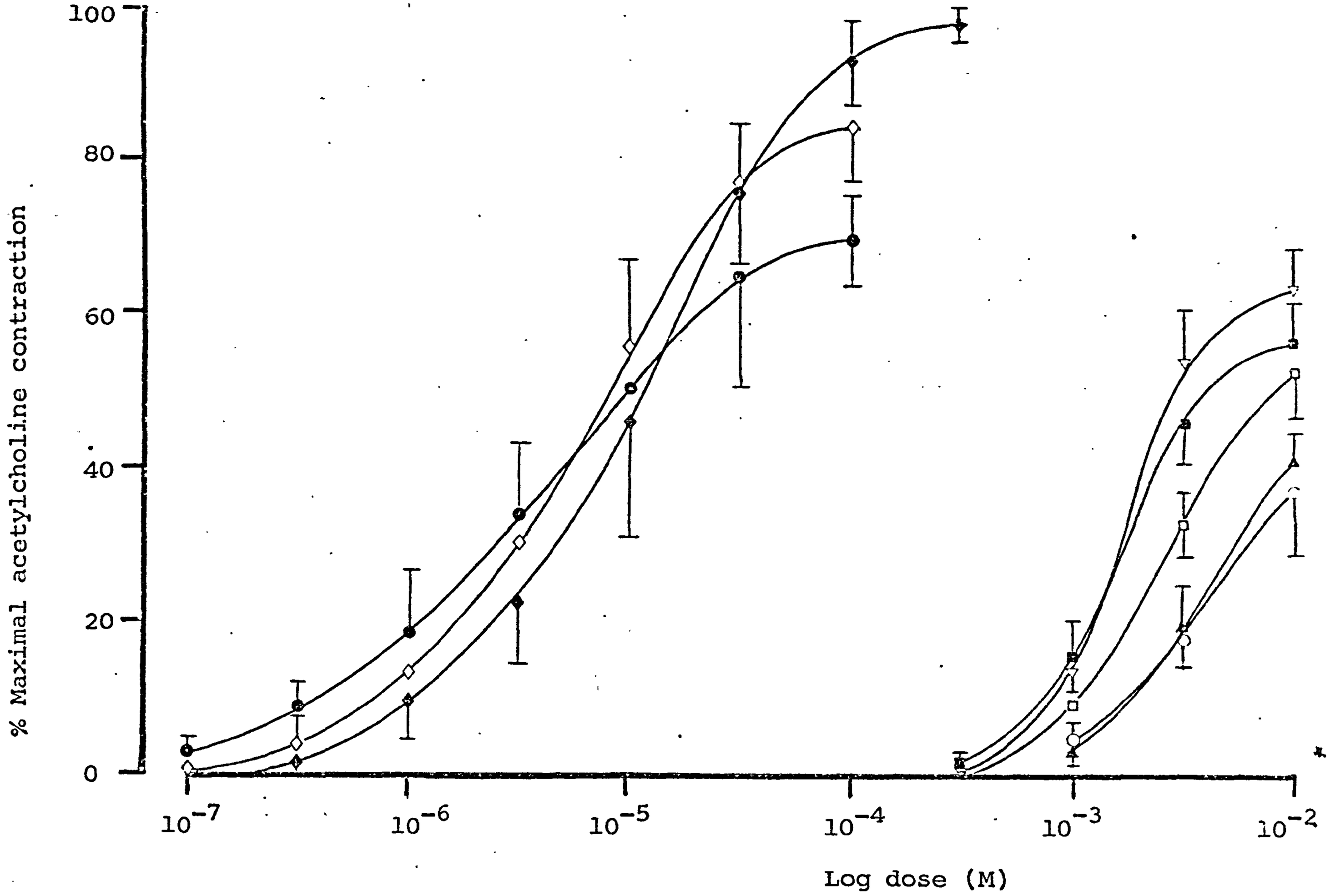


FIGURE 56

Chick biventer cervicis nerve-muscle preparation. Mean dose/response curves to acetylcholine (◆), carbachol (◇), nicotine (⊘), chloroquine (▽), primaquine (■), quinine (□), proguanil (▲) and pyrimethamine (○) on chick isolated biventer cervicis muscle preparations. Each point is the mean of 5 - 8 observations. The vertical bars represent s.e. of means.

potassium and also, Ach-induced contractions of osorinized muscles. Figure 52 illustrates the effect of chloroquine on carbachol-evoked contractions of the tissue. Moderate to high concentrations of the compounds (2.5×10^{-4} - 2.0×10^{-3}M) antagonized, in a non-competitive manner, Ach-induced contractions of the biventer muscle (Figure 53). Relatively high doses of the agents themselves caused dose-dependent contractions (Figures 54 and 55). The effectiveness of high concentrations of the antimalarial drugs in causing sustained contraction of the biventer muscle was compared with that of acetylcholine, carbachol and nicotine, and this is summarized in Figure 56.

Except with quinidine, and occasionally, chloroquine and pyrimethamine, low to medium doses (ie. 2.5×10^{-6} - 2.5×10^{-5}M) of the antimalarial drugs did not produce any noticeable effect on twitches evoked by direct or indirect electrical stimulation. Quinidine, in doses of 2.5×10^{-6} to 1.5×10^{-4}M, and occasionally pyrimethamine in doses of 5.0×10^{-6} to 2.5×10^{-5}M, potentiated muscle twitches induced by direct or indirect electrical stimulation. However, higher concentrations of all the antimalarials (5.0×10^{-4} - 1.0×10^{-2}M) dose-dependently reduced or abolished the twitches (Figure 57). In all cases, higher doses of the antimalarial drugs were required to depress the twitches of the muscle preparations induced by direct electrical stimulation than of indirectly evoked ones. After the

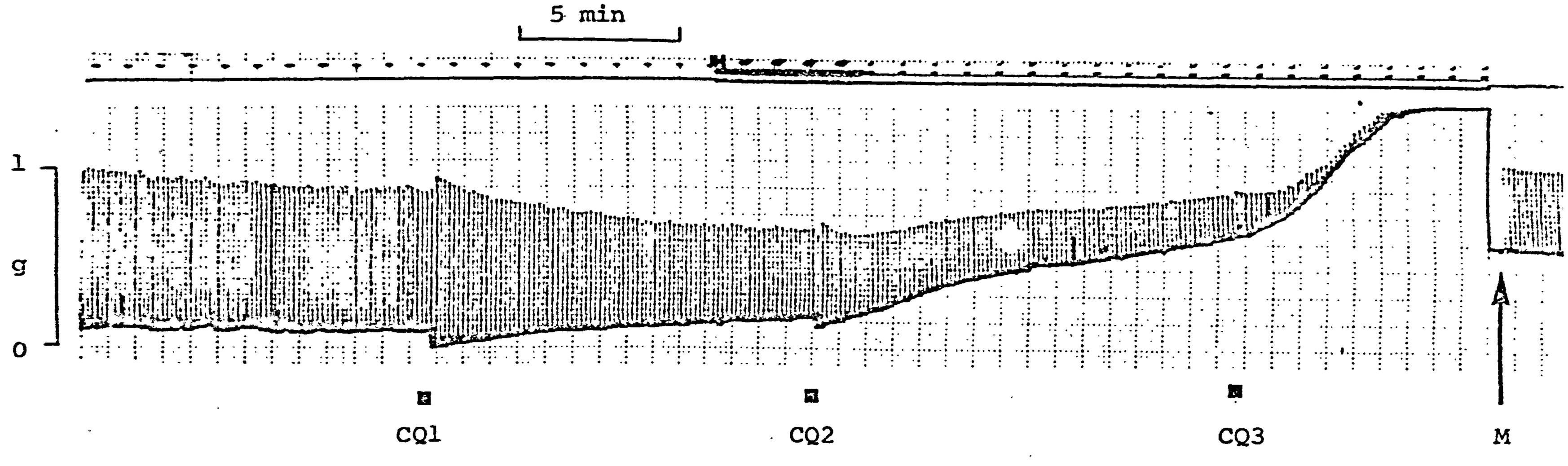


FIGURE 57

Chick biventer cervicis nerve-muscle preparation. Effect of chloroquine (CQ) on maximal isometric twitches of chick biventer cervicis nerve-muscle preparation stimulated indirectly at a frequency of 0.1 Hz. CQ1, CQ2 and CQ3 denote chloroquine 1.0×10^{-4}M, 5.0×10^{-4}M and 1.0×10^{-3}M respectively. The muscle was directly stimulated at M (upward arrow).

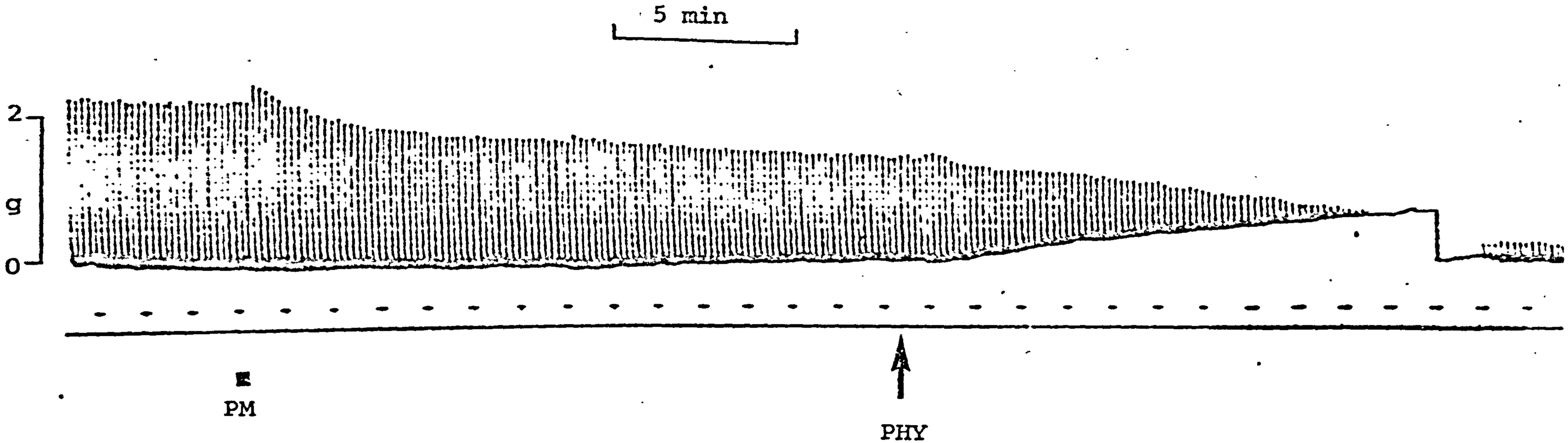


FIGURE 58

Chick biventer cervicis nerve-muscle preparation. Effect of physostigmine (PHY, 5.0×10^{-7}M, at the upward arrow) on pyrimethamine (PM, 5.0×10^{-4}M)-induced depression of maximal twitches of chick biventer cervicis muscle evoked by indirect electrical stimulation once every 10 sec. The neuromuscular blocking action of pyrimethamine was not reversed by physostigmine.

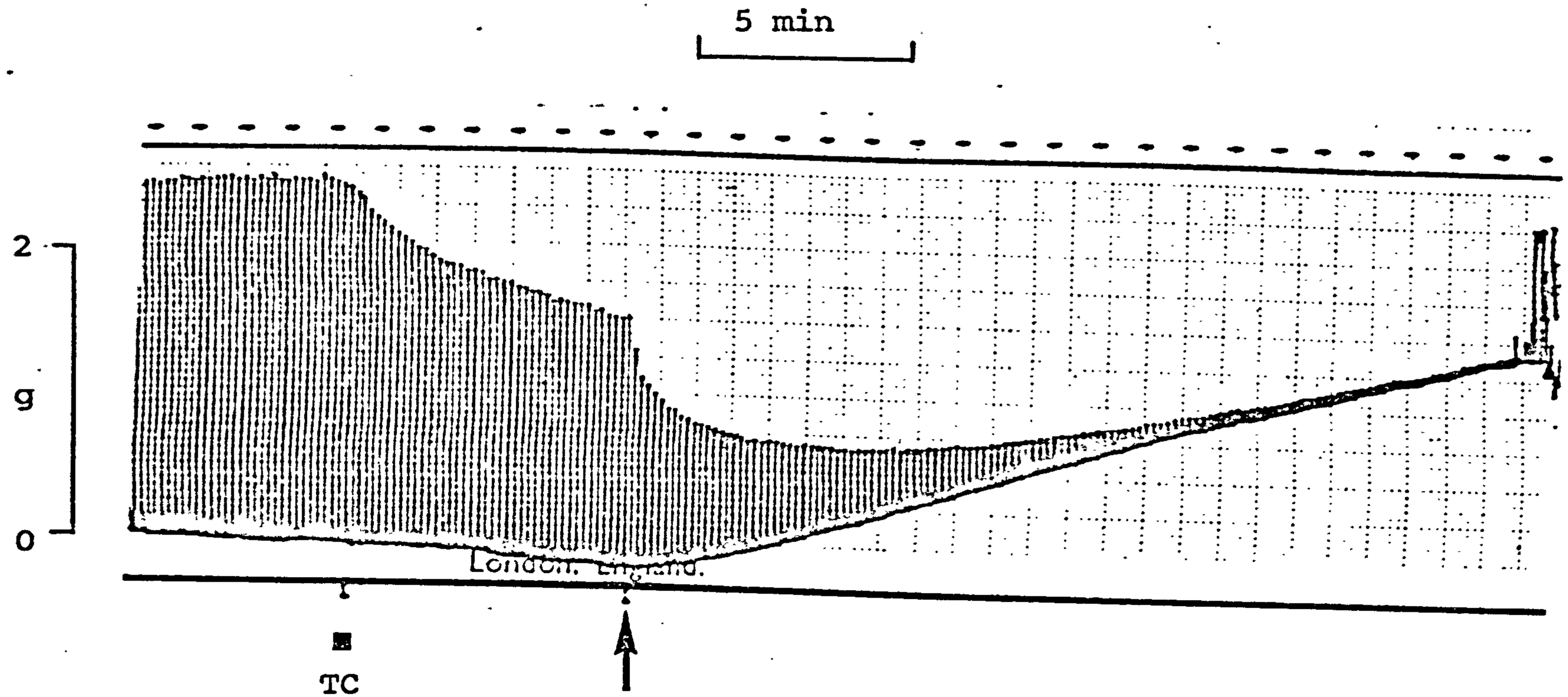


FIGURE 59

Chick biventer cervicis nerve-muscle preparation. Effect of primaquine (PQ, 5.0×10^{-4}M, at the upward arrow) on d-tubocurarine (TC, 1.5×10^{-6}M)-induced depression of maximal isometric twitches of chick biventer cervicis muscle evoked by indirect electrical stimulation at a frequency of 0.1 Hz. Primaquine potentiated the neuromuscular blocking effect of d-tubocurarine on the muscle.

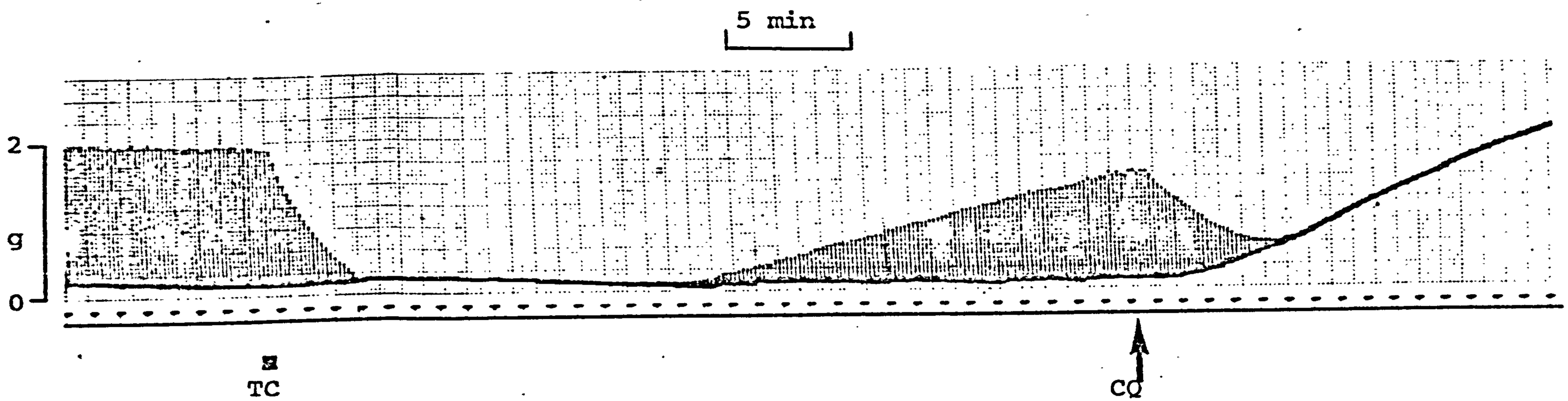


FIGURE 60

Chick biventer cervicis nerve-muscle preparation. Effect of chloroquine (CQ, 5.0×10^{-4}M, at the upward arrow) on d-tubocurarine (TC, 5.0×10^{-6}M)-induced paralysis of maximal twitches of a chick biventer cervicis muscle evoked by indirect electrical stimulation once every 10 sec. Chloroquine produced 'recurarization' of the muscle.

twitches had been completely abolished in the indirectly stimulated muscle preparations, direct stimulation of the same muscles still produced twitches of about 15 - 40 per cent of the initial twitch heights (See Figure 57).

Neuromuscular blockade produced by the compounds was not reversed by physostigmine (Figure 58). Medium to high concentrations of all the antimalarials (2.5×10^{-4} - 1.0×10^{-3}M) markedly potentiated the neuromuscular blocking actions of d-tubocurarine and suxamethonium. Figure 59 shows a typical trace. At the same dose levels, all the antimalarial compounds also produced recurarization of the muscle preparations just recovering from full paralysis, and also of partially curarized muscles (Figure 60).

3.14 Effects on rat phrenic nerve-hemidiaphragm muscle preparation

In all the preparations, quinine produced a biphasic effect on the twitches of the indirectly and directly stimulated hemidiaphragm muscle. Low to medium doses of quinine (2.5×10^{-6} - 2.5×10^{-4}M) markedly increased the twitch heights whilst higher concentrations (5.0×10^{-4} - 2.0×10^{-3}M) depressed the twitches in a dose-dependent manner, (Figure 61). In a few non-curarized preparations (8 - 17 per cent) low concentrations of chloroquine, primaquine, proguanil and pyrimethamine (1.5×10^{-6} - 2.5×10^{-5}M) each slightly potentiated the twitches evoked by indirect and

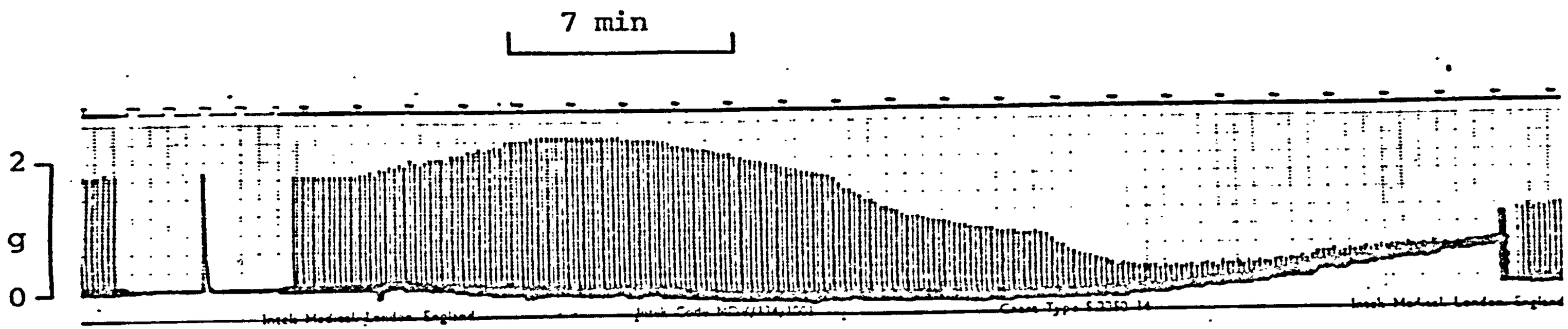


FIGURE 61

Rat phrenic nerve-hemidiaphragm muscle preparation. Effects of quinine (QN) on maximal twitches of rat hemidiaphragm muscle preparation evoked by indirect electrical stimulation at a frequency of 0.1 Hz. QN1, QN2, QN3 and QN4 denote quinine 1.0×10^{-4}M, 2.5×10^{-4}M, 5.0×10^{-4}M and 1.0×10^{-3}M respectively.

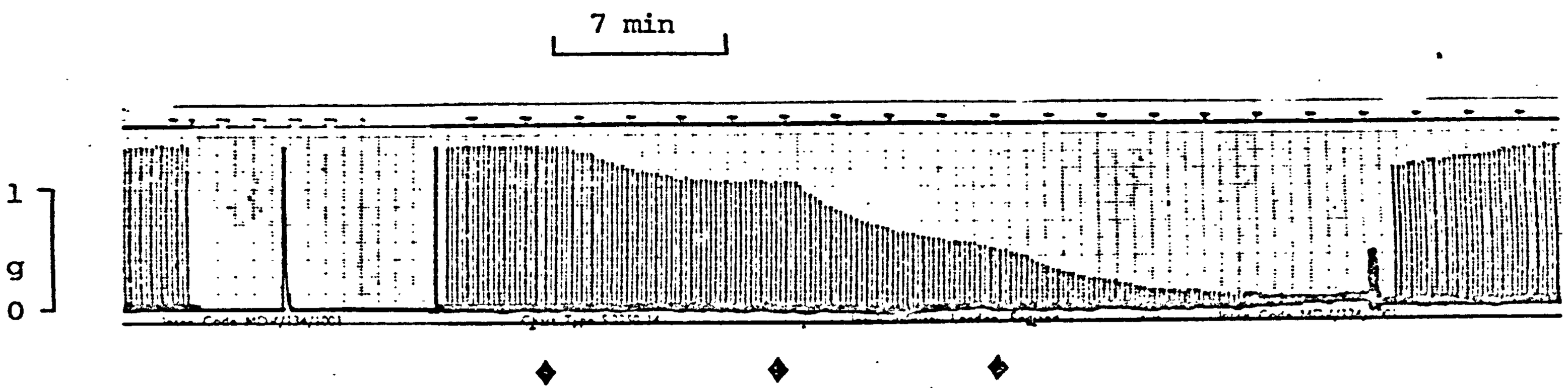


FIGURE 62

Rat phrenic nerve-hemidiaphragm muscle preparation. Effect of primaquine (PQ) on maximal twitches of rat hemidiaphragm muscle preparation stimulated indirectly (0.1 Hz) once every 10 sec. PQ1, PQ2 and PQ3 represent primaquine 2.5×10^{-4}M, 5.0×10^{-4}M and 1.0×10^{-3}M respectively.

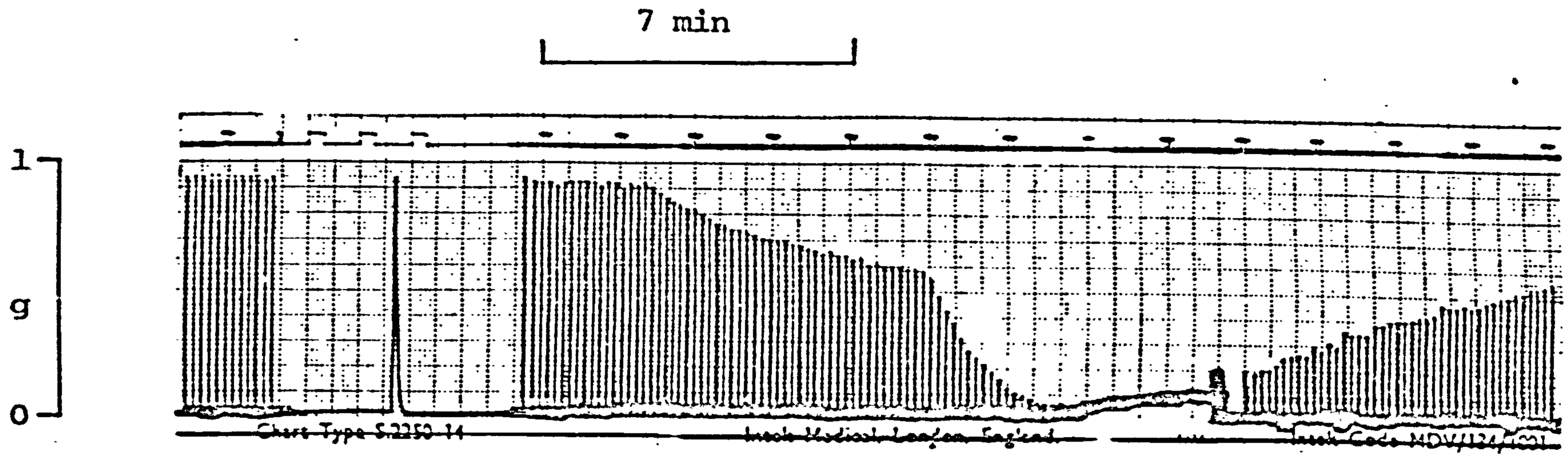


FIGURE 63

Rat phrenic nerve-hemidiaphragm muscle preparation. Effect of chloroquine (CQ) on maximal twitches of rat hemidiaphragm muscle preparation stimulated indirectly at a frequency of 0.1 Hz. CQ1 and CQ2 denote chloroquine, 5.0×10^{-4}M and 2.5×10^{-3}M respectively.

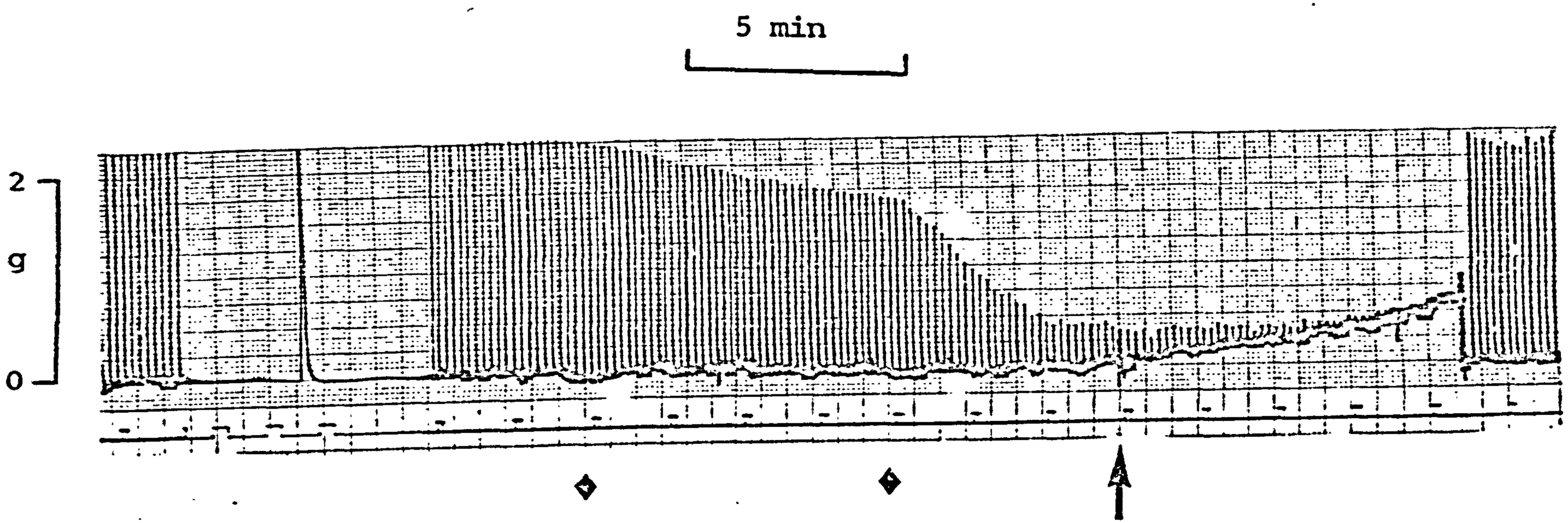


FIGURE 64

Rat phrenic nerve-hemidiaphragm muscle preparation. Effect of physostigmine (PHY, 5.0×10^{-7}M, added at the upward arrow) on neuromuscular blocking action of proguanil (PG). Maximal twitches of the hemidiaphragm muscle was evoked by indirect electrical stimulation (0.1 Hz) once every 10 sec. PG1 and PG2 represent proguanil 5.0×10^{-4}M and 2.5×10^{-3}M respectively. The neuromuscular blockade induced by proguanil was not reversed by physostigmine.

direct stimulation. As with high doses of quinine however, high concentrations of these other antimalarial agents (2.5×10^{-4} - 2.0×10^{-3}M) produced a dose-related, curare-like, action on phrenic nerve-hemidiaphragm muscle preparations. Figures 62 and 63 show typical traces obtained. Higher concentrations of quinine, chloroquine, primaquine, proguanil and pyrimethamine were required to produce marked depression of twitches evoked by direct stimulation than indirect stimulation. However, this blockade was not reversed by physostigmine (Figure 64).

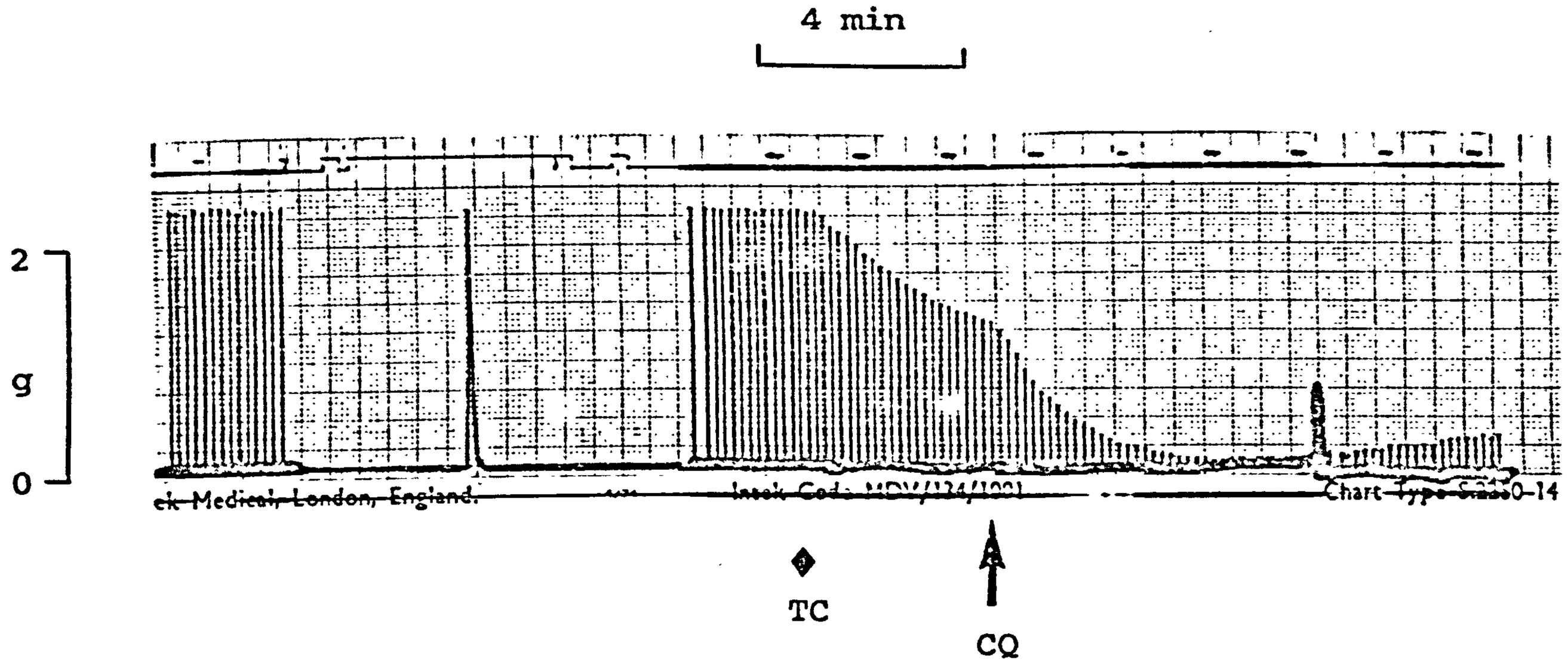


FIGURE 65

Rat phrenic nerve-hemidiaphragm muscle preparation. Effect of chloroquine (CQ 5.0×10^{-4}M, added to the bath at the upward arrow) on neuromuscular blocking action of d-tubocurarine (TC, 1.5×10^{-6}M). The neuromuscular blocking effect of d-tubocurarine was markedly potentiated by chloroquine.

TABLE 3

Anticholinesterase activities of the antimalarial compounds and lignocaine compared with that of physostigmine in chick biventer muscle homogenates. Each figure represents the mean of three determinations.

Agent	Mean molar concentrations (I_{50}) which produced 50% inhibition of chick biventer muscle cholinesterase
Physostigmine salicylate	2.5×10^{-7} M
Chloroquine sulphate	2.7×10^{-4} M
Primaquine phosphate	2.6×10^{-4} M
Quinine Sulphate	2.9×10^{-4} M
Proguanil hydrochloride	4.8×10^{-4} M
Pyrimethamine base (dissolved in equimolar lactic acid)	6.1×10^{-4} M
Lignocaine base (dissolved in acid saline)	9.3×10^{-4} M

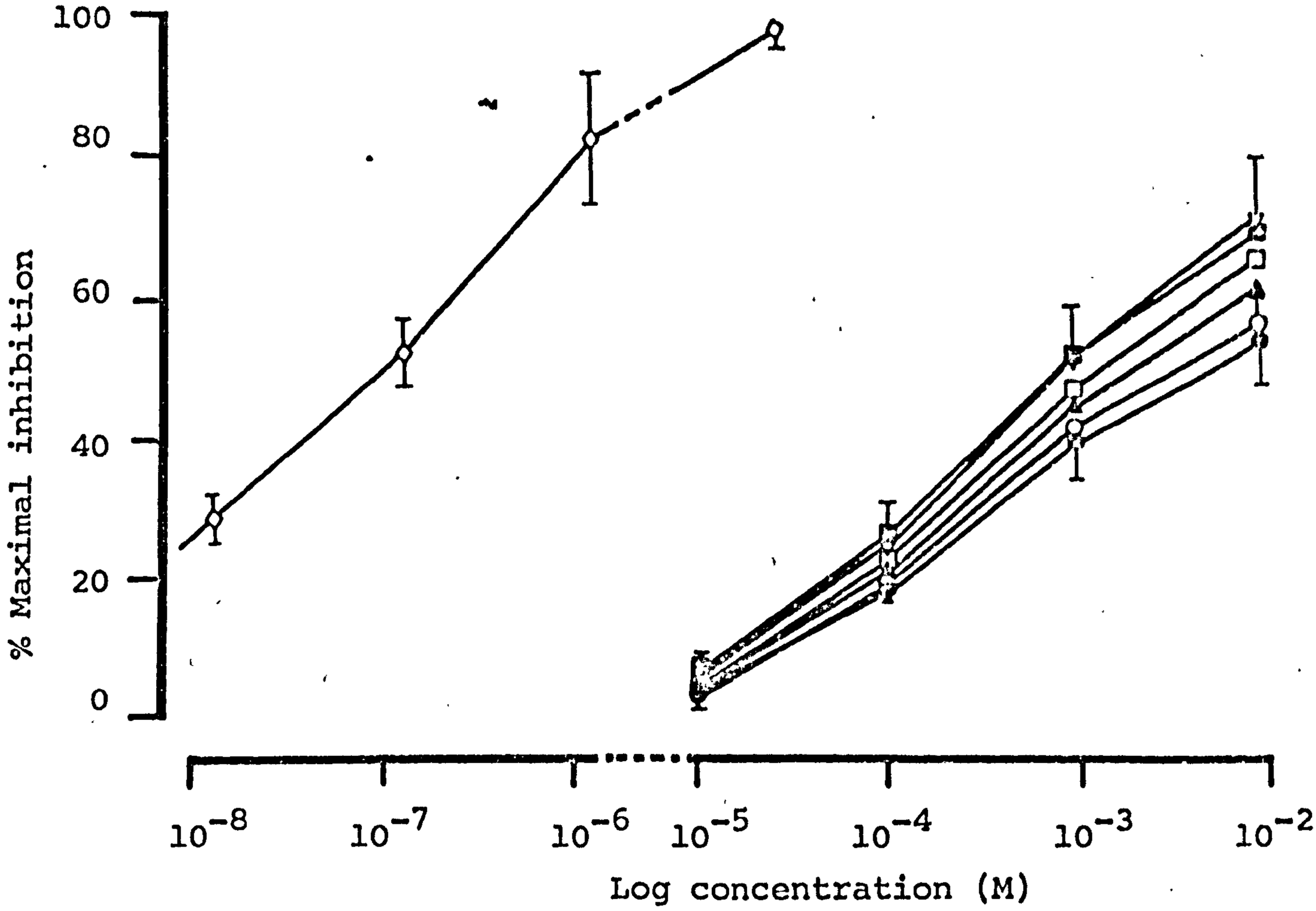


FIGURE 66

Anticholinesterase activity. Inhibition of chick biventer cholinesterase by physostigmine (◇), chloroquine (■), primaquine (▽), quinine (□), proguanil (▲), pyrimethamine (○) and lignocaine (●). All the antimalarial compounds inhibited the chick cholinesterase enzyme in a dose-related manner.

All the antimalarial drugs, in concentrations of 5.0×10^{-4} to 1.0×10^{-3}M, markedly potentiated the effect of d-tubocurarine and suxamethonium on this muscle preparation. Figure 65 illustrates a typical trace. In partially curarized muscles, and also in preparations just recovering from full paralysis, the same moderate to high doses of all the antimalarials (5.0×10^{-4} - 1.0×10^{-3}M) caused re-curarization (as in the chick biventer cervicis muscle preparations). In most preparations (89 per cent) doses of the antimalarial compounds which produced neuromusclar blockade also caused slight but sustained contraction of the hemidiaphragm muscle (see Figures 61 and 64).

3.15 Anticholinesterase determination

The results obtained from colorimetric determinations show that all the antimalarial compounds possessed anticholinesterase activity although physostigmine was far more active. Figure 66 summarises the results obtained and Table3.shows the mean concentration of the antimalarials, lignocaine and physostigmine which produced 50 per cent inhibition of chick biventer cholinesterase.

3.16 Effects on cat soleus muscle

Intravenous injections of low to medium doses of quinine (2 - 8 mg/kg) markedly potentiated the twitch responses of the soleus muscle to indirect electrical stimulation. On the contrary, higher concentrations of the drug (10 - 16 mg/kg)

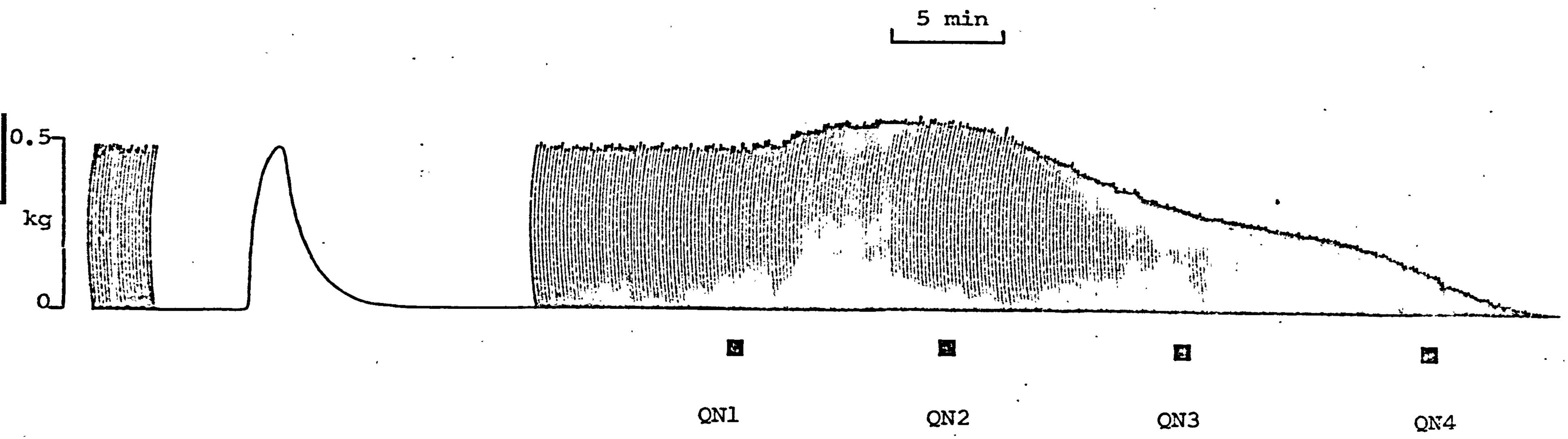


FIGURE 67

Anaesthetized cat. Effects of quinine (QN, iv.) on maximal twitches of a soleus muscle of an anaesthetized cat evoked by electrical stimulation of the motor nerve at a frequency of 0.1 Hz. QN1, QN2, QN3 and QN4 represent intravenous quinine 7.5 mg/kg, 10 mg/kg, 12.5 mg/kg and 15 mg/kg respectively. The twitches were initially augmented before they were depressed by quinine.

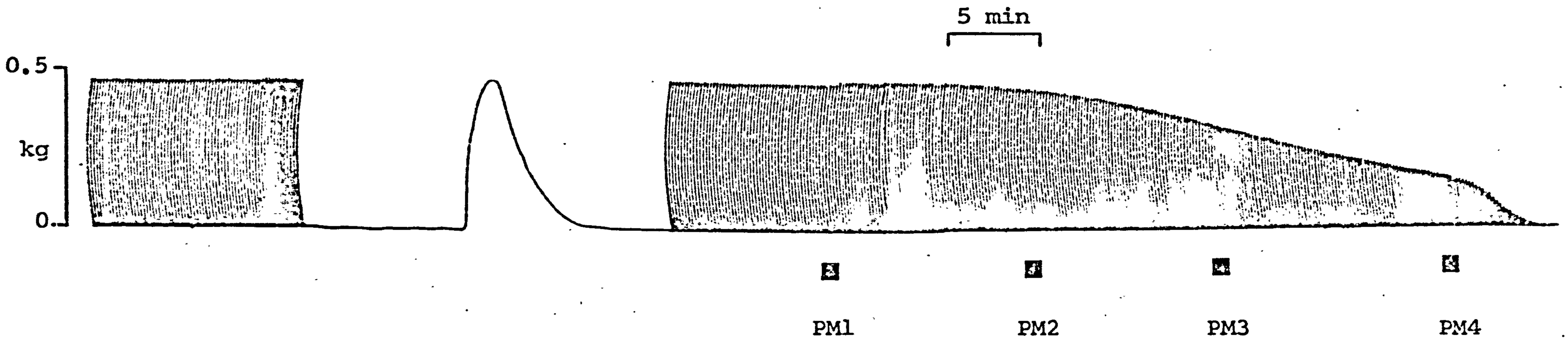


FIGURE 68

Anaesthetized cat. Effect of pyrimethamine (PM, iv) on maximal twitches of a soleus muscle of an anaesthetized cat induced by electrical stimulation of the motor nerve (0.1 Hz) once every 10 sec. PM1, PM2, PM3 and PM4 denote intravenously injected pyrimethamine, 7.5 mg/kg, 10 mg/kg, 12.5 mg/kg and 15 mg/kg respectively.

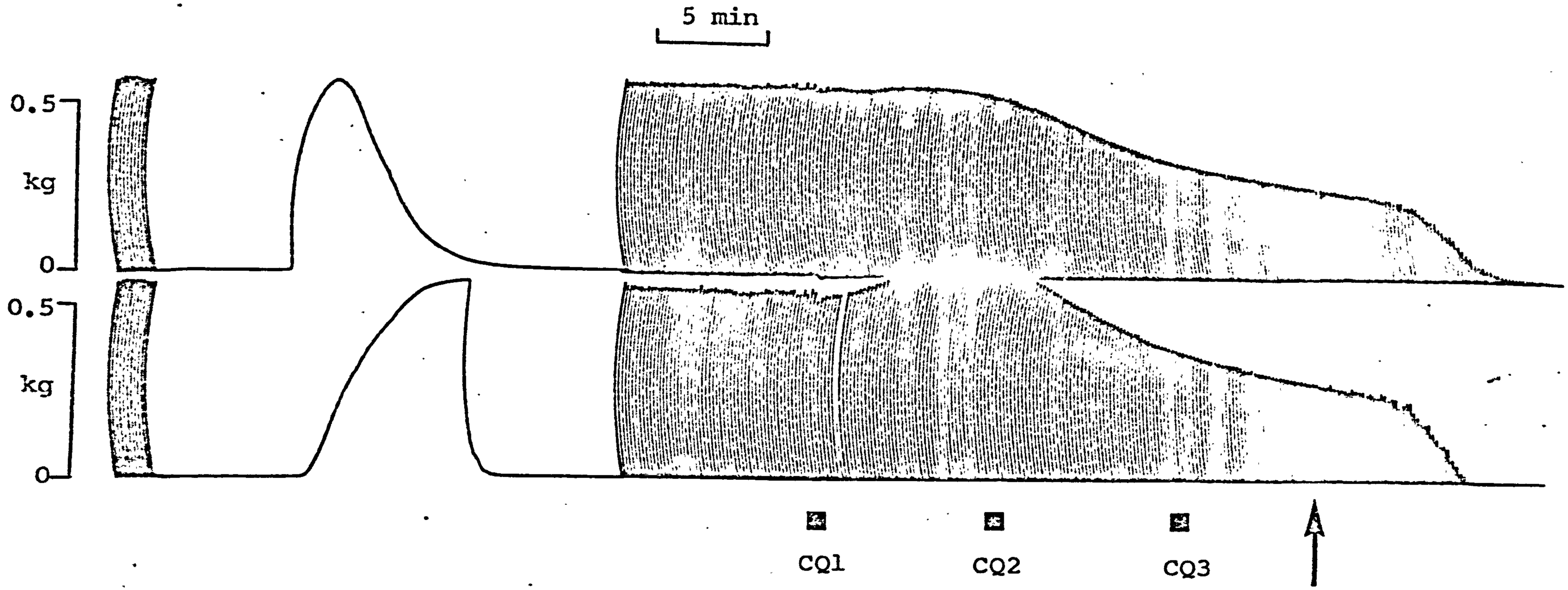


FIGURE 69

Anaesthetized cat. Effect of physostigmine (PHY, 150 µg/kg iv. injected at the upward arrow) on neuromuscular blockade produced by chloroquine (CQ) in a soleus muscle of an anaesthetized cat. Maximal twitches of the soleus muscle were evoked by stimulating the motor nerve (o.1 Hz) once every 10 seconds. CQ1, CQ2 and CQ3 denote intravenous chloroquine, 7.5 mg/kg, 10 mg/kg and 12.5 mg/kg respectively. The lower trace is an integration of the upper one.

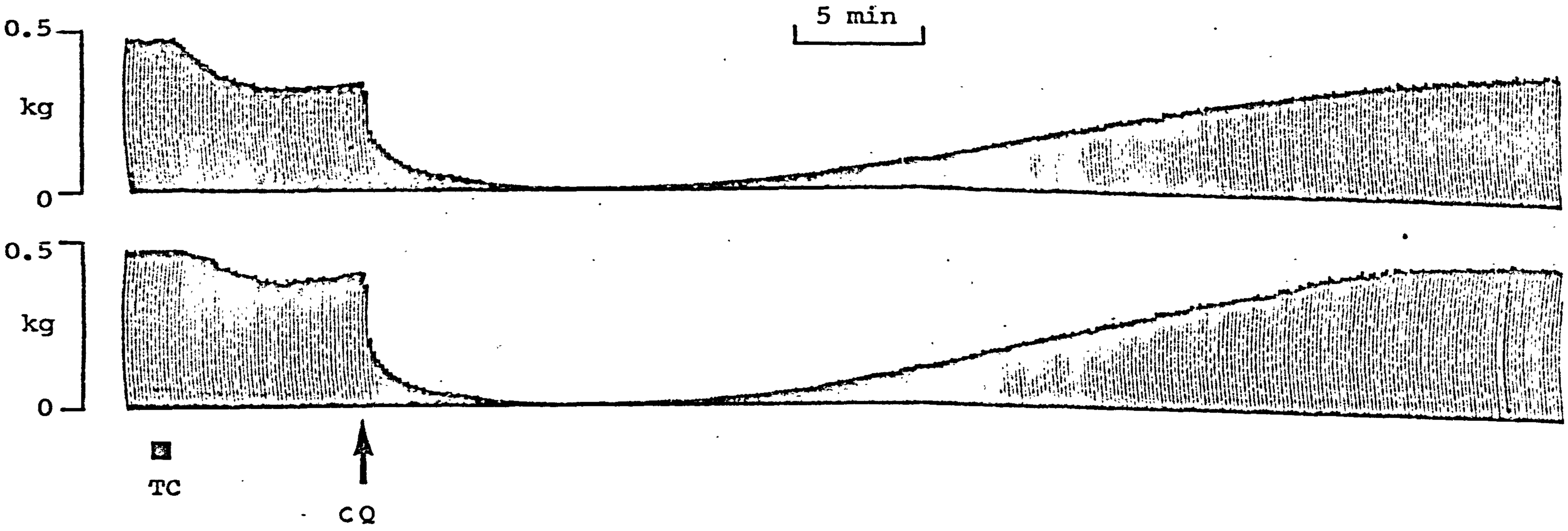


FIGURE 70

Anaesthetized cat. Effect of chloroquine (CQ, 10 mg/kg, injected intravenously at the upward arrow) on partial neuromuscular blockade produced by d-tubocurarine (TC, 150 µg/kg, iv.) in a soleus muscle of an anaesthetized cat. Maximal twitches of the soleus muscle were elicited by stimulating its motor nerve at a frequency of 0.1 Hz. The lower trace shows an integration of the upper one. Chloroquine deepened the neuromuscular block induced by d-tubocurarine.

produced a dose-related reduction of twitch height. In a few preparations low to medium concentrations of primaquine and chloroquine (2 - 8 mg/kg) independently produced slight enhancement of the indirectly evoked twitches of the muscle. However, higher doses of these quinoline antimalarial agents (10 - 16 mg/kg) always depressed the twitch heights in a dose-related manner. Low to medium concentrations of proguanil (1 - 5 mg/kg) and pyrimethamine (1 - 6 mg/kg) produced no obvious effect on the twitches of this muscle. Higher concentrations of the compounds (8 - 16 mg/kg) inhibited twitch height in a similar manner to the quinoline antimalarials (Figures 67 and 68). In all cases, recovery of twitch height depended upon the drug injected and always varied directly with the extent of depression elicited. The order of recovery from twitch depression was pyrimethamine (fastest) $\geq$ proguanil $\geq$ quinine $\geq$ chloroquine $\geqq$ primaquine. Neuromuscular block produced by the antimalarial drugs was not reversed by physostigmine (100 - 200 µg/kg) eg. Figure 69.

Medium to high doses (ie. 8 - 15 mg/kg) of all the antimalarial drugs deepened the neuromuscular blocking effect of intravenously administered d-tubocurarine (150 - 200 µg/kg) and suxamethonium (25 - 30 mg/kg) (Figure 70). At the same dose levels, all the antimalarials also caused recurarization of muscle preparations just recovering from the full effect of d-tubocurarine and suxamethonium (See Figure 71). Furthermore, all the antimalarial compounds

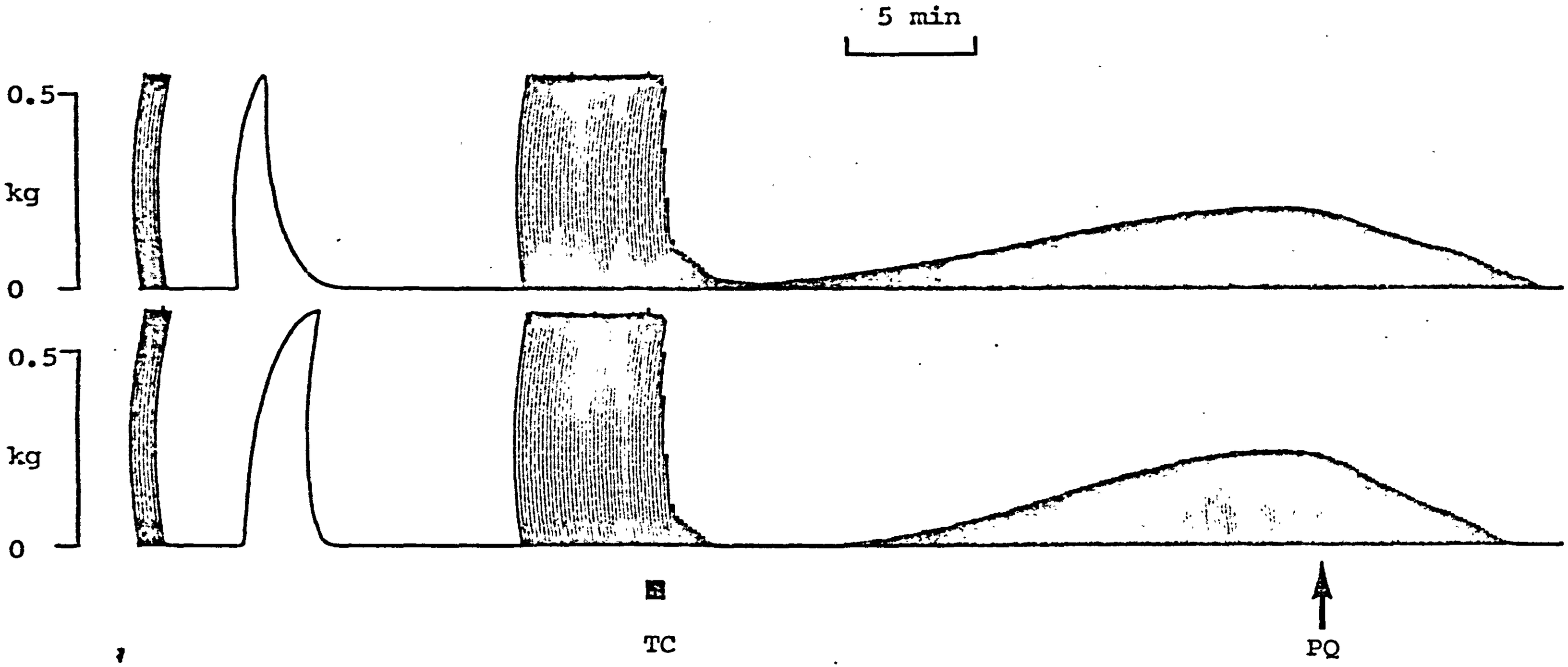


FIGURE 71

Anaesthetized cat. Effect of primaquine (PQ, 10 mg/kg, injected intravenously at the upward arrow) on full neuromuscular blockade produced by d-tubocurarine (TC, 200 µg/kg, iv.) in a soleus muscle of an anaesthetized cat. Maximal twitches of the muscle were evoked by stimulating the motor nerve at a frequency of 0.1 Hz. The lower trace is an integration of the upper trace. Primaquine produced 'recurarization' of the muscle.

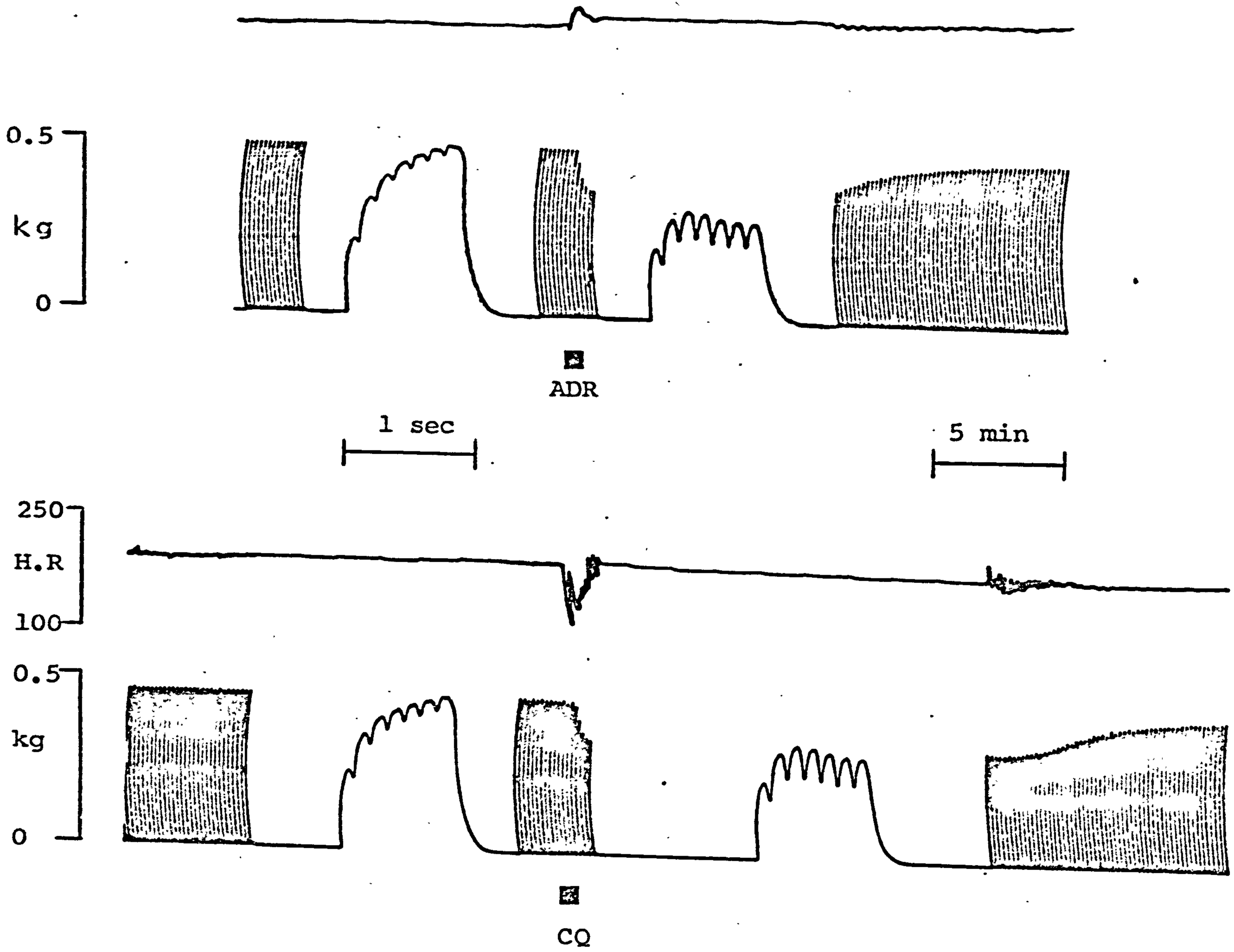


FIGURE 72

Anaesthetized cat. Effects of adrenaline (ADR, 0.3 µg/kg iv. - upper panel) and chloroquine (CQ, 10 mg/kg iv. - lower panel) on incomplete tetanic contractions of soleus muscle evoked by stimulating the motor nerve at a frequency of 7 Hz for 1 sec. every 10 seconds. The twitch height and the degree of fusion of the contractions were reduced by adrenaline and chloroquine.

lengthened the recovery time of the muscle after the partial effect of d-tubacurarine and suxamethonium by 3 - 50 minutes (See Figure 70).

Intravenous injections of moderate to high doses (8 - 16 mg/kg) of all the compounds also reduced the degree of fusion of incomplete tetanic contractions of soleus muscle in a dose-related fashion. Adrenaline (0.3 - 0.5 µg/kg) also reduced the twitch height, and the degree of fusion of incomplete tetanic contractions, of the indirectly stimulated soleus muscle. Figure 72 illustrates atypical trace obtained.

3.17 Effects on cat tibialis anterior muscle

Intravenous injections of low to medium concentrations (2 - 8 mg/kg) of quinine potentiated, in a dose-dependant manner, twitch height of tibialis anterior muscles evoked by indirect electrical stimulation. Higher doses of the drug (10 - 16 mg/kg) depressed the twitches. In some of the preparations, low to medium doses (2 - 8 mg/kg) of chloroquine and primaquine independently produced a slight enhancement of indirectly evoked twitches of the muscle. However, higher concentrations of these amino-quinolines (10 - 16 mg/kg) produced a dose-related depression of twitch height (as in the soleus muscle).

Low to moderate concentrations (1 - 8 mg/kg) of proguanil and pyrimethamine produced no observable effect on the twitches whilst higher doses (10 - 16 mg/kg) dose-dependently inhibited the twitches in a dose-related manner. This result is similar to that obtained in the soleus. Recovery of the muscle twitches was delayed, and depended on the amount of twitch depression induced and on the agent administered. As in the soleus muscle, neuromuscular block elicited by the antimalarial drugs (10 - 20 mg/kg) was not reversed by physostigmine.

As with the effect of low to medium concentrations of quinoline antimalarial compounds, adrenaline (5 - 10 µg/kg) enhanced the twitch amplitude of the tibialis anterior muscle. This action of adrenaline was inhibited or abolished by intravenous administrations of propranolol (300 - 400 µg/kg). Similarly, this dose level of propranolol inhibited the twitch augmentation effect of the low to medium doses of quinoline antimalarials on this muscle. In contrast, the effect of quinine was only reduced by about 10 - 20 per cent.

Medium to high concentrations of all the antimalarial drugs (10 - 16 mg/kg) markedly deepened the neuromuscular blocking actions of d-tubocurarine (150 - 200 µg/kg) and suxamethonium (25 - 30 mg/kg) on the tibialis anterior muscle. In the same dose range, they also caused recurarization of the tibialis anterior muscle preparations which were just

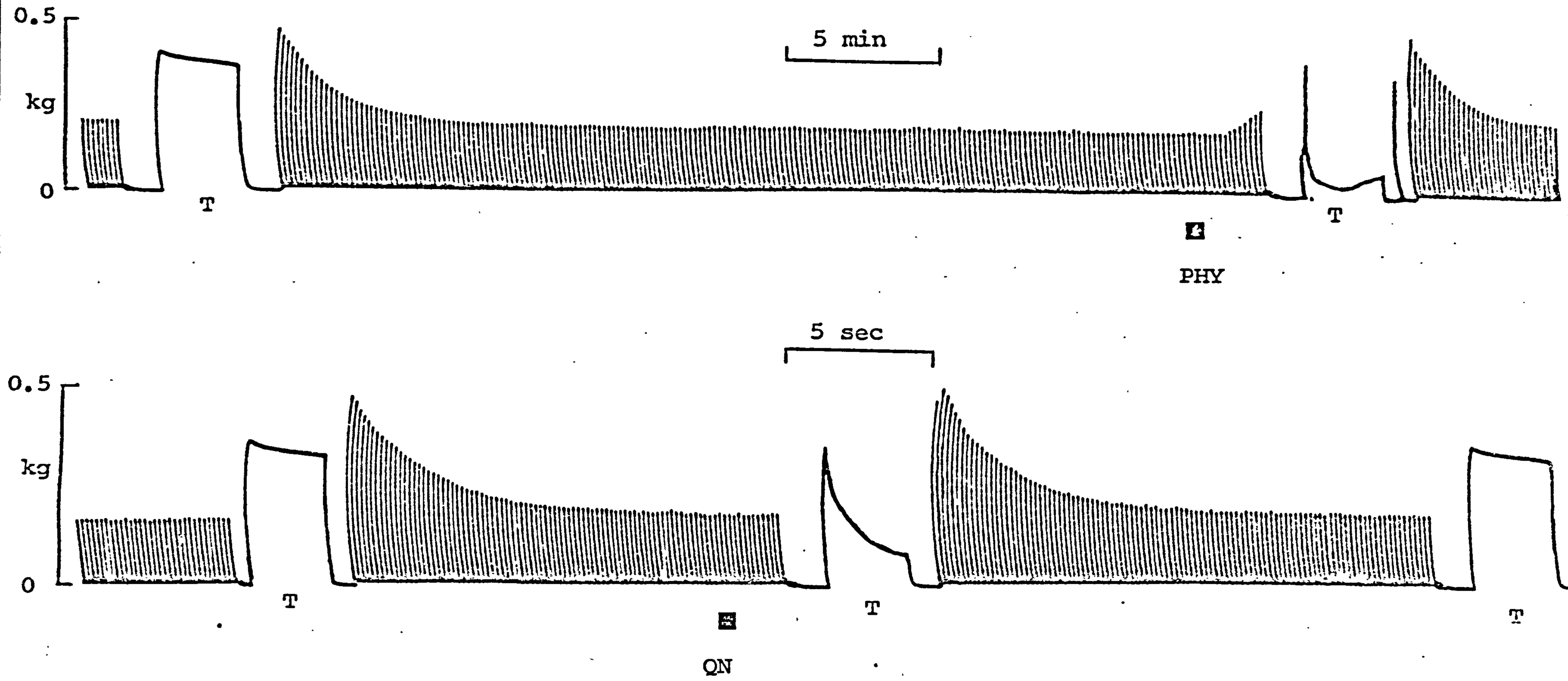


FIGURE 73

Anaesthetized cat. Effects of physostigmine (PHY, 250 µg/kg, iv.) and quinine (QN, 10 mg/kg, iv.) on the twitches and maximal tetanic contractions of a tibialis anterior muscle of an anaesthetized cat in response to electrical stimulation of the motor nerve. At T, a tetanus was elicited by stimulating the motor nerve at 50/sec for 5 sec.

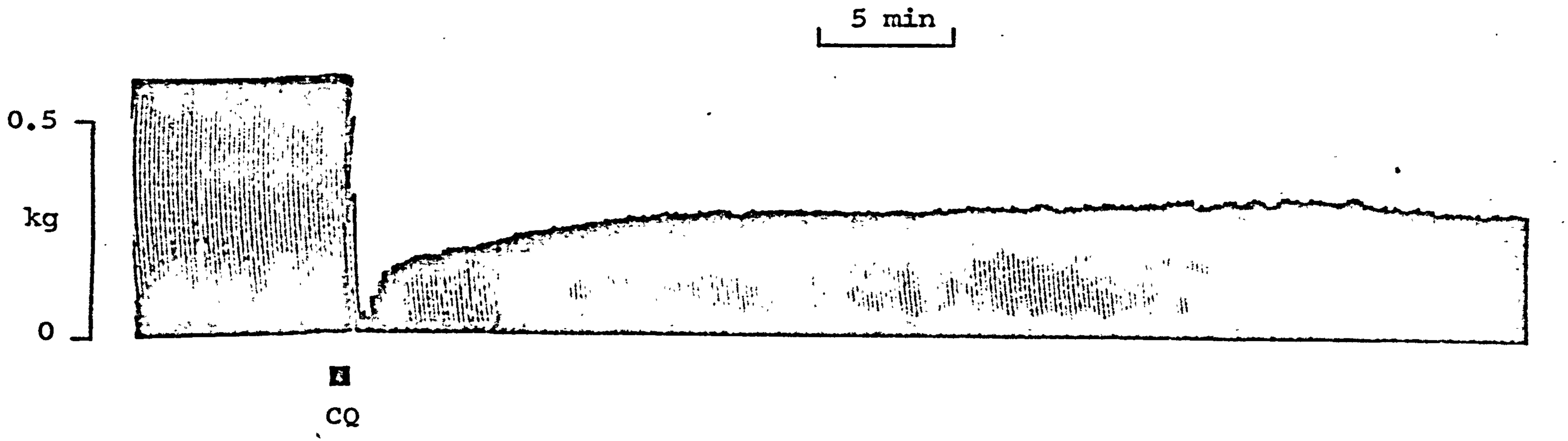


FIGURE 74

Anaesthetized cat. Effect of close-arterial injection of chloroquine (CQ, 10 mg/kg) on maximal twitches of a tibialis anterior muscle evoked by electrical stimulation of the motor nerve (0.1 Hz) once every 10 seconds.

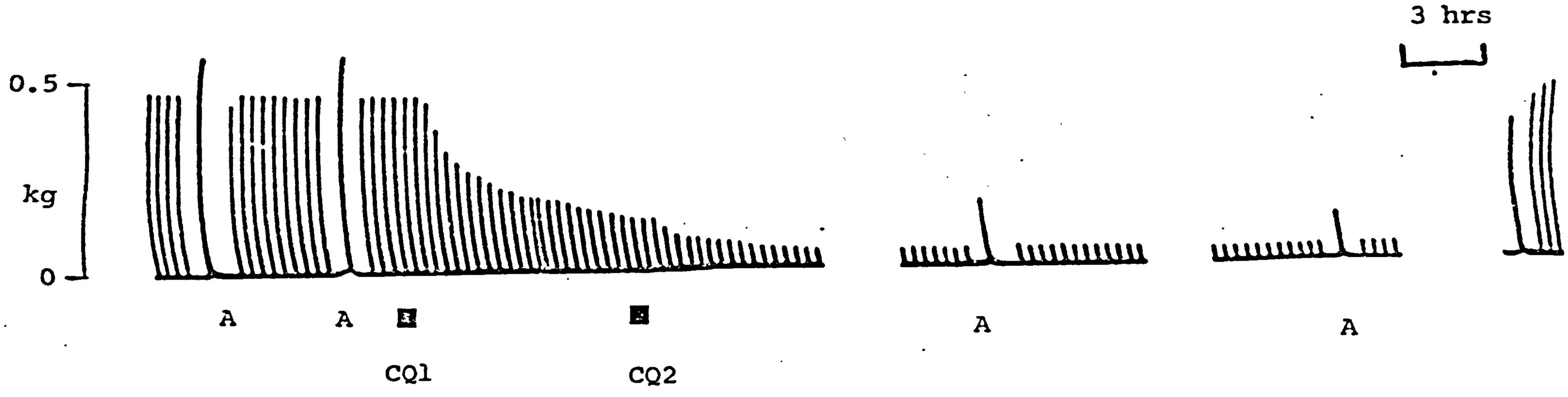


FIGURE 75

Anaesthetized cat. Effect of chloroquine (CQ) on maximal twitches of a tibialis anterior muscle of an anaesthetized cat stimulated via the motor nerve (0.1 Hz) once every 10 sec. At A, electrical stimulation was temporarily stopped and 7 µg of acetylcholine was injected close-arterially. At CQ1 and CQ2, 12.5 mg/kg and 15 mg/kg chloroquine were injected intravenously respectively. During the block of the twitches by chloroquine, the responses of the muscle to intra-arterially injected acetylcholine were inhibited; showing that chloroquine acts post-junctionally.

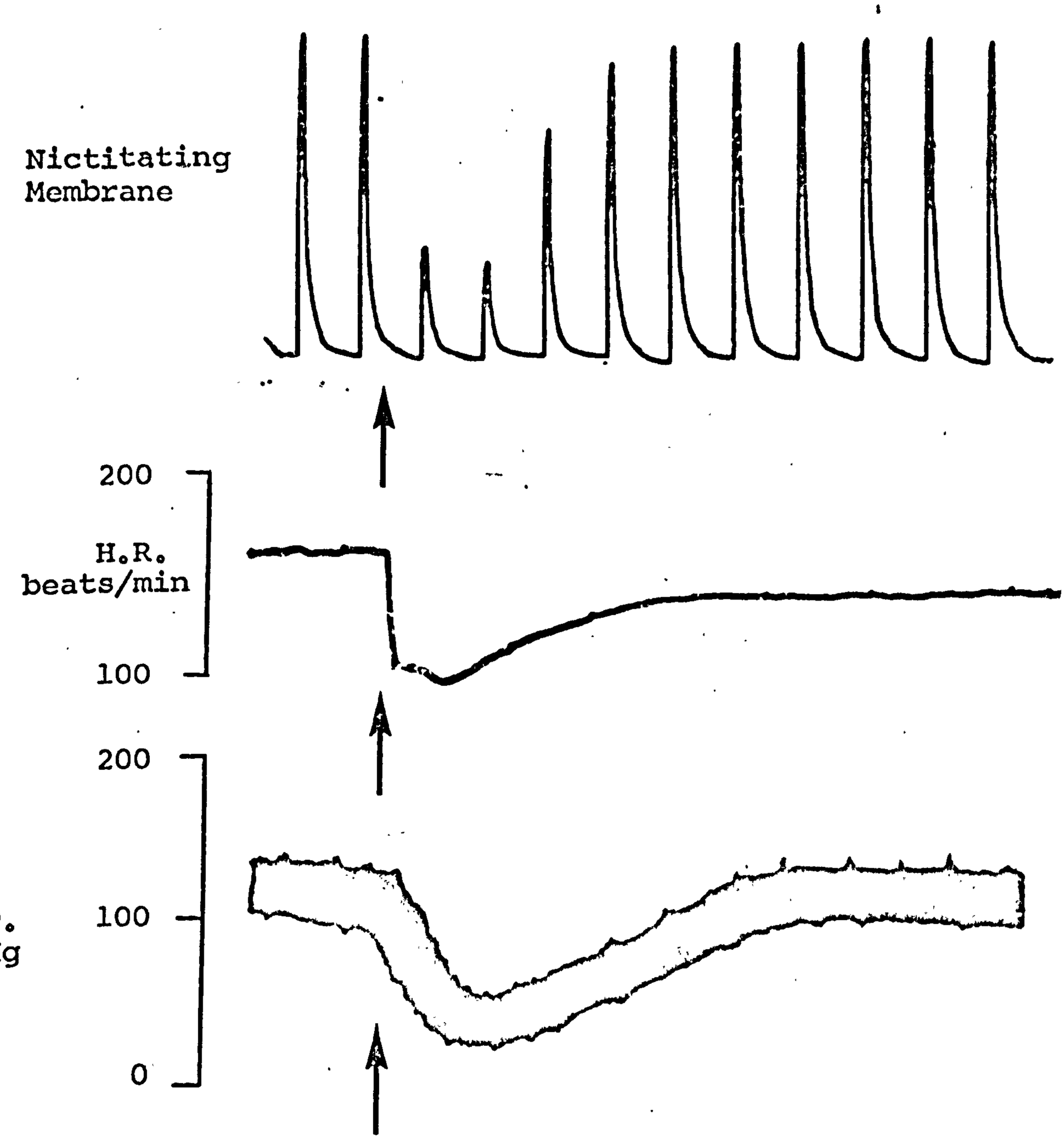


FIGURE 76

Anaesthetized cat. Effect of primaquine (PQ, 10 mg/kg, injected intravenously at the upward arrow) on the electrically-induced contractions of the nictitating membrane (upper panel), and on the heart rate (middle panel) and arterial blood pressure (lower panel) of an anaesthetized cat.

recovering from either partial, or full, blockade by d-tubocurarine and suxamethonium (as in the soleus muscle, Figures 70 and 71). In similar doses, these compounds lengthened the recovery time of the muscle (as in the soleus) by 3 - 45 minutes, depending on the compound injected and the dose administered (see Figure 70). Furthermore, in medium to high doses (8 - 16 mg/kg), all the antimalarials decreased, in a dose-dependent manner, the maximum amplitude of tetanic contractions and also caused a fade of maintained tension (Figure 73).

Close-arterial injections of all the compounds (10 - 16 mg/kg) produced dose-related decreases in twitch height evoked by indirect electrical stimulation. Recovery was much delayed (by 10 - 130 minutes). Figures 74 depicts a typical trace obtained. Moreover, the responses of the muscle to acetylcholine (5 - 7 µg, by close-arterial injection) were inhibited, or abolished, by these doses. (Figure 75).

In these concentrations (10 - 16 mg/kg) all the antimalarial drugs studied reduced the height of contraction of the cat nictitating membrane evoked by indirect electrical stimulation in a dose-related manner. (Figure 76 shows a typical trace obtained) and, at all concentrations (1 - 20 mg/kg), decreased systemic arterial pressure and heart rate. The cardiovascular effects of antimalarial drugs are examined in detail in Section 4 of this thesis.

DISCUSSION

The results obtained in this study demonstrate the pharmacological effects of some antimalarial compounds on various skeletal muscles and shed some light on their possible mechanism of action. The ability of the agents, at relatively low to medium concentrations, to potentiate Ach-induced contractures of the frog rectus abdominis and chick biventer muscles is probably a consequence of their anticholinesterase activity. The results obtained from the anticholinesterase determination lend support to this conclusion, which is in agreement with the work of Blaschko, Chou and Wadja (1947), Wright and Sabine (1948), and other more recent investigators (Garcia, Miyares and Sainz 1971, Ayitey-Smith and Vartanian 1975). However, at those particular dose levels, the agents might also be augmenting Ach-induced contractures of the muscles by inhibiting Ca^{2+} uptake into the sarcoplasmic reticulum (Batra 1974). Nevertheless, the suggestion that the effects are mediated through an effect on cholinesterase is strengthened by the observation that doses of the compounds which potentiated Ach-induced contractures were either without effect on, or inhibited, carbachol-induced contractures of the same muscle preparations.

The non-competitive inhibition of carbachol-and potassium-evoked contractures, as well as of Ach-induced contractures of physostigmine-treated muscles, probably indicates a non-

specific antagonism. This may suggest that the action might not involve a drug-receptor interaction. Similarly, the non-competitive antagonism of Ach-induced contractures by moderate to high doses of all the antimalarials may also be due to a "non-specific" effect of the drugs.

The dose-related contractures of the frog rectus and chick biventer cervicis muscles caused by moderate to high concentrations of all the antimalarial drugs can probably best be explained in terms of Ca^{2+} release from the sarcoplasmic reticulum. It is now generally accepted that in skeletal muscle, contraction is coupled to excitation through the release of calcium from the sarcoplasmic reticulum (Hasselbach 1964; Weber 1966; Sandow 1965; Ebashi and Endo 1968). It has also been suggested that agents that induce contractures, and potentiate twitches, act either directly or indirectly on the sarcoplasmic reticulum to cause a release of Ca^{2+}, or an inhibition of its uptake, thereby increasing the free myoplasmic Ca^{2+} concentration (Carvalho 1968; Fuchs et al., 1968; Weber and Herz 1968; Ogawa 1970, Balzer 1972, and Batra 1974). Of all the antimalarials studied, only quinine and quinidine have been reported to cause a release of Ca^{2+} at moderate to high dose levels, and to cause an inhibition of Ca^{2+} uptake at relatively lower doses. However, since all the antimalarial compounds investigated produced similar effects on skeletal muscle, it is likely that high doses of all the synthetic antimalarials, like quinine and quinidine,

cause a release of Ca^{2+} from calcium stores in the skeletal muscles in an as yet obscure way. This Ca^{2+} releasing effect of the antimalarial agents would account for the contractures of the frog rectus and chick biventer muscles produced by moderate to high doses of the compounds.

All the antimalarial drugs studied affected the twitches of both slow and fast-contracting muscles induced by electrical stimulation. The potentiation of the twitch heights of electrically stimulated chick biventer cervicis and rat hemidiaphragm muscles by relatively low to medium concentrations of quinine has been thought to be probably due to the ability of the drug to inhibit Ca^{2+} uptake, and thereby to increase the free myoplasmic Ca^{2+} concentration. Low to medium doses of other antimalarials that also potentiate the twitch height are also likely to exert their action through a similar mechanism.

High concentrations of quinine and of all other antimalarials caused a dose-dependent depression of the twitches and also induced sustained contractures of the muscles. The depressant curare-like effects of all the compounds on electrically evoked twitches of the muscles is probably related to their local anaesthetic activity (Chinyanga, Greenberger and Vartanian 1971; Chinyanga, Vartanian, Okai and Greenberger 1972; Vartanian and Chinyanga 1972) whilst muscle contraction induced by the drugs is possibly due to a Ca^{2+}-releasing action of the compounds.

Potentiation of the electrically-induced twitches of the cat soleus and tibialis anterior muscles by low to medium doses of quinine (and sometimes chloroquine and primaquine), is again probably due to their ability to interfere with Ca^{2+} fluxes and especially its release and uptake. However, low to medium concentrations of proguanil and pyrimethamine did not augment the twitches of the cat skeletal muscles. It may be that these two latter drugs are not as potent as quinine, chloroquine and primaquine in causing a release of Ca^{2+} from the sarcoplasmic reticulum, or in causing an inhibition of Ca^{2+} uptake. On the other hand, the dose-related curare-like depression produced by high concentrations of all the antimalarials in the cat is probably attributable to the local anaesthetic action of the drugs. This observation is in agreement with the work of Chinyanga et al, (1971, 1972) and Vartanian and Chinyanga (1972).

Medium to high concentrations of all the antimalarial compounds potentiated the action of muscle relaxants and neuromuscular blocking agents. The exact mechanism of this effect is still obscure but may be related to their local anaesthetic action. The recurarization always produced by the antimalarials on skeletal muscles just recovering from either a partial or a full effect of d-tubocurarine may also be due to this action by producing neuromuscular blockade of those muscle fibres spared by d-tubocurarine.

All the five antimalarial drugs studied independently produced a depression of tentanus in the tibialis anterior muscle and also interfered with its maintenance. Similar observations with quinine were made by Harvey (1939). Oester et al., (1939) attributed this effect to a curare-like action, a prolongation of refractory period and a decrease in muscle excitability. However, it is of interest that physostigmine produced similar effects though in much lower concentrations. Since the antimalarial agents have also been shown to possess anti-cholinesterase activity (Wright and Sabine 1948; Blaschko et al., 1947; Garcia et al., 1971; Ayitey-Smith and Vartanian 1975), it is likely that this action on the tension and degree of fusion of tetanic contractions of the cat tibialis anterior muscle is mediated through an anticholinesterase mechanism.

Femoral arterial injections of medium to large doses of all the antimalarials produced two-fold effects on both the soleus and the tibialis anterior muscles of cats:

1. A sharp and dose-related decrease in the twitch amplitude of the indirectly stimulated muscles. This depression persisted for a long time (30 - 120 minutes), especially after primaquine and chloroquine, and is probably caused by a quick depression of neuromuscular transmission;
2. A slowly-developed decline in muscle tension which is presumably due to the effect of the drugs on the biochemical processes of the muscle.

Similarly, all the drugs blocked the response of the tibialis anterior muscle to intra-arterially injected acetylcholine. Since it is usually assumed that any drug that acts post-junctionally (eg. tubocurarine) will block the tibialis anterior muscle response to intra-arterially injected acetylcholine, it is likely that, in medium to high doses, the antimalarials act post-junctionally.

Adrenaline enhanced electrically-induced twitches of the tibialis anterior muscle. This observation is in accordance with the findings of several investigators (West and Zaimis 1949; Brown, Goffart and Vianna-Dias 1950; Goffart and Ritchie 1952; Montagu 1955; Bowman and Zaimis 1955, 1958; Bowman, Goldberg and Raper 1962; Bowman and Raper 1962, 1965, 1966, 1967). It is generally agreed that this effect of adrenaline is independent of neuromuscular transmission and is exerted on the muscle fibres themselves. Because this effect of adrenaline can be blocked by propranolol, it is further agreed that the effect of this catecholamine is mediated through beta-(β-) adrenoceptors, (Bowman and Raper 1967). Relatively low to medium doses of the aminoquinoline antimalarials acted like adrenaline and enhanced electrically-evoked twitches of the tibialis anterior muscle. This effect was partially antagonized by propranolol. It is however unlikely that this action of the antimalarials involves a release of catecholamines, either locally or from the adrenal medulla, because the compounds produced a dose-

dependent decrease in blood pressure and heart rate, whereas adrenaline and noradrenaline produced dose-related increases in blood pressure and in heart rate. It is therefore likely that this effect of low to medium doses of quinine and aminoquinoline antimalarials is either due to a release of Ca^{2+}, or inhibition of Ca^{2+} uptake, or both. It is also probable that propranolol merely antagonizes the effects of the compounds on the muscle in a non-specific way.

During the incomplete tetanic contractions of the soleus muscle, fusion and tension are markedly reduced by adrenaline. These effects are independent of the concomitant blood flow changes in the muscle (Bowman and Zaimis 1958; Bowman and Raper 1962, 1967). Medium to high concentrations of all the antimalarials acted like adrenaline on this muscle. They reduced the twitch heights, tension and the degree of fusion of incomplete tetanic contractions. However, it is unlikely that catecholamines are involved in this action of the antimalarials. This idea is strengthened by the fact that injections of any of the antimalarial compounds produced a dose-related fall in blood pressure and a decrease in heart rate, and also inhibited contractions of the nictitating membrane induced by sympathetic nerve stimulation, whereas noradrenaline and adrenaline produced opposite effects.

All the antimalarial drugs investigated reduced the height of contraction of the cat nictitating membrane evoked by indirect electrical stimulation. This observation is in agreement with the result obtained by Vane (1949) for proguanil. This action is probably linked with their local anaesthetic activity.

From the experimental evidence presented in this section, it appears that all the antimalarial drugs, have a similar action on skeletal muscle. In human subjects, both therapeutic and high doses of the compounds induce neuromuscular weakness. As local anaesthetics, they presumably block neuromuscular transmission by interfering with the release of acetylcholine in response to the nerve action potential. Their ability to potentiate competitive and depolarizing muscle relaxants is also probably mediated through the same mechanism. The antiveratrinic (membrane stabilising) properties of the antimalarials have been investigated by Arora (1955), and it has been shown that amodiaquine (camoquine), chloroquine (avlochlor) and mepacrine are more potent than quinine in this respect. This probably indicates that among the antimalarial drugs, the depressant property is related to this membrane stabilising property (Grewal and Sharma 1960). Quinine is currently being used as a drug of choice in the treatment of various types of myopathies, especially congenital myotonia, because of its membrane stabilising action (Arora 1955). As there seems to be a correlation between

the depressant action of the antimalarials and their membrane stabilising action (Arora 1955), and in view of the fact that chloroquine and primaquine are more potent and have a more prolonged action than quinine, the two aminoquinoline drugs might prove more effective than quinine in the treatment of congenital myotonia and allied clinical conditions of skeletal muscle dysfunction (Arora 1955). It is hereby suggested therefore, that chloroquine and primaquine deserve clinical trials in those diseases.

SECTION 4

CARDIOVASCULAR PHARMACOLOGY OF THE ANTIMALARIAL DRUGS

INTRODUCTION

4.1 Actions of quinidine and quinine on cardiac muscle

There are relatively few documented reports on the cardiovascular actions of the antimalarial drugs in literature. Most of the work done concerns quinidine, the dextrorotatory isomer of quinine, which has long been established as an anti-dysrhythmic drug, and is claimed to be the most active of the cinchona alkaloids in reversing cardiac dysrhythmias (Wenckeback 1923). The renewed interest in quinidine for clinical purposes dates from 1918 when Frey described its effects in auricular fibrillation. Since then, there have been many attempts to analyse these effects in laboratory animals and in man. Many investigators have studied the effects of quinidine on the size of the auricle and ventricle, blood pressure, vagal and sympathetic tone, peripheral circulation, sino-atrial (S-A) and atrio-ventricular (A-V) conduction and the refractory period of the heart.

Lewis (1921) reported that quinidine depressed excitability, slowed conduction velocity and heart rate, increased the refractory period and caused a decrease in vagal tone of the heart muscle. In 1937, Starr, Gamble, Margolios, Donal, Joseph and Eagle found that larger doses of quinidine accelerated the heart rate. This vagolytic effect was felt to be a potential drawback in certain situations where the drug had to be administered to man. Gold (1950) reviewed

the pharmacology and clinical uses of quinidine in the treatment of cardiac dysrhythmias. Subsequent reviews have been published by Sokolow (1951), Conn (1964), Lyon and De Graff (1965), Bellet (1971, 1972) and Avlado and Salem (1975).

The well-known anti-dysrhythmic activity of quinidine has been suggested to be due, at least in part, to its influence on the refractory period. Besides the lengthening of the refractory period induced by quinidine, a reduction of conduction velocity might also play some part (Besch, Marks and Dutta 1969). According to Heistracher (1971), quinidine probably increases the refractory period by reducing membrane permeability for Na^+ and K^+ ions during excitation, thereby inhibiting the Na^+/K^+ - activated ATP-ase. The interference of quinidine with lipids (Mokler and Mathur 1968) has been thought to contribute to the diminished membrane permeability.

Experimental evidence has shown that quinidine has a biphasic action on myocardial contractile force. A positive inotropic effect of quinidine has been reported in rat and guinea-pig isolated right atria (Kruta 1964), and in the rabbit isolated right atrial preparation driven at a low frequency (Kennedy and West 1969). After the administration of low to moderate doses of quinidine (1.0×10^{-4}M), Zetler and Strubelt (1971) and Lamoijer and Zwieten (1974) also obtained an increase in contractile

force of isolated, electrically stimulated guinea-pig atria. Zetler and Strubelt (1971) attributed the positive inotropic action of quinidine on cardiac muscle to the release of endogenous catecholamines. Lameijer and Zwieten (1974) found that the small increase in contractile force brought about by quinidine (a maximum of 10% of the initial value) remained unchanged after 3 mg/kg reserpine pre-treatment, even though this level of reserpinization markedly blocked the action of tyramine (10^{-5} to 10^{-4}M). On the basis of this observation, these workers disagreed with the early hypothesis of Zetler and Strubelt (1971) regarding the possible mechanism of action of quinidine's positive inotropic effect on the heart. Himori and Taira (1976) have similarly reported that low doses of quinidine (0.01 - 0.1 mg) produced a positive inotropic effect in blood-perfused canine papillary muscles. These investigators observed that the positive inotropic effect of quinidine was not modified by a dose of propranolol which inhibited completely the positive inotropic response to noradrenaline, and therefore suggested that the effect of quinidine is unlikely to be mediated via an adrenergic mechanism. The authors thought that the positive inotropic response of lower doses of quinidine might be due to an increase in the availability of calcium ions for the contractile machinery since it has been shown that in the dog heart quinidine causes the release of calcium from the sarcoplasmic reticulum and mitochondria and inhibits the uptake of calcium

ions by these organelles (Fuchs, Gertz and Briggs 1968).

On the other hand, quinidine, in high doses, is generally accepted to be an agent which depresses the contractility of the heart (Parmley and Braunwald 1967; Mierzwiak, Mitchell and Shapiro 1967; Covino and Shannon 1969; Goodman and Gilman 1975; Himori and Taira 1976). According to Himori and Taira (1976) the negative inotropic effects of quinidine may be a consequence of the total imbalance in the extra-cellular or intra-cellular mobilization of calcium ions needed for maintenance of myocardial contraction.

Quinine, the prototype antimalarial agent, shares all the aforementioned pharmacological actions of quinidine.

4.2 Actions of synthetic antimalarial compounds on cardiac muscle

The anti-dysrhythmic and cardiovascular actions of the synthetic antimalarial drugs - the 4- and 8- aminoquinolines and mepacrine, proguanil and pyrimethamine - have also been investigated. The anti-dysrhythmic action of chloroquine, primaquine and mepacrine has been demonstrated, among others, by Arora (1955); Arora, Sharma and Madan (1955); Arora and Madan (1956); Arora, Madan and Pathak (1956); Sanabria (1955); Hoss and Schmidt (1959). The cardiac effects of the antimalarial compounds have been attributed to their membrane stabilising action by Arora (1955) and Arora et al,

(1955). Those authors observed that the antimalarial agents as a group prolonged the conduction time and refractory period as evidenced by lengthening of P-Q and Q-T intervals of the ECG respectively, and also reduced the heart rate.

4.3 Actions of other quinoline antimalarials on cardiac muscle

Agarwal and Arora (1956) found that chloroquine and amodiaquine were effective in halting auricular fibrillation and attributed this effect to their anticholinergic action. Chloroquine has been shown to exert a more effective anti-fibrillatory action in the heart than quinidine (Arora et al, 1955). The fibrillating-combating properties of chloroquine were studied by Arora and his colleagues (1956) in mongrel dogs with atrial flutter induced by the injury-stimulation procedure using quinidine sulphate and procainamide as reference standards. The average total dose of chloroquine producing reversion of the flutter to normal sinus rhythm was found to be 6.2 mg/kg, whilst doses of 21.1 mg/kg of quinidine sulphate and 33 mg/kg of procainamide were required to produce equivalent effects. In a few other reports, amodiaquine and primaquine have been shown to be more effective than quinidine in reversing atrial fibrillation (Arora and Madan 1956 ; Arora, Madan and Pathak 1956; Arora and Madan 1956). In 1960, Arora and Arora showed that amodiaquine possessed quinidine-like

action on the refractory period of isolated rabbit atria and in dogs had a stronger antifibrillatory action on acetylcholine-induced atrial fibrillation than quinidine. These investigators also reported that amodiaquine differed from quinidine in not protecting against epinephrine-induced ventricular dysrhythmias and in causing a greater slowing of conduction rate and less prolongation of the refractory period. In a separate report, Arora and Madan (1956) showed primaquine to be more effective than quinidine in protecting dogs against atrial fibrillation induced by the topical application of aconitine and against atrial flutter caused by crush stimulation of the atria, but did not show protective action against epinephrine-hydrocarbon-induced ventricular dysrhythmias. These workers further showed by studying the electrocardiogram that primaquine increased the refractory period and conduction time of heart muscle more than quinidine. In 1959, Hess and Schmidt showed that intravenous injections of chloroquine were capable of terminating aconite-induced atrial fibrillation in the open-chest dog preparation. These authors claimed that chloroquine and quinidine were equally effective in depressing the resting excitability of isolated cat papillary muscle. The workers further observed that chloroquine depressed conduction velocity very little compared with quinidine, and that although the muscle recovered from the chloroquine-induced depression, it showed little recovery from the effects of quinidine. In experiments by Hess and Haugaard (1956) and Hess (1954)

with rat heart slices and homogenates, it was found that whilst quinidine and quinine strongly depressed oxygen uptake and glucose utilisation, chloroquine and procainamide had no such depressant effects. Sanabria, Carbonell and Soto (1959) studied the actions of chloroquine on the enzymes of the conduction system in the dog heart and found that it inhibited succinic dehydrogenase. Such an inhibition was not apparent after injections of quinidine or procainamide. Since the enzyme should act in the Krebs cycle on transformation of succinic acid to fumaric acid, it was assumed that inhibition of this enzyme would render the energy required for conduction of the cardiac impulse unavailable. These workers therefore suggested that the anti-fibrillatory action of chloroquine could be attributed to this enzyme inhibition effect. Holland and McCutcheon (1962) also observed that amodiaquine and primaquine were as effective as quinidine in shortening the duration of experimentally-induced atrial fibrillation but were more potent anti-dysrhythmic agents than quinidine.

In 1968, Jindal, Pandya and Kelkar found that amodiaquine, and to a lesser extent chloroquine, increased the sensitivity of ventricles to adrenaline-induced dysrhythmias and abolished the cardio-inhibitory response to acetylcholine. These authors attributed the former effect to a release of noradrenaline from tissue amine stores. Several other investigators have also reported the myocardial effects of antimalarial compounds. Chloroquine, chloroquine quinethiolate

and chloroguanide have been shown to depress myocardial contractility, atrial muscle excitability and cardiac output in laboratory animals (Aviado, Inoh and Cho 1968a; Aviado and Bollet 1969; and Aviado, Sadavongvivad and Cambar 1970).

In recent years, the importance of adenosine as an important constituent of blood capable of influencing myocardial contractility and coronary blood flow has become increasingly obvious (eg. Berne 1964). All the antimalarial drugs, although chemically dis-similar, possess anti-dysrhythmic properties (Burno, Burstein and Di Palma 1954; Nottleton, Poyser and Shorter 1974). One mechanism has been offered to explain the cardiac effects of these drugs. Madinaveitia and Raventos (1949) reported that antimalarial drugs can block some of the electrocardiographic effects of adenosine. The status of the anti-dysrhythmic activity of primaquine and other 8-aminoquinolines remained relatively uncertain until quite recently. Burno et al, (1954), reported the ability of pentaquine to prevent experimental dysrhythmias in cats. Arora and Madan (1956) confirmed the anti-dysrhythmic effect of pentaquine in dogs and noted that primaquine and pentaquine had a similar action. The subsequent observations of Angelakos and Hegnauer (1959) failed to indicate any such activity of these 8-aminoquinolines. Bass, Ramirez and Aviado (1972) have demonstrated the anti-dysrhythmic activity of primaquine and other 8-aminoquinolines. Ventricular fibrillation induced by

chloroform inhalation was prevented by pretreatment of the experimental animals with primaquine or any one of the 8-aminoquinolines. These investigators further found that primaquine antagonized some of the cardiac effects of adenosine (bradycardia and atrio-ventricular block) and suggested consequently that the drug might be depressing the heart muscle in a manner reported previously by Madinaveitia and Raventos (1949) and Bass and Aviado (1972). Therefore, Bass et al, (1972) concluded that primaquine like all other 8-aminoquinolines, depressed cardiac muscle. These authors claimed further that primaquine blocked β-adrenergic receptors as shown by its depression of the cardiac muscle response to isoproterenol. They observed a reduction in blood pressure in response to common carotid clamping and to hypoxia and attributed this to depression of a central sympathetic pathway (as also postulated by Moe, Peralta and Seevers (1949). In conclusion, Bass et al, (1972) assumed that primaquine probably interfered with the cardiovascular system at two distinct sites, viz:

(1) β-adrenergic blockade in the heart, and
(2) central blockade of pathways responsible for a rise in blood pressure.

4.4 Actions of non-quinoline antimalarial drugs on cardiac muscle

The actions of non-quinoline antimalarial agents on cardiac muscle have been investigated by a number of workers.

Vane (1949) studied the action of proguanil (paludrine) on the heart and cardiovascular system and found that the drug lengthened the refractory period of cardiac muscle. When he compared the activity of the compound with that of quinidine using Dawes's method, paludrine was found to be approximately one-eighth as active as quinidine in prolonging the refractory period of the myocardium. On the isolated perfused cat heart (Langendorff preparation), paludrine was found to inhibit the amplitude of contraction and rate of beating of the heart in a dose-dependent manner. This inhibition was accompanied by coronary dilatation. Paludrine was also observed to depress both contractility and rate of isolated rabbit auricles. At the same time, the normal inhibitory action of acetylcholine was found to be changed to stimulation. Burn and Vane (1949) further established that acetylcholine would restart auricles whose beating was stopped by paludrine. Anti-fibrillatory effects of proguanil and pyrimethamine have been reported in a number of other heart muscle preparations (Burne et al, 1954; Armitage 1957; Armitage and Burn 1957; and Arora and Madan 1956). Proguanil and pyrimethamine, unlike the quinoline anti-malarial compounds (quinine, quinidine, chloroquine, amodiaquine and primaquine), have not been shown to possess any sympathomimetic effects (see Chen and Anderson 1947; Vane 1949; Gierlotka 1950; Burne et al, 1954; Jindal 1956; Arora and Madan 1956 , 1956 ; and Abdel-Aziz, Karrar and Idris 1971). The findings of these earlier workers have been recently strengthened by Nettleton, Poyser and Shorter

(1974) who obtained neither a rise in blood pressure, nor cardiac acceleration, following the intravenous administration of pyrimethamine (3 - 10 mg/kg) to dogs. Armitage, Burn and Gunning (1957) studied the influence of some anti-malarial substances on electrically-induced ventricular fibrillation of the isolated rabbit heart. These authors found that the antimalarial compounds chloroquine, mepacrine and pyrimethamine arrested persistent ventricular fibrillation and restored a normal rhythm. In 1970, Matsuo, Ruiz, Smith and Aviado demonstrated that pyrimethamine prevented chloroform-induced dysrhythmias in mice, reduced cardiac output in anaesthetized dogs and depressed ventricular function in dog heart-lung preparation. They also found that chronic administration of pyrimethamine prolonged repolarization time, depolarization time and cycle length of the transmembrane action potential of rat atrial muscle. These workers further showed that pyrimethamine had no effect on the catecholamine content in the hearts of mice.

4.5 Influence of ionic fluxes on cardiac muscle effects of antimalarial drugs

The role of acetylcholine in normal and abnormal cardiac dysrhythmias has now been well documented. Acetylcholine can induce auricular fibrillation under certain conditions (Burn, Vaughan Williams and Walker 1955), and according to Briscoe and Burn (1954), the anti-fibrillatory action of quinidine could be mediated through some interference in

the mechanism of acetylcholine. Dawes (1952) observed that a negative after potential in heart muscle was decreased by acetylcholine. Paludrine and quinidine reduced the acetylcholine content of the brain and heart, and gave some protection against minimal electroshock seizures in rats (Bose, Saifi and Sharma 1963). Several authors have shown that acetylcholine could stimulate the efflux of potassium and influx of sodium in myocardial tissue (Bammer 1953; Burgen and Terroux 1953; Conn and Wood 1960; Holland 1957a, 1957b, Johnson and Robertson 1957; and Shanes 1959). It has been reported that quinidine depresses this ionic flux (Conn and Wood 1960; Holland 1957a, 1957b). Holland and McCutcheon (1962) observed that the ionic changes caused by acetylcholine were also depressed by amodiaquine and primaquine. The actions of proguanil and quinidine on isolated rabbit atria, particularly their antagonism of acetylcholine, have been described in detail (Burn and Vane 1949; Briscoe and Burn 1954; and McKendrick and Godfrey 1959). The two chemically unrelated antimalarial compounds apparently antagonized the action of acetylcholine in a similar manner and changed the action of acetylcholine on the atria from the usual inhibitory action to a stimulant one. A few authors have shown that the action of quinidine and other antimalarial substances on cardiac muscle largely depends on the K^+ and Na^+ ion concentrations of the surrounding medium. Armitage (1957) showed that the depression of amplitude and rate of contractions of the isolated perfused rabbit heart and of isolated rabbit atria

produced by pyrimethamine, chloroquine and mepacrine seemed to be due to a diminution of the permeability of the cardiac muscle membrane to K^+, since it could be reversed by lowering the external K^+ ion concentration. The depression caused by paludrine was not reversed in this way. However, this depression, even when it proceeded as far as arrest, was also always reversed by acetylcholine. During exposure to any of the antimalarial compounds tested, Armitage (1957) found that the normal inhibitory action of acetylcholine was converted to a stimulant action. This stimulant action of acetylcholine was assumed to be due to its effect in increasing the permeability of the membrane to K^+. Proguanil therefore resembled quinidine and was thought to produce its action by reducing the permeability of the cell membrane to K^+. Another possible explanation would be that since acetylcholine causes myocardial depression via muscarinic mechanism, and since proguanil and quinidine have been shown to possess atropine-like actions, it is possible that the drugs block muscarinic effects of acetylcholine and thereby make acetylcholine available to cause a release of noradrenaline from cardiac stores. Armitage found that after arrest by chloroquine, mepacrine and pyrimethamine, the atria always failed to respond to electrical stimulation, whereas after arrest by quinidine, they usually responded to electrical stimulation. He concluded that the action of quinidine and other antimalarial compounds in arresting fibrillation might be due to their effects in diminishing the permeability of the cell membrane to K^+, and thus lessening dysrhythmogenic

efflux of K^+. Agarwal and Deshmanker (1960) studied the influence of Na^+ ion concentration on the action of quinidine and some other antimalarial drugs on frog cardiac muscle. They found that quinidine, procainamide, proguanil and chloroquine all depressed frog ventricular contractions, but low Na^+ potentiated the cardiac depressant action of quinidine, procainamide and paludrine and reversed that of chloroquine. These workers further observed that high Na^+ and low K^+ reversed the cardiac depressant action of quinidine.

Sides and Wittels (1975) have demonstrated that primaquine is capable of inhibiting the short-circuit current which has been shown to be a measure of net transepithelial sodium flux (Leaf, Anderson and Page 1958). These authors further found that primaquine specifically inhibited the sodium-potassium sensitive, magnesium-dependent adenosine triphosphatase (Na^+, K^+-ATP ase) that is commonly associated with the transport of sodium (Skou 1965). Erythrocytes exposed to primaquine have been shown to lose K^+ and gain Na^+ in an almost 1:1 exchange without undergoing concomitant changes in their redox systems (Weed, Eber and Rothstein 1961). These findings are compatible with an inhibitory effect of primaquine on erythrocytic Na^+, K^+-ATP ase. Quinidine, which has in common with primaquine the basic quinoline ring structure, has been found to inhibit the Na^+, K^+-ATP ase activity in preparations of rat skeletal muscle (Ellis 1964) and toad cardiac muscle

(Kennedy and Naylor 1965). The ability to inhibit Na^+, K^+-ATP ase, therefore, could be a property shared by the quinoline antimalarial compounds (Sides and Wittels 1975).

4.6 Actions of quinidine and quinine on blood pressure and the peripheral circulation

It has been established that in human beings and experimental animals, especially dogs, the intravenous or oral administration of quinidine produces a fall in blood pressure (Ferrer et al, 1948; Waal 1966; Farmer and Levy 1968). The fall in blood pressure is usually coincident with an increase in leg volume and a decrease in pulmonary arterial pressure (Jackson, Friedlander and Lawrence 1922), and with a decrease in peripheral resistance (Ferrer, Harvey, Werko, Dresdale, Cournand and Richards 1948). In dogs, intra-arterial injections of quinidine reduced perfusion pressure in the perfused gracilis muscle and hind paw (Schmid, Nelson, Mark, Heistad and Abboud 1974). Thus the fall in blood pressure produced by quinidine is largely due to peripheral vasodilation. Himori and Taira (1976) have also demonstrated that quinidine caused a dose-related increase in blood flow of the isolated blood-perfused canine papillary muscle.

Several investigators have attributed an adrenergic mechanism to the action of quinidine on the cardiovascular system. The drug has been shown to reduce pressor responses to adrenaline (Nelson 1928; Hiatt 1950; Lu 1951) and it has

been suggested that quinidine blocks alpha - (α-) adrenoceptors (Conn and Luchi 1964). However, changes in blood pressure responses produced by this drug did not permit Schmid et al, (1974) to make definite conclusions regarding the mechanism of action of the drug on the cardiovascular system. Dreifus, Rabbino and Watanabe (1964) have also attributed the depressant effect of quinidine on the cardiovascular system to an adrenergic blocking activity of the drug. Angelakos, Daniels and King (1965) later concluded that this effect was due to an alpha - (α-) but not a beta - (β-) adrenergic blocking action of quinidine. Additional, but indirect, evidence that quinidine might influence sympathetic control of vascular tone was provided by the observation that the drug increased the volume of innervated, but not denervated, canine hind-limb (Lu 1951; Schmid et al, 1974).

Schmid et al, (1974) found that the intravenous administration of quinidine markedly reduced vasoconstrictor responses to sympathetic stimulation and to noradrenaline in canine gracilis muscle and hind-limb paw. Clinically, these workers observed that therapeutic doses of quinidine in man caused vasodilatation in fore-arms of subjects and significantly reduced constrictor responses to intravenous norepinephrine and to negative pressure in lower part of the body. It also produced hypotension. These authors interpreted their results as indicating that quinidine interfered selectively with vasoconstrictor stimuli (which

presumably activated alpha - (α-) adrenergic receptors) and suggested that this mechanism, as well as a possible direct vasodilator effect of the drug, might have caused the vasodilatation and hypotension observed.

4.7 Actions of other quinoline antimalarial drugs on blood pressure and peripheral circulation

Amodiaquine and chloroquine have been reported to produce both a transient hypotension and a moderate potentiation of the pressor action of injected catecholamines in dogs (Jindal 1956; Jindal and Joseph 1958). In 1968, Jindal and co-workers observed that in anaesthetized dogs pre-treated with either dexamphetamine or methylamphetamine, intravenous injections of amodiaquine and chloroquine always produced a somewhat sustained rise in blood pressure after an initial transient hypotension. They found that this pressor response was severely reduced by the alpha (α-) adrenoceptor blockers tolazoline and dibozane; the additional administration of pronethanol, a beta (β-) adrenoceptor blocking agent, totally abolished the prossor responses. Thus, amodiaquine and chloroquine appeared to evoke pressor responses through an adrenergic mechanism. In conclusion, however, these authors suggested that the pressor effects of these quinoline antimalarials might be mediated (to a large extent) through a release of catecholamines from the tissue stores but seemingly not from the adrenal medulla and that the amines could be

released in the vicinity of receptor sites and not into the general circulation. In separate reports, Jindal and Joseph (1958), and Pandya, Jindal and Kolkar (1969) demonstrated that chloroquine and amodiaquine potentiated the pressor responses of injected adrenaline and noradrenaline in dogs, and suggested that these drugs might be liberating catecholamines from the tissue amine stores and may block noradrenaline re-uptake. Deshpande and Tiwaskar (1964) have also reported the hypotensive effects of chloroquine in experimental animals. The resultant severe decrease in blood pressure following chloroquine administration was not blocked by atropine or antihistaminic drugs, and was long-lasting. These workers further observed that the pressor action of adrenaline was not affected by chloroquine and they therefore thought that the hypotensive effect of chloroquine is likely due to its depressant action on the heart.

4.8 Actions of non-quinoline synthetic antimalarial compounds on blood pressure and peripheral circulation

In anaesthetized animals, Vane (1949) found that intravenous injections of proguanil (paludrine) caused a transient fall in blood pressure. This author further observed that in the dog's hind-leg perfused with blood, the drug produced vasodilatation and increased the outflow of blood. The vasodilatation was diminished by neoantergan, an antihistaminic agent. Vane also found that paludrine antagonized the action of adrenaline, both on dog's perfused

hind-limb and on cat blood pressure. The fall in blood pressure in anaesthetized cats caused by the intravenous administration of paludrine (4 mg/kg) and histamine (10 µg/kg) was reduced by the injection of benadryl. Thus, the antihistaminic agents neoantergan and benadryl, reduced the vasodilatation and hypotension caused by paludrine. The action of adrenaline on dog's perfused leg and cat's blood pressure was also found to be inhibited by paludrine.

4.9 Cardiovascular actions of antimalarial drugs in man

The actions of the commonly used antimalarial agents on the human cardiovascular system are well-documented. Chloroquine has been employed at various times in the treatment of dysrhythmias as an alternative drug for quinidine. Burrell and Martinez (1958) found that chloroquine and hydroxychloroquine were effective in the therapy of cardiac dysrhythmias in man. These authors found that the side effects of chloroquine appeared to be fewer and less pronounced than those of quinidine and quinine. When chloroquine was administered intravenously in two patients with atrial fibrillation, Sanghvi (1956) found that the fibrillation was converted to atrial flutter. He assumed therefore that the slowing of the atrial rate appeared to be due to a vagolytic action and an increase in the refractory period. Sanabria (1955) reported 35 cases of dysrhythmias treated with chloroquine and observed

favourable effects in only about 50% of the population studied. On the other hand, Chen and Chou (1959) reported that in six out of thirteen patients, ectopic rhythm disappeared and there was marked improvement in five others. In the experience of these authors, cases which demonstrated poor response to chloroquine also showed poor response to procainamide and quinidine. Bellet (1961) observed, when the effects of chloroquine and quinidine were compared in the same patients under similar conditions, that chloroquine proved to be less dependable in abolishing or suppressing ectopic beats.

While the toxic effects of chloroquine in man have been intensively studied and recognized, its precise effects on the heart and general circulation in acute poisoning have been poorly documented. This is largely due to its rapid lethal effect (Don Michael and Aiwazzadeh 1970). However, Hess and Schmidt (1959) found that the drug reduced the excitability and conductivity of cardiac muscle but not the velocity of the cardiac impulse. They found that chloroquine had a potent myocardial depressant action. This effect was thought not to reside in the quinoline ring- although the latter was considered as the cause of vasodilatation (Hess 1954). At a cellular level, chloroquine blocked the enzyme succinic dehydrogenase in cells of conducting system and thereby interfered with the Krebs cycle (Di Palma 1960). Although its acute action on the cardiovascular system has been poorly documented, it is a known

anti-dysrhythmic agent and has also been used in the treatment of angina pectoris (Sanabria, 1955). It has been recognized as causing hypotension, and this effect has been attributed to peripheral vasodilatation by Riseman, Steinberg and Altman (1954). The effect on the electrocardiogram (ECG) in acute poisoning in man have not been previously documented, although the effects of administering the drug chronically for 1 to 21 days have been described (Scott 1950). These electrocardiographic effects consisted of T-wave inversion, S-T segment depression, and QT prolongation. These effects were reversed by potassium. According to Don Michael and Aiwazzadeh (1970), the mechanism of death from chloroquine ingestion would appear to be related to a failure of myocardial contraction aggravated by bradycardia and the appearance of ventricular rhythm in the terminal stages rather than to respiratory or vasomotor depression. These authors found that antidotal therapy with adrenaline and atropine was highly effective in patients with sustained cardiac arrest. Considering the reversal of cardiac arrest by adrenaline, they speculated on whether or not pre-treatment with sympathomimetic agents and atropine would protect against chloroquine toxicity in human beings.

Riseman (1959) clinically investigated the influence of some antimalarials in the treatment of angina pectoris. He considered that quinine, chloroquine and pentaquine (an 8-aminoquinoline drug related to primaquine) had a "marked

value" while proguanil (paludrine) was of "little or no value" in the treatment of the disease. Following therapeutic doses of proguanil administered to patients with malaria, some of whom had cardiovascular disorders, Perroni (1953) observed no ill effects in any of the patients and concluded that "proguanil is the antimalarial drug of choice for patients with cardiovascular diseases".

4.10 Aims of this study

Since the results reported in literature by clinicians and laboratory workers are often conflicting, there remains an urgent need for further investigations into the actions of antimalarial drugs on cardiac muscle, systemic blood pressure and the peripheral circulation. The series of experiments described in this section were therefore designed to:

(1) Study the effects of representatives of the major classes of antimalarial drugs on cardiac muscle and on the circulatory system of some laboratory animals;

(2) Attempt to explain the mechanisms of the known effects of the drugs on the heart, and circulation, eg. the anti-dysrhythmic and hypotensive actions of the drugs;

(3) Examine the hypothesis that:

(i) primaquine and other 8-aminoquinoline anti-malarials possess β-adrenoceptor blocking activity on the myocardium, and

(ii) that quinidine (and quinine) block either α - or β- (or both) adrenoceptors in

vascular smooth-muscle.

(4) Find out if the chronic toxic effects of the agents which often result in death could be explained in terms of their action on the cardio-vascular system.

METHODS

A: 'IN VITRO' EXPERIMENTS

4.11 Isolated cardiac muscle preparations

All the isolated cardiac muscle preparations were set up in 10 ml organ baths. The tissues were suspended in Krebs-Henseleit salt solution of the following composition (in mM): NaCl 118; $NaHCO_3$ 25; $CaCl_2$ 2.52; KCl 4.7; $MgCl_2$ 1.2; NaH_2PO_4 1.28; and glucose 5.55, bubbled with carbogen-5% carbon-dioxide and 95% oxygen and maintained at 32°C. The preparations were allowed to equilibrate, under an applied resting tension of 0.5 - 1.0 g, until the force and/or rate of contractions were stable (usually 30 - 60 minutes after setting up). The force of contractions were recorded isometrically by means of Devices force-displacement transducers (Type UF1), pre-amplifiers (Model M2P) and a two-channel pen recorder (Model M2R).

4.12 Guinea-pig isolated atria

(a) Spontaneously-beating preparations

Male and female guinea-pigs of the Dunkin Hartley strain weighing between 300 and 650 g. were killed by a sharp blow on the back of the head and bled out. The hearts were quickly excised and placed in a petri-dish containing oxygenated Krebs-Henseleit solution at room temperature. Intact right and left atria were carefully dissected out in one piece free from ventricular and connective tissues,

and avoiding damage to the pace-maker region. They were then suspended in 10 ml. organ baths containing Krebs-Henseleit solution maintained at 32°C and continuously aerated with carbogen. Each preparation (mean weight 235 ± 21 mg) was subjected to a resting tension of 0.75 g. and left in the organ bath until the spontaneous contractions were stable before challenging with drugs. Drugs were usually added to the bath in a cumulative manner and washed out three times after the maximum response was attained. Doses were repreated where possible at regular intervals of 10 - 15 minutes after the last washing.

The spontaneous amplitude and rate of contractions, as well as drug-evoked responses of the tissue were recorded isometrically as indicated earlier. The rate of contraction of the atrial strips in the absence, and in the presence, of drugs was estimated at a paper speed of 10 mm sec^{-1}.

(b) Electrically-driven preparations

The left atrium was carefully dissected free from ventricular, connective and right atrial tissues, impaled on a thin platinum wire electrode and placed in a 10 ml organ bath containing Krebs-Henseleit solution at 32°C continuously gassed with 95% O_2 - 5% CO_2 mixture. The atrium was driven electrically with square wave pulses of 5 msec duration at a frequency of 2 Hz and with supramaximal voltage of 5 - 10 volts delivered from SR1 stimulators. Paired preparations (one control and one treated) were set up to allow for changes in sensitivity. The tissues were placed under a

resting tension of 0.5 - 0.75 g and allowed to equilibrate (usually for a period of 30 - 45 minutes after mounting) until the force of contractions became stable before they were treated with drugs. Drugs were usually administered to the bath cumulatively and doses were repeated where possible at regular intervals of 10 - 15 minutes after three washings.

The electrically-induced contractions, and drug-evoked responses of the tissues, were recorded isometrically as described above. Fast recordings were made at a paper speed of 10 mm sec^{-1}.

4.13 Reserpinized guinea-pigs

Some of the experiments were carried out on atria taken from guinea-pigs treated with reserpine (5 mg/kg body weight injected intraperitoneally) 18 - 24 hours before sacrifice. Satisfactory reserpinization was confirmed by the absence of positive inotropic responses to the indirectly acting sympathomimetic amine tyramine (3×10^{-5}M).

4.14 Measurement of the properties of antimalarial compounds in antagonising the effects of isoprenaline, noradrenaline and calcium in electrically-driven left atria

Cumulative dose-response curves (usually 3) were obtained with either isoprenaline, noradrenaline or calcium until reproducible responses were obtained. Dose-response curves

to the particular agonist were then repeated, thirty minutes after the administration of the antimalarial agent to the organ bath. Three concentrations of antimalarial drug were used in each preparation. The responses were expressed as a percentage of the maximum of each agonist cumulative-dose-response curve and dose-ratios between the third control value and the curves obtained in the presence of the test drug were calculated at the 50 per cent level. Special note was made of any change in the absolute maximum response obtained with the agonists. Calculations of pA_2 values for antagonism of the positive inotropic response to (-) noradrenaline and (-) isoprenaline were carried out by the method of Arunlakshana and Schild (1959) and the slope of the plots for each agonist/antagonist pair calculated by computer regression analysis. The responses to calcium were treated similarly to give 'apparent pA_2 values' in order to give some indication of relative antagonist potency of the antimalarial drugs against this agent.

4.15 Rabbit isolated papillary muscle

Rabbits of either sex weighing between 1.8 and 4.0 kg were killed by a blow on the back of the head and bled out. The hearts were quickly removed from the thorax and placed in a beaker containing Krebs-Henseleit solution at room temperature. Papillary muscles, ranging in length from 4.1 to 6.2 mm (mean 5.3 mm) and weighing between 2.2 and

4.9 mg, were excised from the right ventricles. The isolated papillary muscles were impaled on thin platinum wire electrodes and suspended in 10 ml organ baths containing Krebs-Henseleit solution, maintained at 32°C and continuously aerated with 5% carbon-dioxide in oxygen. The muscle preparations were electrically stimulated with rectangular wave pulses of 5 msec duration at a frequency of 2 Hz and supramaximal voltages of 5 - 10 volts delivered from SR1 stimulators. The isolated muscles were subjected to a resting tension of 0.75 - 1.0 g and allowed to equilibrate for a period of 30 - 45 minutes until the force of contractions were stable before they were exposed to drugs. Drugs were usually applied cumulatively followed by three washings. Where possible, doses were repeated at regular intervals of 10 - 20 minutes after the last washing. Paired muscle preparations were always set up. In some cases, papillary muscles were isolated from rabbits pretreated with reserpine (5 mg/kg body weight as described earlier for guinea-pigs). The electrically-evoked contractions, and drug induced responses, of the isolated muscles were recorded isometrically. Fast recordings were made at a paper speed of 10 mm sec^{-1}.

4.16 Determination of antidysrhythmic activity of antimalarial compounds

The antidysrhythmic activity of the antimalarial drugs was assessed by measuring the effects of the compounds on

maximum driving frequency (MDF) and on effective refractory period (ERP) of guinea-pig isolated electrically driven left atrial preparations.

Guinea-pig left atria were isolated, set up and stimulated as described above. The method used was essentially that described by Dawes (1946) and consisted of increasing the stimulation frequency until the atrium could no longer respond to each separate stimulus. This maximum driving frequency (MDF) is inversely related to the effective refractory period (Ellis 1956). In this investigation, control runs (at a paper speed of 10 mm sec^{-1}) were carried out at 30 minute intervals until consistent measurements of MDF were obtained. The test drug was then added to the organ bath and the procedure repeated 5, 10, 20 or 30 minutes later. Changes in maximum driving frequency were expressed as percentages of the control value. Quinidine was used as the standard drug and lignocaine was also used for comparison.

4.17 Calculations

The maximum driving frequency (MDF) and the effective refractory period (ERP) were calculated as follows:

Suppose the control stimulation frequency at which atrial contraction no longer followed the frequency of stimulation = 7.0 Hz, then the maximum driving frequency (MDF) = 7.0 sec^{-1} and the effective refractory period (ERP) = $\frac{1}{7.0}$ sec = 143 msec.

Drugs which decreased the maximum driving frequency and increased the effective refractory period were regarded as possible antidysrhythmic agents.

4.18 Ouabain-induced dysrhythmias (Somani and Lum 1965)

Cardiac dysrhythmias were induced in male and female guinea-pigs (350 - 700 g) and cats (1.5 - 3.5 kg) anaesthetized with intraperitoneal injections of sodium pentobarbitone (60 mg/kg). The method used in these experiments was adopted from that of Somani and Lum (1965). The jugular veins of the guinea-pigs and femoral veins of the cats were cannulated for heparin (200 units/kg) and drug administrations. In all cases, ouabain-induced ventricular tachycardia was elicited by an initial loading dose of 50 µg/kg intravenously. This was followed 10 to 20 minutes later by additional (10 - µg/kg) doses, administered at five minute intervals, until a persistent ventricular tachycardia developed. In experiments involving alprenolol, propranolol or antimalarial compounds, the drug was administered intravenously 10 minutes after the ventricular tachycardia was established. Each dose of ouabain or the blocking agent was washed in with 0.2 ml of 0.9% $^{w}/_{v}$ sodium chloride solution (normal saline). The electrocardiogram (ECG) was recorded (using limb lead I or II) by means of an eight-channel ink-jet writing recorder (Elema-Schönander Mingograph 81) and monitored visually with the aid of an RI oscilloscope (type 9383).

B: 'IN VIVO' EXPERIMENTS

4.19 Anaesthetized cats

Cats of both sexes weighing between 1.5 and 3.5 kg were anaesthetized with an intra-peritoneal injection of sodium pentobarbitone (60 mg/kg). The trachea was cannulated but the animal was allowed to breathe spontaneously. When necessary, the animals were artificially respired with room air using a Palmer positive-pressure ventilation pump (rate 20/min; stroke volume 40 - 60 ml). The volume of the pump was adjusted to give an arterial pO_2 between 80 and 100 mm. Hg (measured by using a calibrated Radiometer electrode - for details, see Parratt 1973). A certain degree of hyperventilation was necessary (arterial pCO_2 26 $\pm$ 2 mm. Hg). In spontaneously breathing cats this is 33 $\pm$ 1 mm Hg (Parratt 1973). The right femoral vein was cannulated (for drug administration) and heparin (200 units/kg) was given intravenously. Additional small doses of the anaesthetic agent (sodium pentobarbitone) were administered intravenously when necessary during the course of the experiment to maintain anaesthesia. Electro-cardiogram (ECG) was recorded (using limb lead I or II) and visually monitored with the aid of an oscilloscope (RI Oscilloscope type 9383).

Systemic arterial blood pressure was recorded with a capacitance transducer (Elema-Schönander EMT 35) from a catheter inserted by way of the carotid or femoral artery such that the tip lay in the descending aorta or in the aortic arch.

The Elema-Schönander differentiator was calibrated by measuring the slope of the upstroke of the pressure pulse (using a paper speed of 250 mm/sec) and both systolic and diastolic blood pressures were recorded. Mean arterial pressure was obtained either by electronic integration or calculated by applying the formula: mean blood pressure = diastolic pressure + $\frac{1}{3}$ pulse pressure. Heart rate was calculated by counting the pulses from the arterial pressure (or from the ECG) record over a period of 10 seconds.

In the experiments using open chest cats, a left thoracotomy was performed between the fourth and fifth ribs and positive-pressure ventilation commenced. The chest wall was retracted to expose the heart. An incision was made in the pericardium which was then pulled upwards and retracted with forceps. This procedure caused the apex of the heart to be projected towards the opening in the chest. A suture was passed through the anterior surface of the ventricle or through the apical region. This allowed the heart to be gently pulled while a wide-bore steel needle catheter was inserted into the lumen of the left ventricle by direct puncture of the wall. Alternatively, in the closed-chest cats, a polythene catheter was manoeuvred into the left ventricle via the right carotid artery. In either case, left ventricular pressure was recorded with a capacitance transducer (EMT 34) both at high gain (10 mm Hg = 25 mm; 1 mm Hg = 1.33 mbar), for the accurate assessment of the left ventricular end-diastolic

pressure (LVEDP), and at a lower gain (100 mm Hg = 25 mm), for recording the full left ventricular pressure (LVP) pulse. As an index of myocardial contractility, the rate of rise of left ventricular pressure with time (dP/dt) was determined with an Elma Schönander differentiating circuit, and to correct for changes in after load (Mason 1969), plots of dP/dt at different isovolumic pressures were obtained at a paper speed of 1000 mm/sec. Right atrial pressure (RAP) was measured using a third capacitance transducer (EMT 33, range 0 to 33 mm Hg) from a catheter inserted by way of the right external jugular vein or femoral vein. The gain on the final amplifier was such that systemic blood pressure and ventricular pressure could be measured with an accuracy of 2 mm Hg, right atrial pressure to 0.1 mm Hg, and left ventricular end-diastolic pressure to 0.2 mm Hg. The P-R interval and QRS duration were measured from the ECG record at a paper speed of 250 mm/sec. All the above-mentioned cardiovascular and haemodynamic parameters were recorded by means of an eight-channel ink-jet writing recorder (Elema-Schönander Mingograph 81).

Rectal and mid-oesophageal temperatures were recorded with direct recording thermocouples (Ellab, Copenhagen type TE3). Mid-oesophageal temperature was taken as being representative of body temperature and was maintained between 36 and 38°C with heaters situated under the operating table.

Some of the experiments were carried out in vagotomized cats. In these animals, bilateral vagotomy was performed by sectioning the two vagal trunks in the cervical region of the animal.

Drugs were injected intravenously through the femoral vein in volumes not exceeding 0.5 ml and washed in with 0.2 ml of 0.9% $^{w}/_{v}$ sodium chloride solution (normal saline). Most of the drugs used were disolved in normal saline, hence intravenous injections of 0.9% $^{w}/_{v}$ sodium chloride solution were used as controls. In cases where the drugs were dissolved in vehicles other than normal saline, intravenous injections of such vehicles were used as controls.

4.20 Anaesthetized guinea-pigs

Adult guinea-pigs of either sex (400 - 800 g) were anaesthetized by intra-peritoneal injections of pentobarbitone sodium (60 mg/kg), or occasionally, 0.6 ml/100 g body weight of a 25% $^{w}/_{v}$ solution of urethane (ethylcarbamate), and allowed to breathe spontaneously. The right femoral vein was cannulated (for drug administration) and heparin (60 units/kg) was immediately administered. The right carotid artery was cannulated for the purpose of recording arterial blood pressure.

Systemic blood pressure (recorded by means of a capacitance transducer EMT 35) and electro-cardiogram (using limb lead I or II) were recorded by means of an Elema-Schönander

Mingograph 81. Mean blood pressure was obtained from electronic integration of the pulse pressure or calculated from the formula:

$$\text{mean blood pressure} = \text{diastolic pressure} + \frac{1}{3}\ \text{pulse pressure.}$$

Heart rate was calculated from the ECG record or from the arterial pulse and expressed in beats/minute.

Drug and control solutions were administered intravenously as described earlier for the cats.

RESULTS

A: 'IN VITRO' EXPERIMENTS

4.21 Isolated cardiac muscle preparations

On the basis of their actions on cardiac muscle, all the antimalarial drugs studied have been separated into two groups. The quinoline antimalarial compounds (chloroquine, primaquine, quinine and quinidine) form a distinct family of compounds with identical actions on the myocardium. On the other hand, proguanil and pyrimethamine form another class of compounds with strikingly similar actions on cardiac muscle. In the figures shown in this section, therefore, representative samples have been chosen from each of the two groups in order to avoid duplication and repetition.

4.22 Effects on rate of contraction of isolated spontaneously-beating paired atria

Both proguanil and pyrimethamine at all dose levels used (1.0×10^{-8} - 1.0×10^{-3}M) caused dose-related and relatively transient decreases in the rate of beating of guinea-pig paired atria (Figure 77). On the other hand, each of the quinoline antimalarial compounds (primaquine, chloroquine and quinine) caused a slight positive chronotropic effect (+5 - 14% of control values) at relatively low concentrations (7.5×10^{-8} - 5.0×10^{-5}M) and produced a negative chronotropic effect of long duration at concentrations greater than 7.5×10^{-5}M. This depressant response of high concentrations of the quinoline antimalarials was still

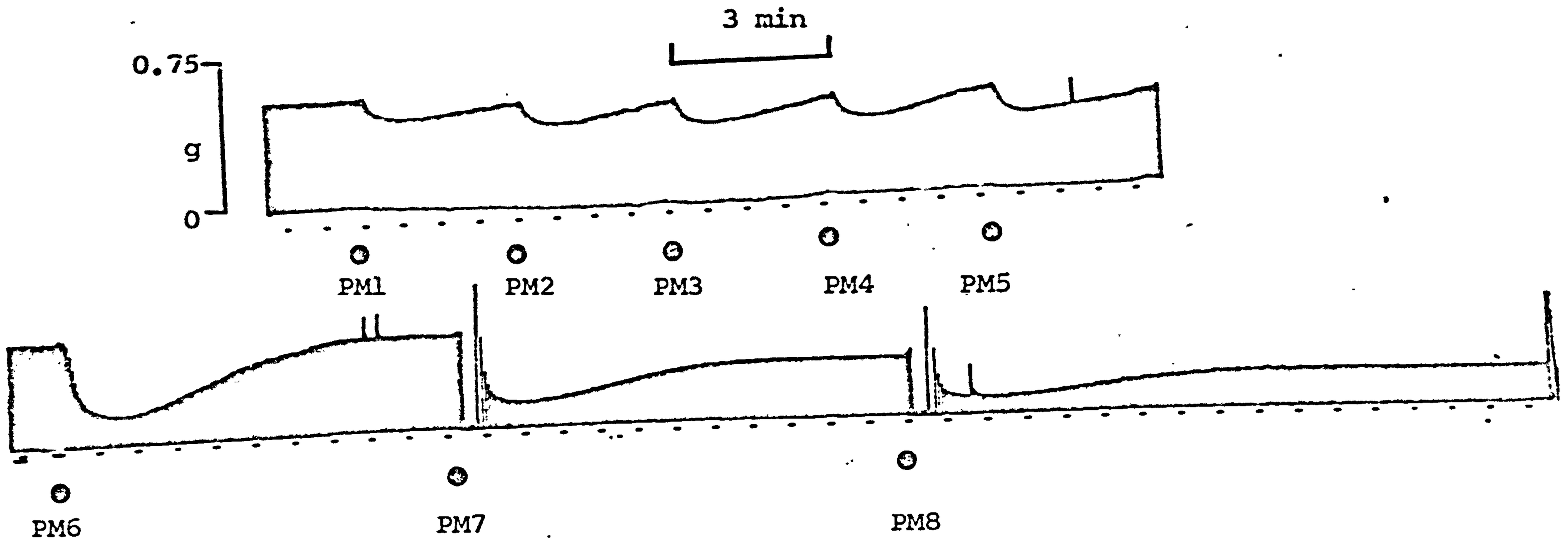


FIGURE 77

Guinea-pig isolated spontaneously-beating paired atria. Effect of pyrimethamine (PM) on isolated spontaneously-beating guinea-pig atria. PM1, PM2, PM3, PM4, PM5, PM6, PM7 and PM8 represent pyrimethamine, 5.0×10^{-8}M, 1.0×10^{-7}M, 5.0×10^{-7}M, 1.0×10^{-6}M, 5.0×10^{-6}M, 1.0×10^{-5}M, 5.0×10^{-5}M and 1.0×10^{-4}M respectively. The maximum effect occurred within 1 minute of administration.

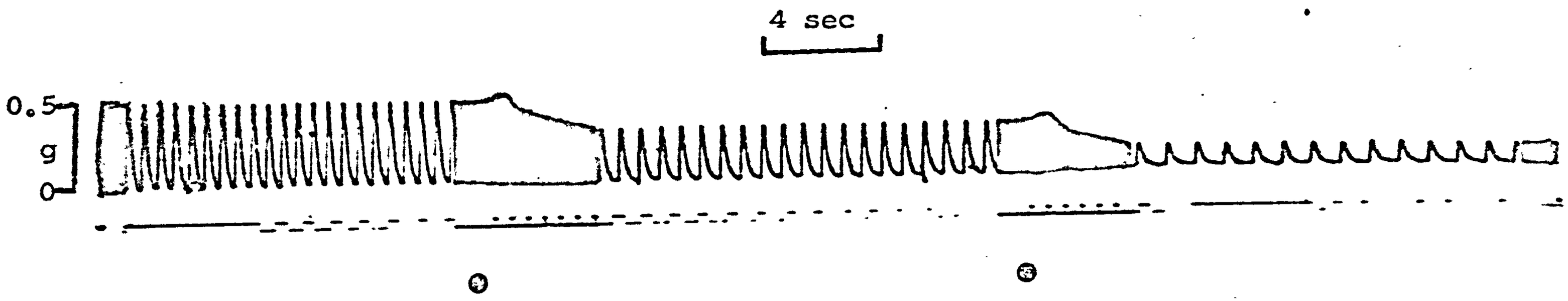


FIGURE 78

Guinea-pig isolated spontaneously-beating atria. Chronotropic effect of chloroquine (CQ) on isolated spontaneously-beating guinea-pig atria. CQ1 and CQ2 denote chloroquine, 1.0×10^{-4}M and 5.0×10^{-4}M respectively.

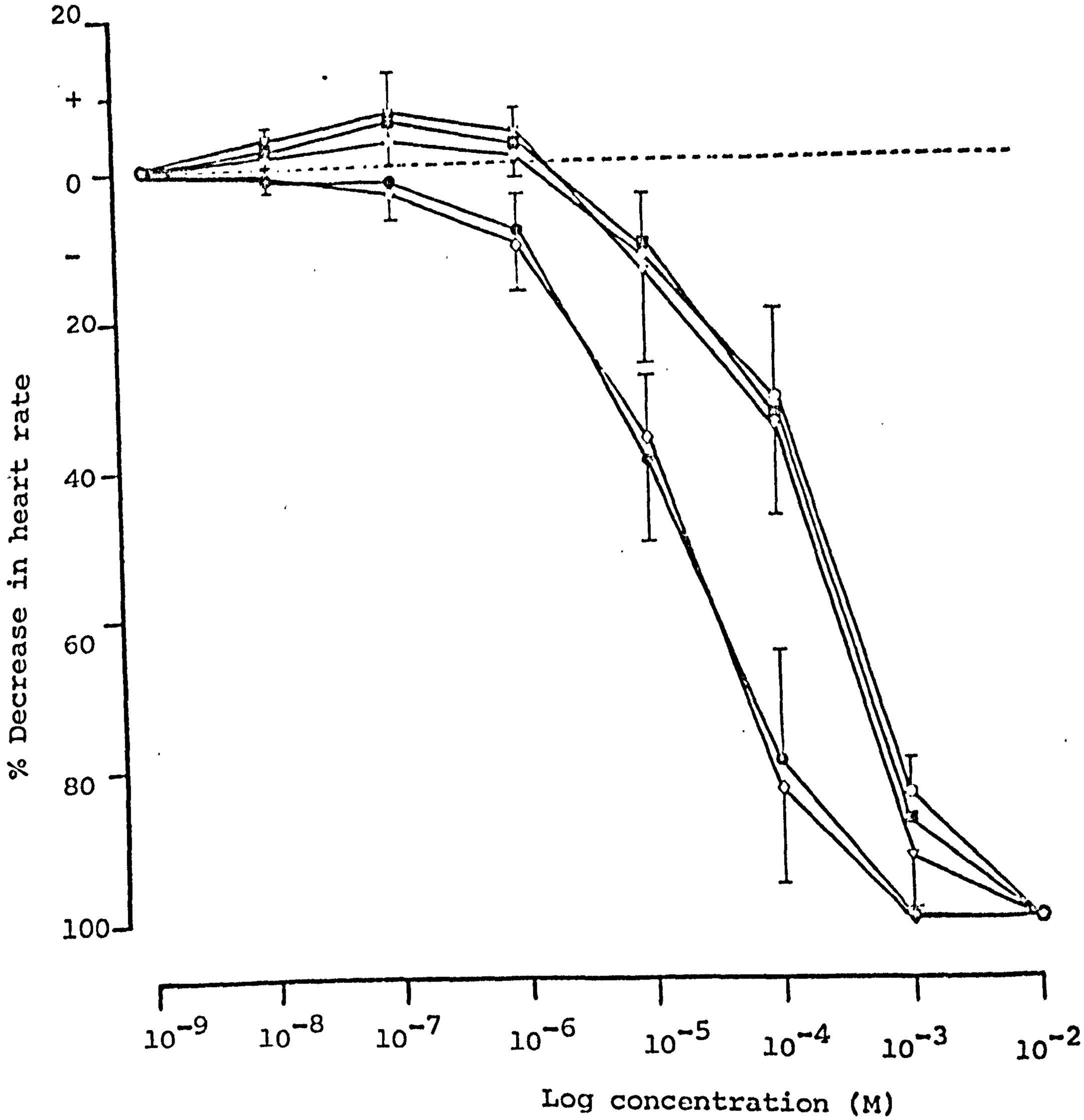


FIGURE 79

Guinea-pig isolated spontaneously-beating atria. The chronotropic effects of primaquine (▽), chloroquine (◘), quinine (○), proguanil (◐) and pyrimethamine (◇) on spontaneously-beating guinea-pig atria. Each point is the mean of 5 - 8 observations. The vertical bars represent s.e. of means.

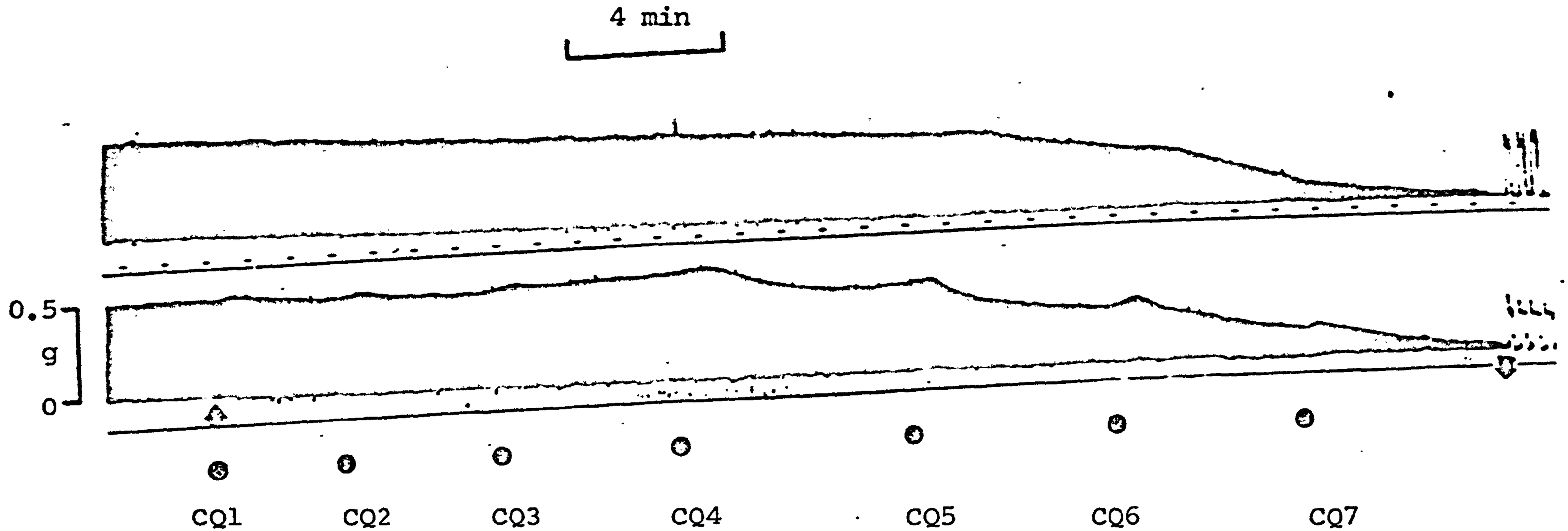


FIGURE 80

Guinea-pig isolated electrically-driven left atria. Effects of chloroquine (CQ) on electrically-driven left atria taken from guinea-pigs pretreated with reserpine (top trace) and saline (bottom trace). CQ1, CQ2, CQ3, CQ4, CQ5, CQ6 and CQ7 represent chloroquine, 1.0×10^{-6}M, 1.0×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M and 2.5×10^{-3}M respectively. Note that the initial stimulant effect of chloroquine (in saline pretreated guinea-pig) was abolished by reserpinization.

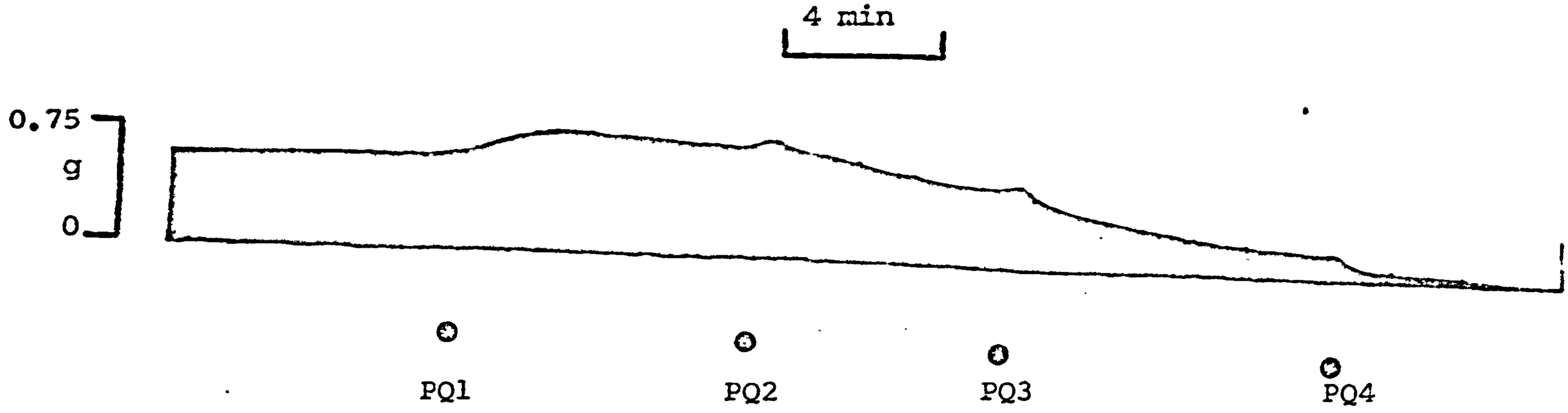


FIGURE 81

Guinea-pig isolated electrically-driven left atria. Effects of primaquine (PQ) on the tension developed by isolated electrically-driven guinea-pig left atria. PQ1, PQ2, PQ3 and PQ4 denote primaquine, 5.0×10^{-5}M, 1.0×10^{-4}M, 5.0×10^{-4}M and 1.0×10^{-3}M respectively.

preceded by a transient stimulant effect (See Figure 78). This positive chronotropic effect was abolished by dl-propranolol (2.5×10^{-6}M) and was not seen in atria taken from reserpinized animals. Treatment of the atria with cocaine ($1.5 - 5.0 \times 10^{-6}$M) before the administration of the quinoline compounds, did not alter the positive chronotropic effects of the drugs. Furthermore, the decreases in the rate caused by all the antimalarials was not affected by previous administration of either atropine ($1.- 2.5 \times 10^{-6}$M) or dipyridamole (2.1×10^{-6}M). Figure 79 summarises the effects of each antimalarial agent on this isolated tissue.

4.23 Effects on tension developed by electrically-driven left atria

Chloroquine, primaquine and quinine caused a positive inotropic response at low doses ($5.0 \times 10^{-7} - 5.0 \times 10^{-5}$M). This stimulant response was abolished by dl-propranolol ($2.5 - 5.0 \times 10^{-5}$M) and by reserpine pre-treatment (Figure 80). In addition, prior administration of cocaine (2.5×10^{-6}M) inhibited the positive inotropic response caused by the quinoline antimalarials as well as that evoked by tyramine ($5.0 \times 10^{-6} - 3.0 \times 10^{-5}$M). Higher doses of the quinoline drugs (concentrations over 7.5×10^{-5}M) caused a dose-dependent negative inotropic response which was preceded by a transient and slight stimulant effect (Figure 81).

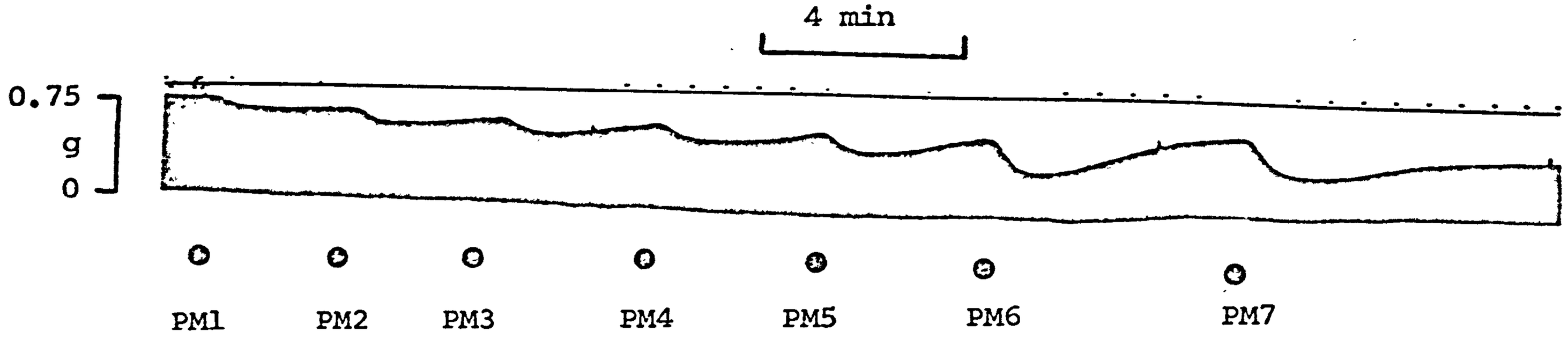


FIGURE 82

Guinea-pig isolated electrically-driven left atria. Effect of pyrimethamine (PM) on the tension developed by isolated electrically-driven guinea-pig left atria. PM1, PM2, PM3, PM4, PM5, PM6 and PM7 represent pyrimethamine, 1.0×10^{-8}M, 5.0×10^{-8}M, 1.0×10^{-7}M, 5.0×10^{-7}M, 1.0×10^{-6}M, 5.0×10^{-6}M and 1.0×10^{-5}M respectively. Pyrimethamine always produced dose-dependent negative inotropic effect.

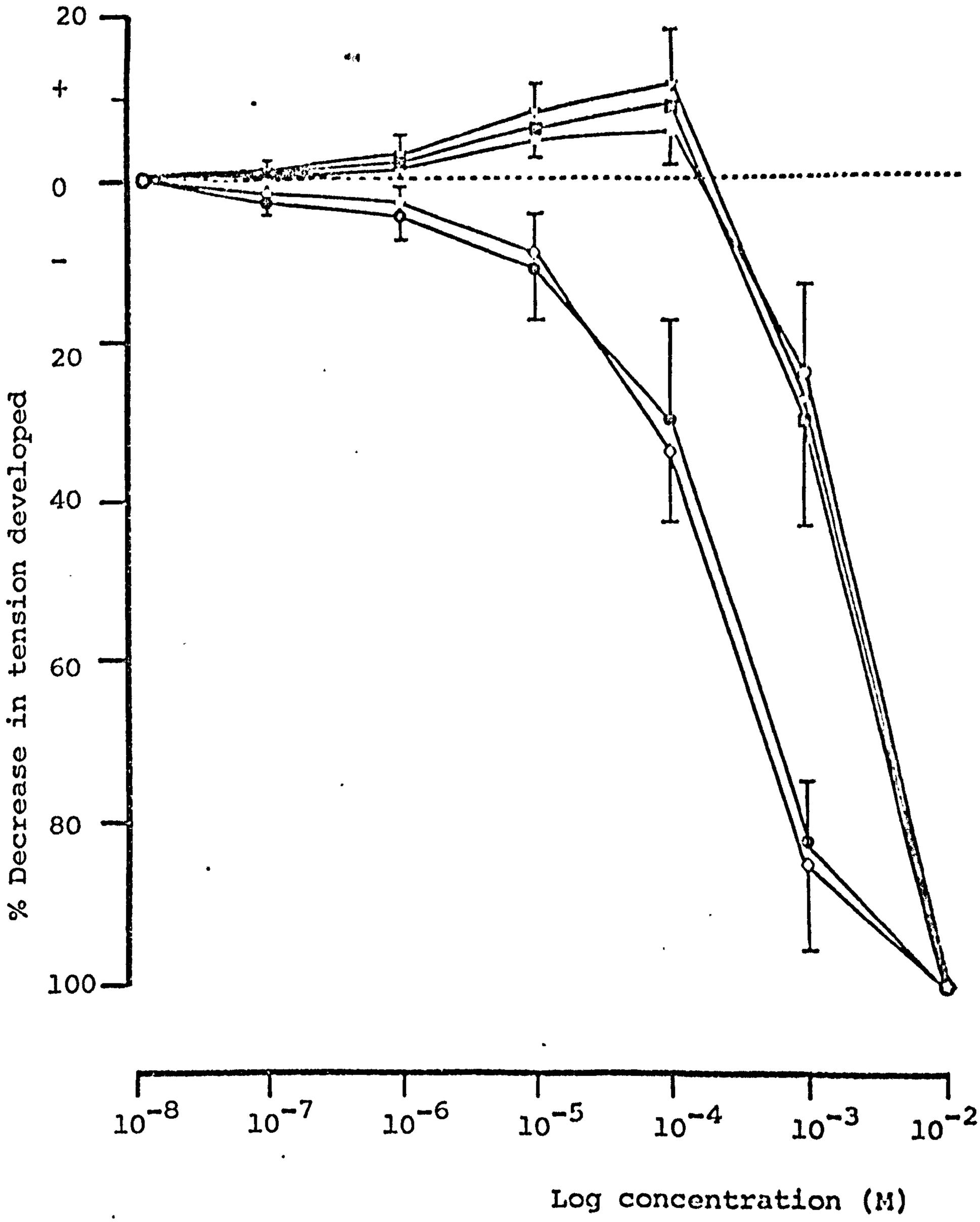


FIGURE 83

Guinea-pig isolated electrically-driven left atria. The inotropic effects of primaquine (▽), chloroquine (□), quinine (○), proguanil (●) and pyrimethamine (◇) on electrically-driven guinea-pig left atria. Each point is the mean of 6 - 8 observations, and the vertical bars denote s.e. of means.

TABLE 4

Calculated pA_2 values and slopes for the antagonist action of the antimalarial drugs on the positive inotropic effects of isoprenaline, noradrenaline and calcium.

Antagonist	Isoprenaline n=4		Noradrenaline n=6		Calcium n=6	
	pA_2	Slope	pA_2	Slope	pA_2	Slope
Primaquine	7.7 ± 0.3	1.13	7.3 ± 0.1	1.32	8.2 ± 0.1	1.03
Chloroquine	7.4 ± 0.4	1.18	7.0 ± 0.1	1.35	8.0 ± 0.1	1.08
Quinine	7.0 ± 0.5	1.32	6.7 ± 0.2	1.71	7.5 ± 0.2	1.11
Proguanil	5.5 ± 0.2	1.83	5.9 ± 0.1	2.23	6.7 ± 0.1	1.56
Pyrimethamine	5.1 ± 0.4	1.97	6.1 ± 0.1	2.29	7.0 ± 0.1	1.35
Propranolol	8.8 ± 0.2	1.02	8.4 ± 0.1	1.00	6.5 ± 0.3	1.65

At no dose level employed did proguanil or pyrimethamine (1.0×10^{-8} - 1.0×10^{-3}M) cause a positive inotropic effect. Instead, they always produced, short-lasting, negative inotropic responses (Figure 82). The negative inotropic actions of all the antimalarial drugs were unaffected by atropine (2.5×10^{-6}M) and dipyridamole (2.1×10^{-6}M). Figure 83 summarises the effects of all the antimalarials studied on guinea-pig isolated driven left atria.

4.24 Effects of antimalarial drugs on the positive inotropic actions of isoprenaline, noradrenaline and calcium

The effects of the antimalarial compounds on the increases in developed tension induced by isoprenaline, noradrenaline and calcium are summarised in Table 4 . The quinoline compounds (ie. primaquine, chloroquine and quinine) were some 10 - 30 times more potent than either proguanil or pyrimethamine in inhibiting the effects of isoprenaline and noradrenaline. In addition, the slopes of the Schild plots for proguanil and pyrimethamine suggested that these drugs were not acting as competitive ß-adrenoceptor antagonists. Indeed, only the antagonism of isoprenaline by primaquine, chloroquine (and propranolol) could be described as competitive as judged by the Schild plots. None of the antimalarial drugs affected the maximum response produced by the agonists. All the antimalarial compounds tested also inhibited, in a dose-dependent manner, the positive inotropic effects of calcium. Indeed their apparent potency

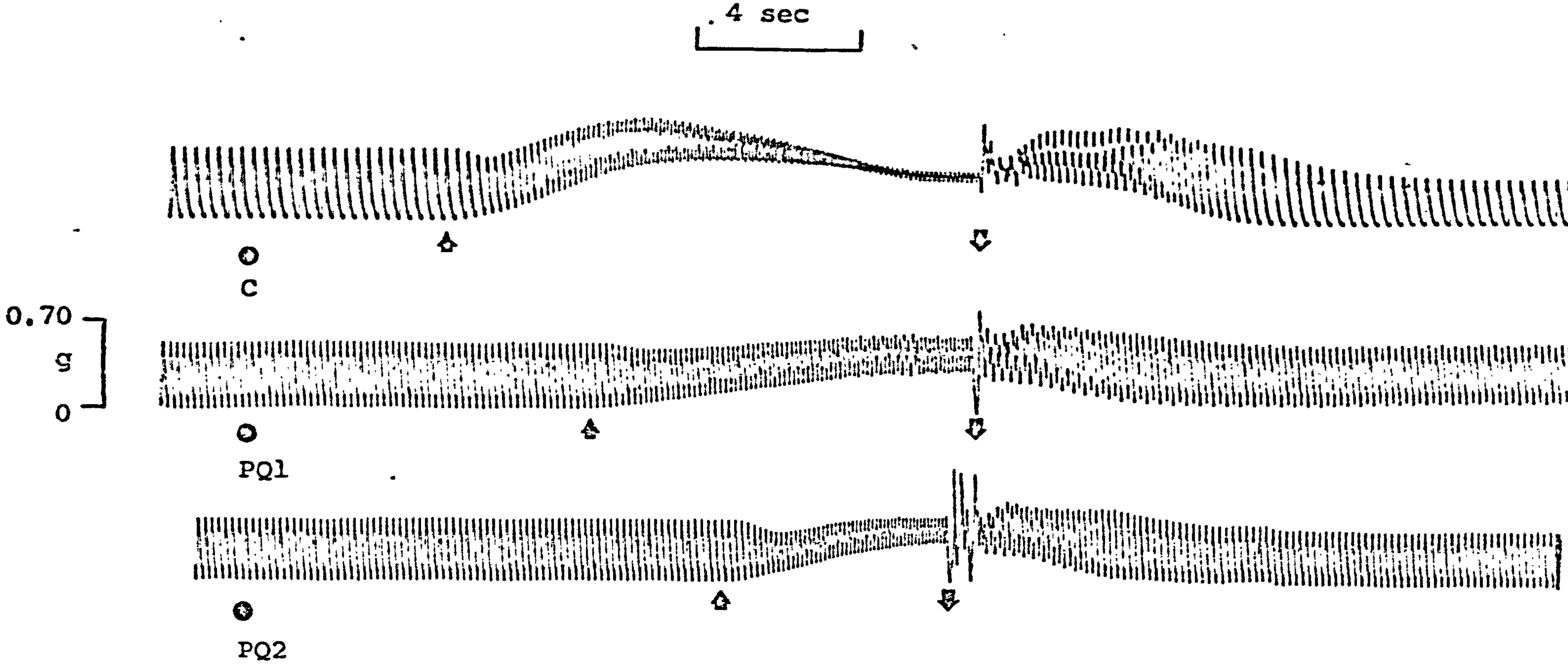


FIGURE 84

Guinea-pig isolated electrically-driven left atria. Effect of increasing the frequency of stimulation on the rate of contraction of electrically-stimulated guinea-pig left atria. At the upward arrows, the frequency of stimulation was gradually increased until the rate of contraction of the atria no longer followed the rate of stimulation (indicated by the downward arrows). C (upper trace) denotes saline treated control, whilst PQ1 (middle trace) and PQ2 (lower trace) represent drug treatment (primaquine, 1.5×10^{-5}M and 2.5×10^{-5}M respectively). All the anti-malarial compounds examined dose-dependently decreased the maximum driving frequency of the driven left atria.

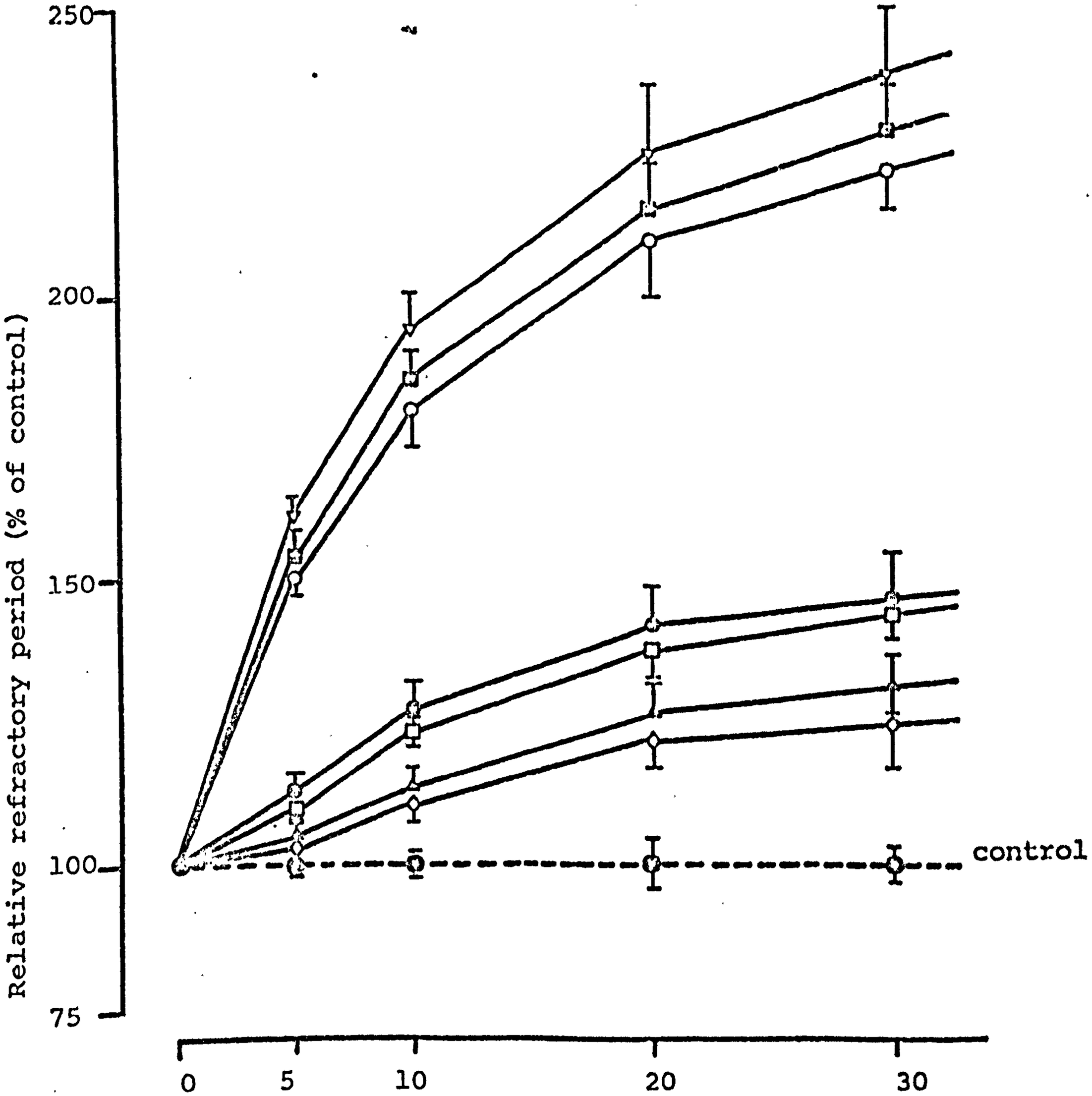


FIGURE 85

Guinea-pig isolated electrically-driven left atria. Effect of contact time on the actions of primaquine (▽), chloroquine (◘), quinidine (O), quinine (●), lignocaine (□), proguanil (▲) and pyrimethamine (◇) (All 3 x 10^{-5}M) in decreasing the maximum driving frequency of electrically-driven guinea-pig atria. The control plot (⊙) was obtained with saline administration. Each point is the mean of 6 - 10 observations. The vertical bars denote s.e. of means.

TABLE 5

Effects of some antimalarial drugs, and lignocaine, on the effective refractory period of guinea-pig isolated electrically-driven left atria. The drugs were left in contact with the tissue for 30 minutes. The number in brackets indicate number of observations.

Drug	Mean ED_{50} (in Molar)	
Primaquine	7.5×10^{-5}	(8)
Chloroquine	9.0×10^{-5}	(6)
Quinidine	1.0×10^{-4}	(8)
Quinine	2.0×10^{-4}	(7)
Lignocaine	3.0×10^{-4}	(5)
Proguanil	1.0×10^{-3}	(7)
Pyrimethamine	1.5×10^{-3}	(6)

in inhibiting calcium was greater than that in blocking isoprenaline or noradrenaline. Propranolol also inhibited calcium-induced contractions but concentrations about 100 times the β-adrenoceptor blocking concentrations were required (Table 4).

4.25 Effects on maximum driving frequency of electrically-driven left atria

All the antimalarial drugs, as well as lignocaine, caused a dose-dependent decrease in the maximum driving frequency of guinea-pig electrically-driven left atria. Figure 84 illustrates some typical traces obtained. The order of potency was found to be: primaquine ≥ chloroquine ≥ quinidine >> quinine = lignocaine ≥ proguanil ≥ pyrimethamine. The magnitude of the effect on maximum driving frequency was dependent on the contact time of the drugs with the tissues (Figure 85). The ED_{50} values of the drugs are presented in Table 5.

4.26 Effects on rabbit isolated electrically-driven papillary muscle

On rabbit papillary muscle, all the antimalarial drugs tested produced effects which are qualitatively and quantitatively similar to those on guinea-pig isolated driven left atria. Figures 86 and 87 show typical traces obtained with chloroquine and pyrimethamine respectively.

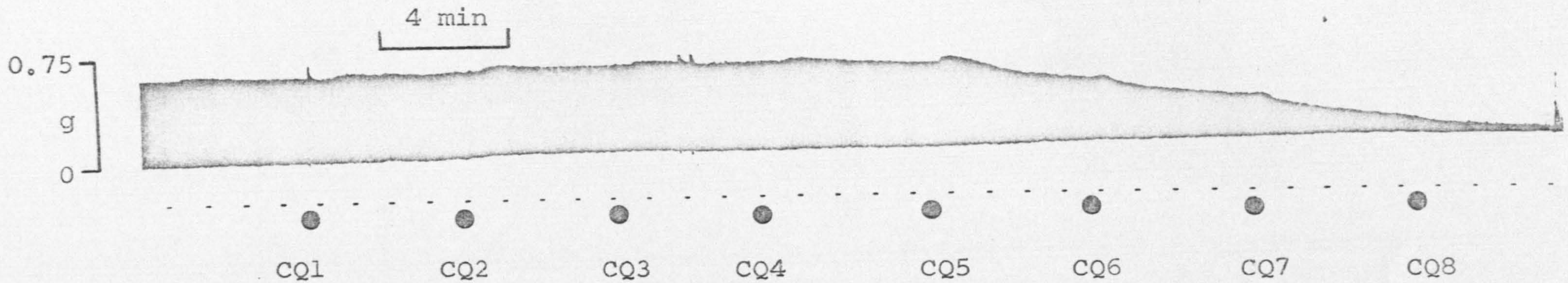


FIGURE 86

Rabbit isolated electrically-driven papillary muscle. Effects of chloroquine (CQ) on the tension developed by electrically-driven rabbit papillary muscle. CQ1, CQ2, CQ3, CQ4, CQ5, CQ6, CQ7 and Q8 - denote chloroquine, 1.0×10^{-6}M, 5.0×10^{-6}M, 1.0×10^{-5}M, 5.0×10^{-5}M, 1.0×10^{-4}M, 5.0×10^{-4}M, 1.0×10^{-3}M and 2.5×10^{-3}M respectively.

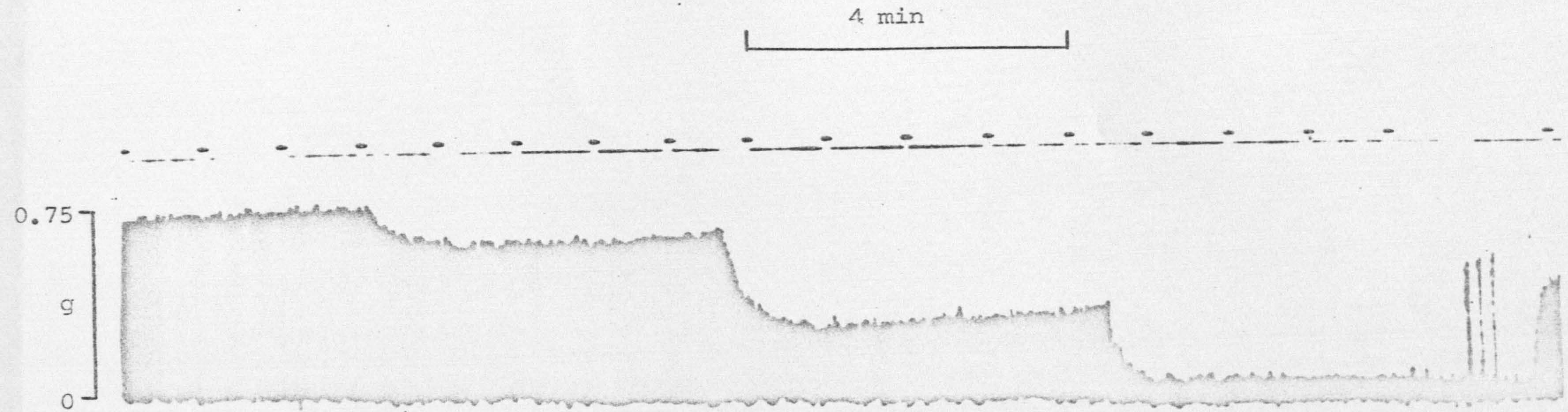


FIGURE 87

Rabbit isolated electrically-driven papillary muscle. Effect of pyrimethamine (PM) on the tension developed by electrically-driven rabbit papillary muscle. PM1, PM2 and PM3 represent pyrimethamine, 2.5×10^{-5}M, 1.0×10^{-4}M and 2.5×10^{-4}M respectively.

Ouabain-induced dysrhythmias

The dose of ouabain required to establish persistent ventricular tachycardia was 80 ± 10 µg/kg. This dysrhythmia usually persisted for 1 - 2 hours.

The β-adrenoceptor blocking agents, alprenolol and propranolol (4 - 20 µg/kg) abolished the dysrhythmia and restored normal sinus rhythm within 3 - 10 min. of the injection. In contrast, all the antimalarial drugs studied, in doses between 1 and 10 mg/kg, failed to suppress the ouabain-induced dysrhythmia and restore normal sinus. Additional (15 mg/kg) doses of the compounds also not only failed to abolish ventricular tachycardia, but actually augmented the dysrhythmia and were usually lethal.

B: 'IN VIVO' EXPERIMENTS

4.27 Effects of antimalarial drugs in pentobarbitone-anaesthetized cats

Intravenous injections of all the five antimalarial drugs studied, at doses ranging between 1.0 and 10.0 mg/kg, always caused dose-dependent reductions in systemic arterial blood pressure and heart rate in all the preparations. The cardiovascular effects of the two major groups of antimalarial compounds are described in more detail below.

4.28 Cardiovascular effects of chloroquine and primaquine

These two quinoline antimalarials produced strikingly similar effects in pentobarbitone-anaesthetized cats. Intravenous administration of chloroquine or primaquine (2.5 - 10 mg/kg) always produced dose-related reductions in the heart rate, in the arterial systolic and diastolic blood pressures, in the left ventricular pressure (LVP), and in the left ventricular dP/dt max. The same doses of the compounds usually caused dose-dependent increases in right artrial pressure and in left ventricular end-diastolic pressure (LVEDP), and always produced multi-phasic effects on the electro cardiogram (ECG), especially on the QRS wave. The electro cardiographic effects of intravenous injections of the agents (5 - 10 mg/kg) usually consisted of an initial transient increase in the height of QRS wave (reaching a peak 7 - 25 seconds after the injection - see Figures 94 and 95), followed by a gradual reduction in the

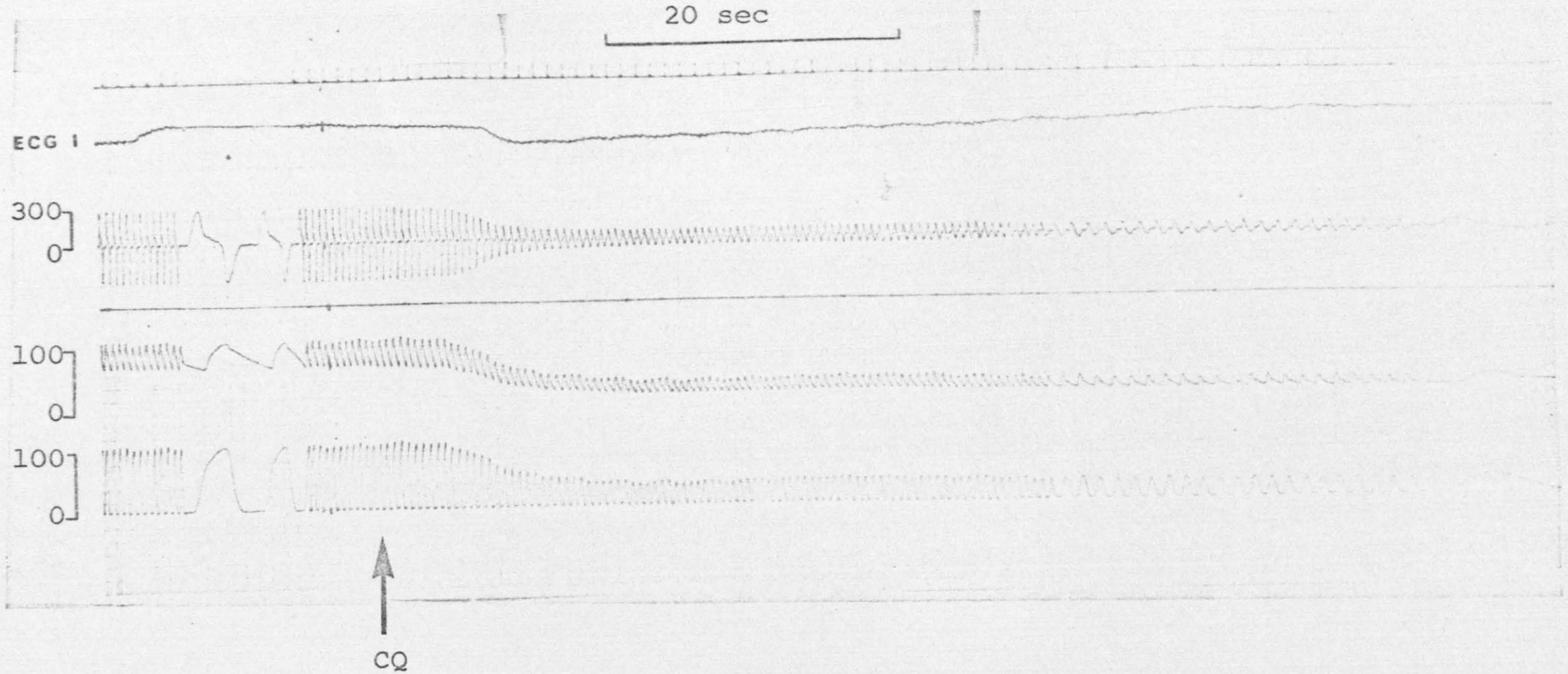


FIGURE 88

Pentobarbitone-anaesthetized cat. Cardiovascular effects of intravenously injected chloroquine (CQ, 10 mg/kg, at the upward arrow) in a pentobarbitone-anaesthetized cat.

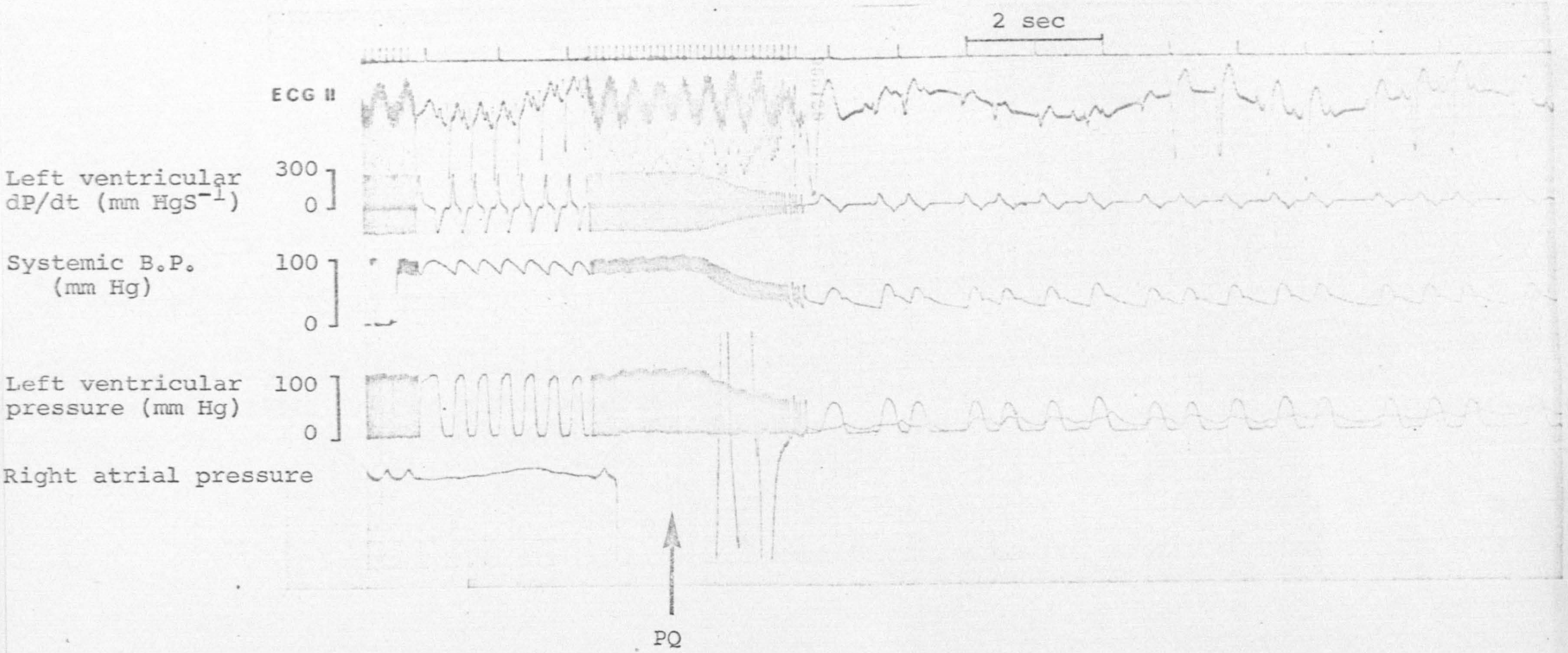


FIGURE 89

Anaesthetized cat. Cardiovascular effects of intravenously administered primaquine (PQ, 8 mg/kg, injected at the upward arrow) in a pentobarbitone-anaesthetized cat. From top to bottom, the parameters are: ECG (lead II), left ventricular dP/dt, systemic blood pressure, left ventricular pressure, and right atrial pressure respectively. (Note the rise in right atrial pressure).

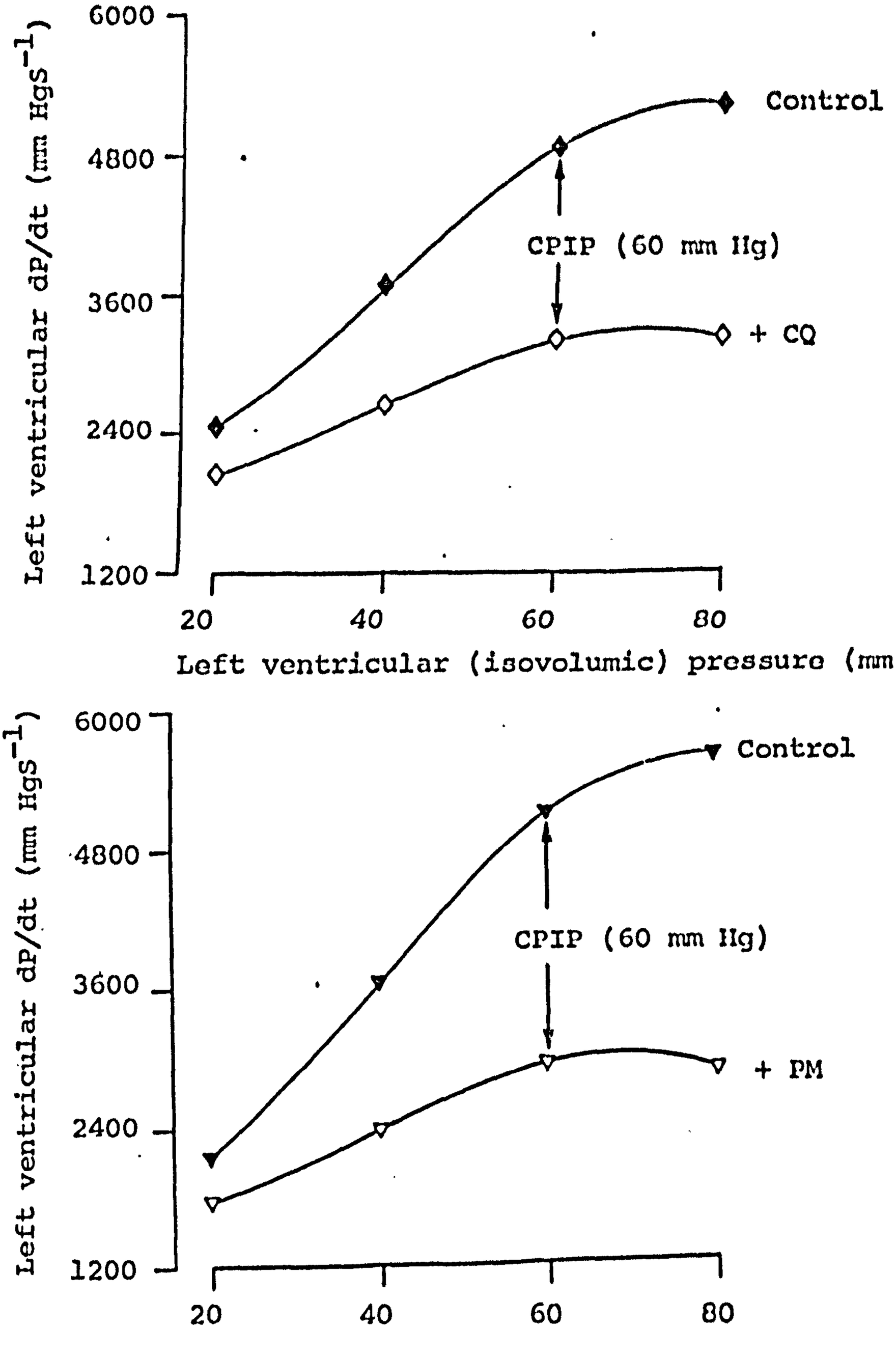


FIGURE 90

Pentobarbitone-anaesthetized cats. The effects of chloroquine (CQ, 8 mg/kg iv., upper panel) and pyrimethamine (PM, 8 mg/kg iv., lower panel) on the relationship between left ventricular dP/dt and left ventricular pressure during the period before the opening of the aortic valves (66 mm Hg in the control situation). The lower ratio of dP/dt peak common developed isovolumic pressure (CPIP at 60 mm Hg) obtained after the administration of the drugs indicates a decrease in the contractile state of the ventricle.

TABLE 6

Anaesthetized cats. Cardiovascular effects of intravenously injected chloroquine (2.5 - 10 mg/kg) in pentobarbitone-anaesthetized cats. The values represent the mean changes from the controls ± s.e. of 5 observations (number in parenthesis).

	Mean change from control (± s.e.) induced by chloroquine (dose in mg/kg)			
	Control	2.5	5.0	10.0
Systolic blood pressure (mm Hg)	118±8 (5)	-28±8 (5)	-84±10 (5)	-112±6 (5)
Diastolic blood pressure((mm Hg)	86±6 (5)	-20±8 (5)	-61±6 (5)	-82±4 (5)
Mean blood pressure (mm Hg)	94±7 (5)	-22±6 (5)	-65±8 (5)	-90±4 (5)
Heart rate (beats/minute)	198±10 (5)	-20±13 (5)	-98±10 (5)	-182±8 (5)
Left ventricular systolic pressure (mm Hg)	110±12 (5)	-17±15 (5)	-68±12 (5)	-100±10 (5)
Left ventricular end-diastolic pressure (mm Hg)	3.0±0.5 (5)	+0.8±0.4 (5)	+2.5±0.5 (5)	+4.2±0.4 (5)
Left ventricular dP/dt max (mm Hgs^{-1})	3480±420 (5)	-920±360 (5)	-3120±420 (5)	-3420±40 (5)
Mean right atrial pressure (mm Hg)	0.5±0.1 (5)	+0.5±0.2 (5)	+1.7±0.2 (5)	+2.8±0.1 (5)
P - R interval (m sec)	115±12 (5)	+15±10 (5)	+60±11 (5)	+156±12 (5)
QRS complex duration (m sec)	62±5 (5)	+12±10 (5)	+63±13 (5)	+135±10 (5)

TABLE 7

Anaesthetized cats. Cardiovascular effects of intravenously injected primaquine (2.5 - 10 mg/kg) in pentobarbitone-anaesthetized cats. The values represent the mean changes from the controls ± s.e. of 3 - 7 observations (number in parenthesis).

	Mean change from control (± s.e.) induce by primaquine (dose in mg/kg)			
	Control	2.5	5.0	10.0
Systolic blood pressure (mm Hg)	122±6 (7)	-32±8 (7)	-88±10 (7)	-114±6 (5)
Diastolic blood pressure (mm Hg)	90±6 (7)	-26±7 (7)	-64±8 (7)	-86±4 (5)
Mean blood pressure (mm Hg)	102±4 (7)	-30±6 (7)	-73±8 (7)	-96±6 (3)
Heart rate (beats/minute)	200±12 (7)	-21±15 (7)	-96±12 (7)	-180±16 (7)
Left ventricular systolic pressure (mm Hg)	112±10 (7)	-20±12 (7)	-70±16 (7)	-98±10 (4)
Left ventricular end-diastolic pressure (mm Hg)	3.6±0.4 (7)	+0.5±0.3 (7)	+2.4±0.4 (7)	+3.8±0.5 (4)
Left ventricular dP/dt max (mm Hgs^{-1})	3640±440 (7)	-960±420 (7)	-3040±560 (7)	-3600±40 (3)
Mean right atrial pressure (mm Hg)	0.6±0.1 (4)	+0.3±0.2 (4)	+1.5±0.2 (4)	+2.6±0.2 (3)
P - R interval (m sec)	110±10 (7)	+16±13 (7)	+55±12 (7)	+150±10 (5)
QRS complex duration (m sec)	60±7 (7)	+10±8 (7)	+66±10 (7)	+130±8 (5)

height of the complex, and inversion of the QRS complex (1 - 5 minutes after the injection). Occasionally, there was elevation of the T wave and S-T segment, reduction of P wave, and prolongation of the P-R interval as well as QRS duration. The initial transient increases in the height of the QRS wave usually lasted for 2 - 5 minutes and were observed more frequently with high concentrations of the drugs (5 - 10 mg/kg). Furthermore, the increase in the QRS complex height was usually well marked when the animal received the high dose for the first time, whereas subsequent doses of the compound were always less effective. However, low doses of the compounds (1 - 2.5 mg/kg) usually produced no effect on heart rate but always caused short-lasting hypotension. Figures 88 and 89 illustrate typical traces while Tables 6 and 7 summarise the results obtained in the pentobarbitone-anaesthetized cats.

Because of the marked changes in after-load, measurements were also made of ventricular dP/dt at fixed isovolumic left ventricular pressures (Mason 1969) before and after the drugs. The results of such experiments with chloroquine (and pyrimethamine) are illustrated in Figure 90 . The lower ratio of dP/dt peak common developed isovolumic pressure (CPIP) obtained after the administration of the drugs indicated a decrease in the contractile state of the ventricle.

TABLE 8

Anaesthetized cats. Cardiovascular effects of bilateral vagotomy alone in pentobarbitone-anaesthetized cats. The values represent the means ± s.e. of five observations (number in parenthesis).

	Before Vagotomy	After Vagotomy	
Systolic blood pressure (mm Hg)	120 ± 4 (5)	116 ± 4 (5)	P > 0.05
Diastolic blood pressure (mm Hg)	88 ± 4 (5)	82 ± 4 (5)	P > 0.05
Mean arterial blood pressure (mm Hg)	96 ± 4 (5)	92 ± 4 (5)	P > 0.05
Heart rate (beats/minute)	194 ± 7 (5)	206 ± 10 (5)	P > 0.05
Left ventricular dP/dt max (mm Hgs^{-1})	2198 ± 94 (5)	2288 ± 114 (5)	P > 0.05

TABLE 9

Anaesthetized cats. Effects of bilateral vagotomy on the cardiovascular responses of pentobarbitone-anaesthetized cats to intravenously injected primaquine (5 mg/kg). The values represent the mean changes from the controls ± s.e. of five observations (number in parenthesis).

	Before Vagotomy		After Vagotomy	
	Control (before primaquine)	Change (after primaquine)	Contrcl (before primaquine)	Change (after primaquine)
Systolic blood pressure (mm Hg)	116 ± 10	-84 (5)	112 ± 12	-86 (5)
Diastolic blood pressure (mm Hg)	86 ± 10	-66 (5)	80 ± 8	-64 (5)
Mean blood pressure (mm Hg)	92 ± 12	-92 (5)	88 ± 10	-76 (5)
Heart rate (beats/minute)	196 ± 12	-92 (5)	208 ± 16	-96 (5)
Left ventricular dP/dt max (mm Hgs^{-1})	2160 ± 240	-2040 (5)	2220 ± 360	-2100 (5)

TABLE 10

Anaesthetized cats. Cardiovascular effects of intravenous noradrenaline (0.25 and 0.5 µg/kg) before, and one hour after, the intravenous injection of chloroquine (5 mg/kg) in pentobarbitone-anaesthetized cats. The values represent the mean changes from controls ± s.e. of four experiments.

	Before chloroquine			After chloroquine (5 mg/kg)		
	Change from control (mean ± s.e. of mean) induced by noradrenaline (dose in µg/kg)			Change from control (mean ± s.e. of mean) induced by noradrenaline (dose in µg/kg)		
	Control	0.25	0.5	Control	0.25	0.5
Systolic blood pressure (mm Hg)	118±16	+50±8	+120±17	116±12	+6±8**	+18±12*
Diastolic blood pressure (mm Hg)	86±14	+48±6	+96±12	88±10	+2±7**	+10±8*
Heart rate (beats/minutes)	204±12	+8±4	+20±16	203±8	-10±4	-6±7
Left ventricular dP/dt max (mm Hgs^{-1})	3600±340	+960±204	+2096±280	3640±520	+20±60**	+64±240*

**P < 0.01; *P < 0.05 compared with change before chloroquine

4.29 Effect of bilateral vagotomy

Bilateral vagotomy had no marked haemodynamic effects in pentobarbitone-anaesthetized cats (Table 8) nor did it modify the cardiovascular effects of chloroquine or primaquine (Table 9).

4.30 Effect of noradrenaline

Noradrenaline (0.05 - 0.5 µg/kg) produced pressor cardiovascular effects in the pentobarbitone-anaesthetized cats. These pressor effects were inhibited by chloroquine and primaquine in a dose-related fashion (Table 10). The antagonism between the compounds and noradrenaline was found to be non-competitive, as indicated earlier in the isolated cardiac muscle preparations.

4.31 Cardiovascular effects of pyrimethamine and proguanil

Pyrimethamine and proguanil (1 - 10 mg/kg) produced similar effects in pentobarbitone-anaesthetized cats. These two non-quinoline antimalarials always caused dose-related reductions in arterial blood pressure, in left ventricular pressure, in left ventricular dP/dt max, and in heart rate. In all preparations, medium to high concentrations of pyrimethamine or proguanil (4 - 10 mg/kg) usually produced slight but dose-dependent increases in right atrial and left ventricular end-diastolic pressures. Low doses of the compounds (1 - 2 mg/kg) did not alter the heart rate,

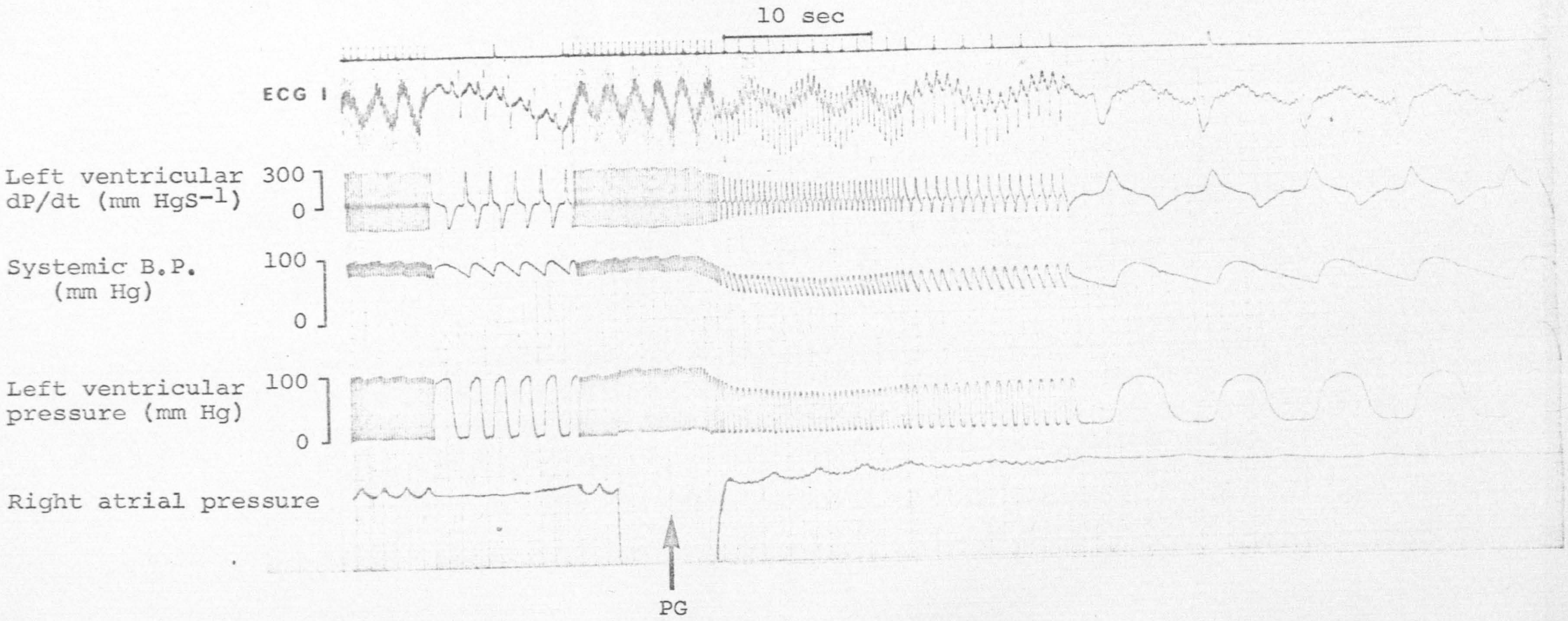


FIGURE 91

Anaesthetized cat. Cardiovascular effects of intravenous proguanil (PG, 5 mg/kg iv., injected at the upward arrow) in a pentobarbitone-anaesthetized cat.

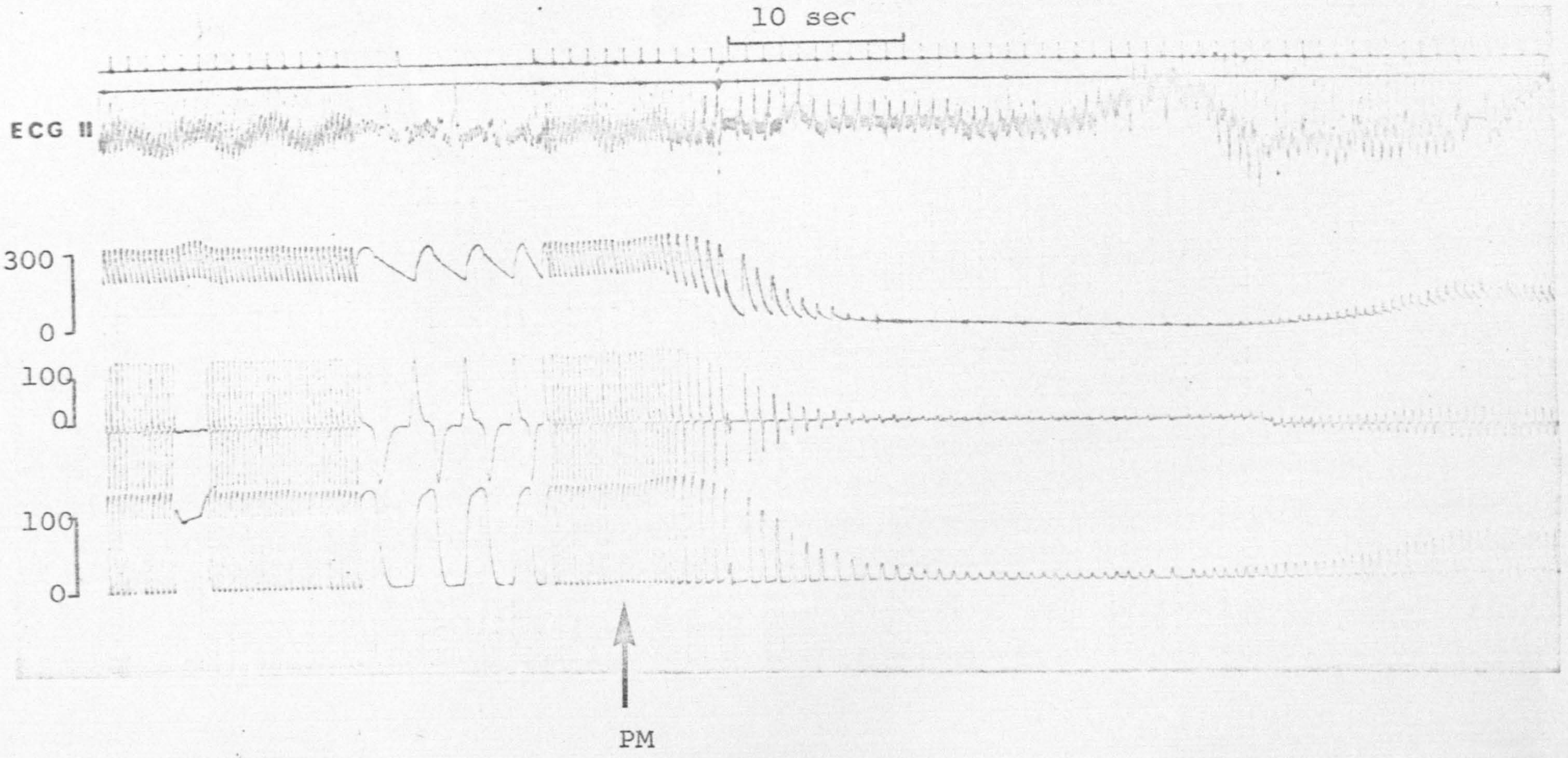


FIGURE 92

Anaesthetized cat. Cardiovascular effects of intravenously injected pyrimethamine (PM, 10 mg/kg iv., at the upward arrow) in a pentobarbitone-anaesthetized cat. From top to botom, the parameters are: ECG (lead II), systemic blood pressure, left ventricular dP/dt and left ventricular pressure respectively.

TABLE 11

Anaesthetized cats. Cardiovascular effects of intravenously injected pyrimethamine (2.5 - 10 mg/kg) in pentobarbitone-anaesthetized cats. The values represent the mean changes from the controls ± s.e. of 4 - 7 observations (number in parenthesis)

	Mean change from control (± s.e.) induced by pyrimethamine (dose in mg/kg)							
	Control		2.5		5.0		10.0	
Systolic blood pressure (mm Hg)	120±7	(6)	-20±8	(6)	-66±10	(6)	-112±8	(5)
Diastolic blood pressure (mm Hg)	88±7	(6)	-14±10	(6)	-50±12	(6)	-80±8	(5)
Mean blood pressure (mm Hg)	96±6	(5)	-18±7	(5)	-54±12	(5)	-86±10	(4)
Heart rate (beats/minute)	196±12	(7)	0	(7)	-18±15	(7)	-173±20	(7)
Left ventricular systolic pressure (mm Hg)	112±8	(6)	-12±10	(6)	-28±16	(6)	-98±12	(6)
Left ventricular end-diastolic pressure (mm Hg)	2.8±0.6	(6)	0	(5)	+0.5±0.4	(6)	+3.1±1.0	(6)
Left ventricular dP/dt max (mm Hgs^{-1})	3660±480	(7)	-180±120	(7)	-988±360	(7)	-3120±600	(7)
Mean right atrial pressure (mm Hg)	0.8±0.2	(4)	0	(4)	+0.3±0.3	(4)	+2.0±0.6	(4)
P - R interval (m sec)	105±12	(5)	+3±2	(5)	+12±10	(5)	+96±17	(4)
QRS complex duration (m sec)	55±8	(5)	+2±2	(5)	+9±8	(5)	+83±20	(4)

TABLE 12

Anaesthetized cats. Cardiovascular effects of intravenous noradrenaline (0.05 - 0.5 µg/kg) before, and one hour after, the intravenous injection of pyrimethamine (5 mg/kg) in pentobarbitone-anaesthetized cats. The values represent the mean changes from controls ± s.e. of five observations

	Before pyrimethamine			After pyrimethamine (5 mg/kg)		
	Change from control (mean ± s.e. of mean) induced by noradrenaline (dose in µg/kg)			Change from control (mean ± s.e. of mean) induced by noradrenaline (dose in µg/kg)		
	Control	0.25	0.5	Control	0.25	0.5
Systolic blood pressure (mm Hg)	120±14	+54±10	+112±16	118±16	+20±16*	+66±18
Diastolic blood pressure (mm Hg)	88±16	+50±8	+98±12	86±10	+8±10*	+34±14
Heart rate (beats/minute)	200±18	+12±6	+18±4	204±12	0	+4±3
Left ventricular dP/dt max (mm Hgs^{-1})	3420±220	+880±240	+2160±320	3540±360	+68±40*	+240±180

*Means $P < 0.05$ compared with change before pyrimethamine

but higher concentrations (4 - 10 mg/kg) always reduced the heart rate in a dose-related manner. Figures 91 and 92 show typical traces, and Table 11 summarises the results obtained with pyrimethamine.

Low concentrations of pyrimethamine or proguanil (1 - 2 mg/kg) did not produce any marked effect on the electrocardiogram, but higher doses (4 - 10 mg/kg) affected the ecg in a dose-rated way. These effects included reductions in the height of the P and QRS waves, inversion of the QRS complex, occasionally, elevation of the T wave and prolongation of the P-R interval and QRS complex duration. The initial transient increase in the height of the QRS wave usually produced by medium to high doses of quinoline antimalarials (5 - 10 mg/kg) was never observed with pyrimethamine or proguanil at the same dose levels.

4.32 Effects of bilateral vagotomy and noradrenaline

As with chloroquine and primaquine, bilateral vagotomy did not modify the cardiovascular effects of pyrimethamine or proguanil in anaesthetized cats. Similarly, the pressor effects of noradrenaline (0.05 - 0.5 µg/kg) were inhibited by pyrimethamine (or proguanil). Table 12 summarises the effects of pyrimethamine on noradrenaline-induced cardiovascular changes.

In general, the depressant cardiovascular effects of pyrimethamine and proguanil were found to be more marked,

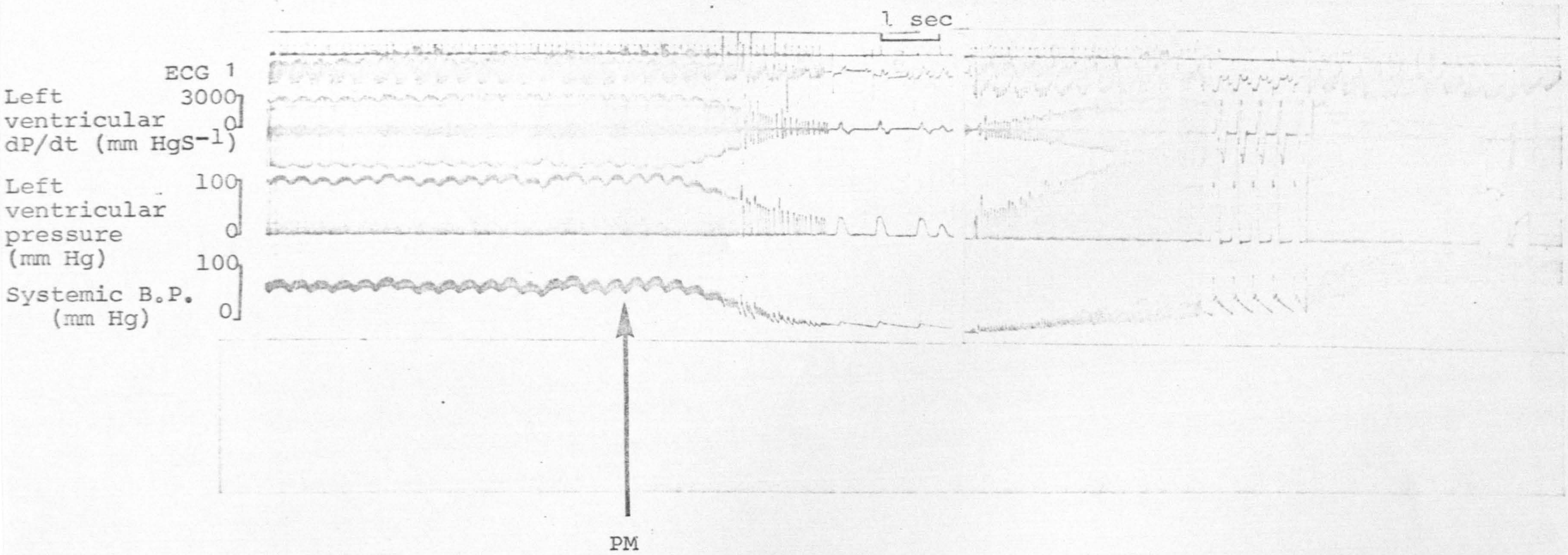


FIGURE 93

Anaesthetized cat. Effects of intravenous pyrimethamine (PM, 10 mg/kg i.v; at the upward arrow) on the ECG (lead I), left ventricular dP/dt, left ventricular pressure, and systemic blood pressure of a pentobarbitone-anaesthetised cat. (Note the secondary hypertensive phase).

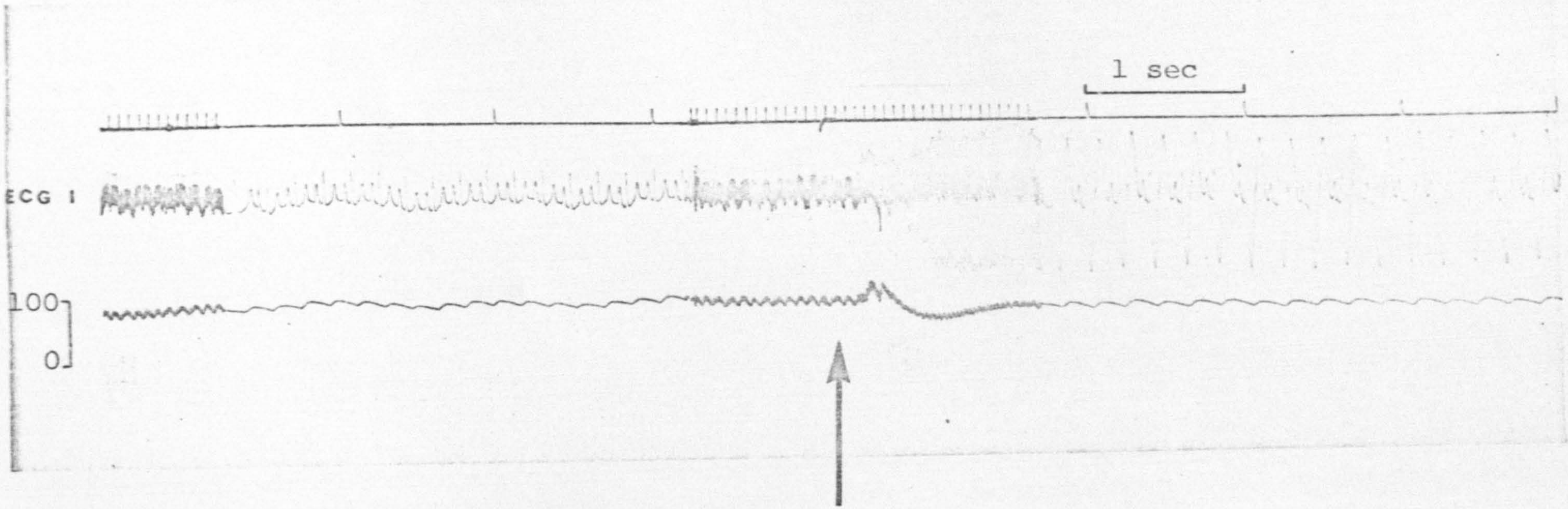


FIGURE 94

Anaesthetized guinea-pig. Effects of intravenously injected chloroquine (CQ, 10 mg/kg iv., at the upward arrow) on the ECG (lead I) and systemic blood pressure of a pentobarbitone-anaesthetized guinea-pig. (Note the marked effect on the ECG).

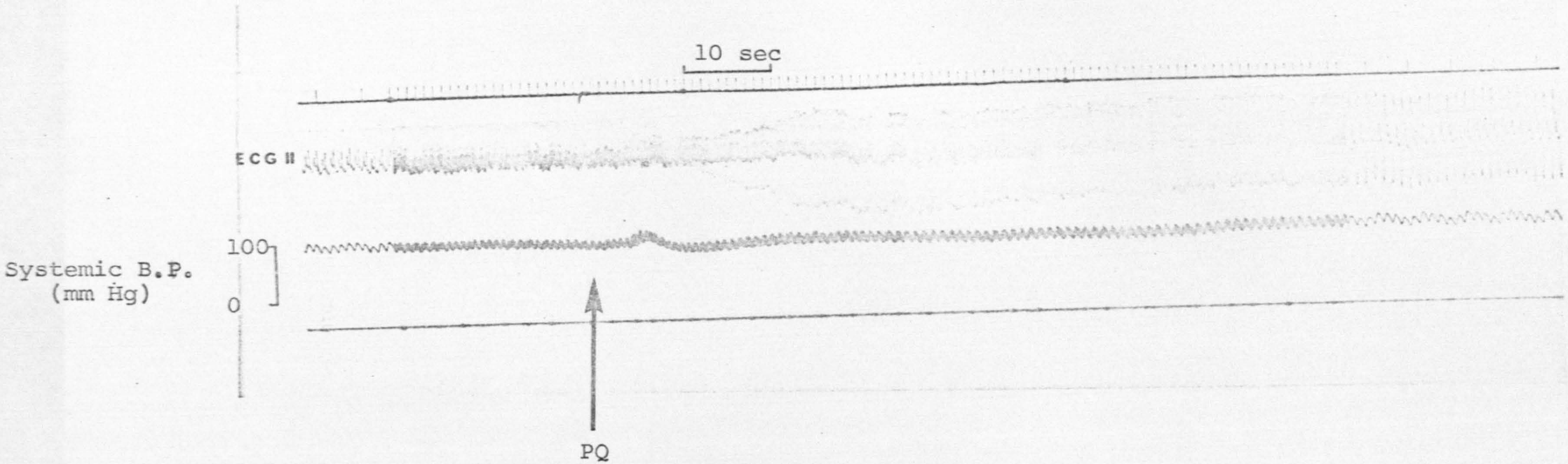


FIGURE 95

Anaesthetized guinea-pig. Effects of intravenous primaquine (PQ, 5 mg/kg i.v., at the upward arrow) on the ECG (lead II) and systemic blood pressure of a pentobarbitone-anaesthetized guinea-pig.

TABLE 13

Anaesthetized guinea-pigs. Cardiovascular effects of intravenous chloroquine (2.5 - 10 mg/kg) in pentobarbitone-anaesthetized guinea-pigs. The values represent the mean changes from the controls ± s.e. of 5 - 8 observations (number in parenthesis)

	Mean change from control (± s.e.) induced by chloroquine (dose in mg/kg)			
	Control	2.5	5.0	10.0
Systolic blood pressure (mm Hg)	112 ± 12 (8)	-22 ± 10 (7)	-76 ± 15 (8)	-102 ± 10 (5)
Diastolic blood pressure (mm Hg)	94 ± 11 (8)	-28 ± 13 (7)	-70 ± 12 (8)	-88 ± 6 (5)
Mean blood pressure (mm Hg)	101 ± 13 (7)	-31 ± 12 (5)	-73 ± 16 (6)	-96 ± 4 (6)
Heart rate (beats/minute)	336 ± 40 (8)	-23 ± 18 (7)	-225 ± 65 (5)	-320 ± 16 (8)

TABLE 14

Anaesthetized guinea-pigs. Cardiovascular effects of intravenous primaquine (2.5 - 10 mg/kg) in pentobarbitone-anaesthetized guinea-pigs. The values represent the mean changes from the controls ± s.e. of 7 - 10 observations (number in parenthesis)

	Mean change from control (± s.e.) induced by primaquine (dose in mg/kg)			
	Control	2.5	5.0	10.0
Systolic blood pressure (mm Hg)	118 ± 16 (10)	-30 ± 12 (10)	-78 ± 18 (10)	-112 ± 5 (8)
Diastolic blood pressure (mm Hg)	98 ± 12 (10)	-34 ± 17 (10)	-76 ± 14 (10)	-93 ± 4 (8)
Mean blood pressure (mm Hg)	104 ± 11 (7)	-35 ± 18 (7)	-78 ± 10 (8)	-92 ± 12 (7)
Heart rate (beats/minute)	420 ± 21 (9)	-38 ± 20 (7)	-250 ± 48 (9)	-401 ± 16 (9)

TABLE 15

Anaesthetized guinea-pigs. Cardiovascular effects of intravenous pyrimethamine (2.5 - 10 mg/kg) in pentobarbitone-anaesthetized guinea-pigs. The values represent the mean changes from the controls ± s.e. of 5 experiments.

	Mean change from control (± s.e.) induced by pyrimethamine (dose in mg/kg)			
	Control	2.5	5.0	10.0
Systolic blood pressure (mm Hg)	114 ± 12	-18 ± 10	-72 ± 12	-103 ± 10
Diastolic blood pressure (mm Hg)	90 ± 10	-14 ± 12	-60 ± 17	-84 ± 6
Mean blood pressure (mm Hg)	102 ± 14	-17 ± 15	-63 ± 18	-96 ± 6
Heart rate (beats/minute)	380 ± 38	0	-44 ± 21	-308 ± 50

although of much shorter duration (reaching a peak 3 - 10 seconds, and wearing off 25 - 50 seconds, after the injection) than those of chloroquine and primaquine. In the case of pyrimethamine, however, there was always a secondary hypertensive phase (lasting for 10 - 36 seconds) following full recovery (Figure 93). This hypertensive phase was not observed with the quinoline compounds, chloroquine and primaquine, and is probably reflex in origin.

EFFECTS OF ANTIMALARIAL DRUGS IN PENTOBARBITONE-ANAESTHETIZED GUINEA-PIGS

4.33 Cardiovascular effects of chloroquine and primaquine

The effects of these two quinoline antimalarials on the electrocardiogram, heart rate and systemic blood pressure of pentobarbitone-anaesthetized guinea-pigs were found to be very similar to those described earlier for pentobarbitone-anaesthetized cats. Typical traces obtained are shown in Figures 94 and 95, while the results are summarized in Tables 13 and 14.

4.34 Cardiovascular effects of pyrimethamine and proguanil

The effects of these two non-quinoline compounds on the ecg, heart rate and systemic blood pressure of pentobarbitone-anaesthetized guinea-pigs were similar to those described earlier in anaesthetized cats. The results obtained with pyrimethamine are summarized in Table 15.

DISCUSSION

4.35 Cardiac muscle

The results obtained in these studies suggest that the actions of the quinoline antimalarials (chloroquine, primaquine and quinine) on isolated cardiac muscle differ from those of proguanil and pyrimethamine. At low concentrations, primaquine, chloroquine, quinine (and quinidine) caused transient positive chronotropic and inotropic responses which are likely to be due to their ability to release noradrenaline. This initial sympathomimetic response evoked by the quinoline compounds was never observed with proguanil or pyrimethamine which at all concentrations tested, caused transient dose-dependent decreases in rate and force. Higher concentrations of the quinolines also caused sustained dose-related negative chronotropic and inotropic responses. Nevertheless, these negative inotropic effects were still preceded by a transient sympathomimetic effect. Although many workers have described the *in vitro* negative inotropic effects of the quinoline group of antimalarials (eg. Armitage 1957; Agarwal and Deshmanker 1960; Lee 1954) other workers have demonstrated a positive inotropic effect (Kruta, Braveny, Hlavkova-Stejskalova and Husakova 1963; Kennedy and West 1969; Lameijer and Van Zwieten 1974). In their study using electrically-driven rabbit atria, Kennedy and West (1969) showed that quinidine caused either a positive, *or* a negative, inotropic response according to the frequency of stimulation used, the concentration of

drug administered and the time of measurement after quinidine. These workers as well as others (Thorpe 1973; Harrow and Dhalla 1975) have attributed the positive inotropic effects of quinidine to an increased calcium availability via either an inhibitory effect of the drug on calcium sequestration (Nayler 1966; Shinebourne, White and Hamer 1969) or by a release of microsomal bound calcium (Bondani and Karler 1966). Fuchs et al, 1968) observed that in the dog heart, quinidine caused the release of calcium from the sarcoplasmic reticulum and mitochondria, and also inhibited the uptake of calcium ions by these organelles. Furthermore, quinidine is known to exert a profound effect on the passive fluxes of potassium and sodium ions through cell membranes (Vaughan Williams 1964) and this may result in interference with calcium movements. Himori and Taira (1976) have also demonstrated that low doses of quinidine caused a dose-related increase in coronary blood flow. These workers observed that the positive inotropic response of quinidine was not modified by a dose of propranolol which completely inhibited the positive inotropic response to noradrenaline. They thus concluded that the positive inotropic effect of quinidine is not likely to be mediated by an adrenergic mechanism, and suggested that the positive inotropic response to lower doses of the drug may be produced by an increase in the availability of calcium ions for the contractile machinery. Results of the studies presented in this section suggest that under the experimental conditions used, the transient

positive inotropic effect of low doses of quinidine and other quinoline compounds was due to a tyramine-like action in releasing noradrenaline. This evidence substantiates and extends the previous findings of Zetler and Strubelt (1971) with quinidine, but is in contrast to those of Lameijer and Van Zwieten (1974) who found that pre-treatment with reserpine (at a lower dose than used in the present study) did not affect the positive inotropic actions of quinidine. In order to check whether the sympathomimetic action of the quinoline antimalarials was peculiar to atrial muscle or to the guinea-pig, the effects of the compounds were studied on rabbit isolated electrically-driven papillary muscles. For comparative purposes, the effects of proguanil and pyrimethamine were also investigated on the same muscle preparations. The results obtained with all the antimalarials studied were quantitatively and qualitatively similar to those produced by the drugs in guinea-pig driven left atria.

Although high concentrations of all the antimalarial agents caused dose-related negative chronotropic and inotropic effects, those produced by proguanil and pyrimethamine were of much shorter duration than those produced by the quinoline antimalarials. The exact mechanism of the cardio-depressant actions of all the antimalarial compounds is still controversial, but the present studies have shown that it does not involve mediation of acetylcholine or adenosine and is more probably due to a direct action of these drugs in stabilising membranes, inhibiting calcium flux and blocking

sodium and potassium channels. The negative inotropic responses induced by the drugs may be a consequence of a decrease in the intracellular concentration or mobilization of calcium ions needed for maintenance of myocardial contraction, and this view is strengthened by the work of Himori and Taira (1976). It is of interest to find that all the antimalarial drugs investigated were more potent in antagonising the positive inotropic effect of increasing the extracellular calcium concentration than they were in antagonising the effects of noradrenaline or isoprenaline. Similar strong antagonism between calcium and chloroquine has been reported by Van Breemen, Farinas and others (1973). These workers observed that the antagonism appeared to be due to either a direct chelating effect of chloroquine on calcium ions, or an effect of chloroquine on cellular membranes involved in calcium storage or mobilization (as postulated by Feinstein and Paimre 1969). The ability of the antimalarial drugs to antagonise the cardiovascular actions of isoprenaline has led some investigators to assign β-adrenoceptor antagonist activity to primaquine (Bass et al, 1972) and quinidine (Dreifus et al, 1964). The results obtained in this study suggest that the blocking effects of the quinolines and other antimalarial drugs on responses of cardiac muscle to β-adrenoceptor agonists are not due to the competitive blockade of β-adrenoceptors but are more probably due to direct effects on muscle contractile machinery. It is also of interest that the order of potency of the drugs tested as antagonists of calcium-

induced inotropic responses was remarkably similar to their relative potency in decreasing maximum driving frequency of guinea-pig driven atria.

The actions of pyrimethamine were of special interest since there is little information available in the literature on the cardiac effects of this compound. Its profile of activity resembled that of proguanil in that the two compounds were some hundred times less active than the quinolines (primaquine, chloroquine and quinidine) in decreasing maximum driving frequency and inhibiting responses to calcium, noradrenaline or isoprenaline. In addition, the decreases in force and rate produced by proguanil and pyrimethamine were relatively transient and were in contrast to the sustained depression caused by the quinoline antimalarials. Pyrimethamine or proguanil did not cause any initial sympathomimetic effects, a finding which agrees with the observations of Nettleton et al, (1974) in the anaesthetized dog. In common with the other drugs tested, pyrimethamine decreased maximum driving frequency of electrically-driven guinea-pig left atria, but was some 300 times less active than primaquine, chloroquine and quinidine.

In recent years, the sympathetic influence on dysrhythmias produced by cardiac glycosides has been both claimed and denied (Roberts, Ito, Reilly and Cairoli 1963; Erlij and Mendez 1964; Somani and Lum 1965). Reserpine pre-treatment

was reported to increase the amount of ouabain required to induce dysrhythmias, and this has led to the conclusion that the dysrhythmias produced by small doses of the glycoside involve the release of catecholamines (Roberts et al, 1963). Rather more recently, however, procedures which diminished adrenergic influences (reserpinization, sympathectomy and adrenalectomy) were reported to increase the lethal dose of cardiac glycosides and alter the mechanism of death from ventricular fibrillation to cardiac arrest (Erlij and Mendez, 1964). Nevertheless, the data of the studies reported in this Section do not preclude the possibility of an adrenergic influence in the genesis of glycoside-induced dysrhythmias. The ability of alprenolol and propranolol to suppress the ouabain-induced dysrhythmia and to restore normal sinus rhythm probably indicates that the glycoside-induced dysrhythmia is mediated via an adrenergic mechanism (especially β-adrenoceptors).

Most of the antimalarial compounds used in the present studies have been reported to abolish cardiac dysrhythmias produced by local application of acetylcholine or aconitine, or electrical stimulation after crushing the auricle; and dysrhythmias elicited by chloroform or a combination of pentolether and epinephrine (Arora 1955; Arora et al, 1955; Arora and Madan 1956; Arora et al, 1956; Sanabria 1955; Hess and Schmidt 1959) but not ouabain-induced dysrhythmias. The result obtained in the present study agrees with those findings. Although the actual mechanism of the anti-

dysrhythmic action of the compounds is still unknown, it is likely that the antimalarial agents exert this action, at least in part, through their membrane-stabilising, local anaesthetic activity as first suggested by Arora (1955) and Arora et al, (1955), rather than via a specific adrenoceptor blockade (Bass et al, 1972).

4.36 Anaesthetized animals

The results obtained from the anaesthetized cats and guinea-pigs are consistent with those obtained with isolated cardiac muscle preparations. The in vivo studies demonstrate that intravenous administrations of chloroquine, primaquine, quinine, quinidine, proguanil and pyrimethamine (2.5 - 10 mg/kg) cause dose-related reductions in systemic arterial blood pressure. These observations are in agreement with the work of Jackson et al, (1922), Ferrer et al, (1948), Vane (1949), Jindal (1956), Jindal et al, (1960), Deshpande and Tiwaska (1964), Waal (1966), Nakano and McCloy (1967), Farmer and Levy (1968), Jindal et al, (1968) and Nettleton et al, (1974). These investigators showed that the various antimalarial drugs produce hypotensive effects in experimental animals and in man. Apart from causing hypotension, however, all the antimalarial compounds studied produced dose-related reductions in myocardial contractility. Apart from quinidine, the effect of the antimalarial agents on myocardial contractility has not been previously reported in the literature.

All the compounds studied inhibited, in a dose-dependent manner, the cardiovascular responses of anaesthetized cats to intravenously injected noradrenaline. This inhibitory action of the antimalarial drugs on the pressor effects induced by intravenous noradrenaline is in keeping with the work of Nelson (1928), Vane (1949), Hiatt (1950), Lu (1951) and Schmid et al, (1974). These investigators found that quinoline and non-quinoline antimalarial agents inhibited the pressor actions of noradrenaline on the cardiovascular system. Nevertheless, the present observation is at variance with the reports of Jindal (1958) and Pandya et al, (1969) who showed that some quinoline antimalarials potentiated the pressor effects of noradrenaline on the cardiovascular system.

Reynolds and Blackenberg (1927), Halsey, Reynolds and Blackenberg (1928), Gold and Modell (1932), Kwit and Gold (1934), and Rowe, Emanuel, Maxwell, Brown, Castellio, Schuster, Murphy and Crumpton (1957) observed that quinidine increased heart rate in dogs with normal sinus rhythm whilst Cattell and Cattell (1926), Cohen and Levy (1921) and Dick, McCawley, Voiss and Krueger (1958) found a decrease or no change in heart rate in frogs and dogs respectively. In human subjects, Starr et al, (1937) also found an increase in heart rate following quinidine, whereas Ferrer et al (1948) showed that in 5 out of 7 patients, heart rate remained unchanged, but in 2 patients, heart rate increased as systemic blood pressure decreased. The

present study showed that smaller doses (less than 4 mg/kg) of quinoline antimalarial compounds (and quinidine) either caused a slight increase in, or did not affect heart rate, whilst larger doses (more than 5 mg/kg) decreased heart rate in a dose-related fashion. The mechanism responsible for the initial increase in heart rate caused by quinidine has been ascribed to its anticholinergic action by Dale (1921), Briscoe and Burn (1954), and James and Nadeau (1964). The initial increase in heart rate induced by low to moderate doses of the quinoline antimalarials investigated is likely to be mediated through the same mechanism.

The mechanism responsible for the hypotensive effects of all the antimalarial drugs (and of quinidine) may be attributed to the dual cardiovascular actions of the compounds i.e. (1) the direct and/or indirect peripheral vasodilatation action and (2) reduction in cardiac output - possibly due to the myocardial depressant effect of the drugs. Jackson et al, (1922) and Nelson (1927) stated that the hypotensive effect of quinidine might be due mainly to peripheral dilatation resulting from "depression of the sympathetic nerve endings" as well as of the vascular smooth muscle. Lu (1951) also attributed the peripheral vasodilatation induced by quinidine to three mechanisms, viz: (1) depression of the sympathetic receptor, (2) depression of sympathetic ganglia, and (3) direct relaxation of vascular smooth muscle. Because all the antimalarial compounds studied possess many pharmacological actions in common with

quinidine, it is likely that the agents produced their cardiovascular effects through a similar mechanism. Carney, Ross and Cooper (1962) showed that a large dose (30 mg/kg) of quinidine caused a marked decrease in aortic pressure when systemic blood flow was kept constant with a flow pump, implying a decrease in peripheral resistance. They were uncertain, however, whether this vasodilatation was due to direct smooth muscle depression, or to blockade of the vasomotor centre and/or sympathetic outflow. Nevertheless, the workers demonstrated that both arterial and venous systems appeared to participate in the dilatation. In experiments in which the central effects of quinidine were dissociated by using hind-limb preparations, Nakano and McCloy (1967) showed that intra-arterial injections of quinidine dilated the femoral artery without any apparent change in heart rate, systemic arterial pressure and myocardial contractile force. It was concluded that the vasodilator effect of quinidine is not mediated through beta-(β-) adrenergic stimulation (Ahlquist, 1948), sympathetic-cholinergic vasodilator stimulation (Folkow and Uvnas 1948), or a histamine release mechanism (Paton 1957). Some of the early workers (Nelson 1927; Lu 1951) have postulated that the vasodilator action of quinidine is due to "the depression of sympathetic receptor", presumably implying that quinidine would block alpha-(α-) adrenoceptors in vascular smooth muscle. However, the results obtained by Nakano and McCloy (1967) are not in agreement with this hypothesis, since quinidine did not modify the vasoconstrictor

action of noradrenaline in the hind-limb preparation. It is likely that all the antimalarial drugs examined share the above effects with quinidine and produce their cardiovascular effects through the same mechanisms.

The hypotension and bradycardia induced by the antimalarial drugs investigated in the present study were not modified by vagotomy. This finding probably indicates that the cardiovascular effects of the compounds, like those of quinidine, are not mediated through a cholinergic mechanism. This suggestion is further strengthened by the observation that the hypotension and bradycardia induced by the agents are resistant to the action of atropine (Deshpande and Tiwaska 1964). Since a cholinergic mechanism is unlikely to account for the hypotensive effects of the compounds, it is possible that the agents exert their hypotensive action via one or more of the mechanisms described above for quinidine. In addition, the ability of the compounds to antagonize the effects of noradrenaline, isoprenaline, calcium (and other drugs) on the heart could contribute to their hypotensive effect.

The effect of quinidine (but not of other antimalarials) on myocardial contractility has been previously studied (Cohn and Levy 1921; Cattell and Cattell 1926; Briscoe and Burn 1954; Armitage 1957; Carney, Ross and Cooper 1962; Nakano and McCloy 1967). Using the myocardiographic technique, Cohn and Levy (1921) demonstrated that quinidine

increased the amplitude of myocardial contraction whereas Briscoe and Burn (1954) and Armitage (1957) found a decrease in myocardial contraction. Working with a Walton-Brodie strain gauge, Carney et al, (1962) found that the administration of 10 mg/kg of quinidine only produced a transient decrease in myocardial contractile force whilst 30 mg/kg of the drug markedly decreased contractile force for a prolonged period of time. Using a strain gauge and measuring left ventricular dP/dt, Nakano and McCloy (1967) showed that the administration of doses less than 6.4 mg/kg of quinidine caused only a slight decrease, or no change at all, in myocardial contractility whereas higher doses (more than 12.8 mg/kg) decreased myocardial contractility. The biochemical mechanisms responsible for the quinidine-induced decrease in myocardial contractility has been studied by several workers (Armitage 1957; Webb, Saunders and Nakamura 1951; Uyeki, Coiling and Du Bois 1954; Ells 1964; Holland 1944; Klein, Holland and Tinsley 1960; Suarez-Roldan and Santos-Martinez 1964) but the precise mechanism remains uncertain. Webb et al (1951), Uyeki et al (1954) and Ells (1964) observed that quinidine inhibited ATP-ase activity in cardiac and skeletal muscles. Uyeki et al (1954) also found that quinidine uncoupled oxidative phosphorylation in the mitochondria of the heart and liver, implying that quinidine might decrease the production of high energy phosphate in the heart. Like quinidine, moderate to high concentrations of the anti-malarial drugs examined always decreased myocardial

contractility in a dose-dependent manner, presumably through mechanisms similar to those analyzed above for quinidine. The lower ratio of dP/dt peak common developed isovolumic pressure (CPIP at 60 mm Hg) obtained after intravenous administrations of chloroquine, primaquine and pyrimethamine (2.5 - 10 mg/kg) indicates that the drugs decreased the contractile state of the heart. This decrease in the contractile state probably also contributes to the hypotensive effects of the compounds.

According to Nakano and McCloy (1967), the effect of quinidine on cardiac output appears to be governed by three haemodynamic parameters: (1) myocardial contractility, (2) venous return, and (3) peripheral resisistance. Cattell and Cattell (1926) reported that in frog isolated heart, low concentrations of quinidine produced an increase in cardiac output whilst high concentrations decreased it. However, Reynolds and Blackenberg (1927) and Halsey et al, (1928) found that quinidine increased cardiac output in anaesthetized dogs. Ferrer et al, (1948) also showed that in normal human subjects, quinidine decreased systemic arterial pressure without any change in cardiac output whereas in patients with low output (or heart failure), quinidine increased cardiac output. Nakano and McCloy (1967) found that quinidine had a biphasic effect on cardiac output in dogs; an initial transient increase followed by a secondary, more prolonged, decrease. These workers attributed the initial transient increase

to the decrease in peripheral resistance, since the dose (6.4 mg/kg) of quinidine administered did not depress myocardial contractility markedly. Nakano and McCloy (1967) further found that with larger doses (more than 6.4 mg/kg) myocardial contractile force decreased markedly and both mean pulmonary arterial and mean left atrial pressures decreased significantly. This probably indicates a decrease in systemic venous return (Carney et al, 1962). Because cardiac output was not determined in the present study, it would not be possible to compare this cardiovascular effect of quinidine with that of other antimalarial agents studied. However, moderate to high doses (5 - 10 mg/kg) of all the antimalarials examined caused dose-related increases in right atrial and left ventricular end-diastolic pressures. These haemodynamic responses would be difficult to explain since they are not peculiar to antimalarial or anti-dysrhythmic drugs along.

Moderate to high doses (above 5 mg/kg) of all the antimalarial agents studied (as well as quinidine) increased the P-R interval and QRS complex duration in a dose-dependent manner. Many other antidysrhythmic agents are known to increase the P-R interval and QRS complex duration (Szekeres and Papp, 1971).

Except for quinidine, reports on blood levels of anti-malarial compounds are poorly documented in the literature. However, the plasma quinidine levels (following the

administration of quinidine) have been studied by Wegria and Boyle (1948); Sokolow (1951); Sokolow and Edgar (1950); Sokolow and Ball (1956); Kahmansohn and Sampson (1950); Swisher, Wedell, Cheng, Sutton and Sutton (1954); Ditlefsen and Knutsen (1956); Scherlis, Gonzalez and Bossman (1961); and Nakano and McCloy (1967). Wegria and Boyle (1948) stated that the intensity of the cardiovascular effects of quinidine and its plasma concentrations were not qualitatively parallel except for its effect on the electrocardiogram. These authors further observed that the plasma level of the drug usually decreased faster than its cardiac effects. However, according to the observations made by the other investigators (mentioned above), the doses of quinidine administered seem to determined its plasma concentrations at a specific time. Nakano and McCloy (1967) demonstrated that both the electrocardiographic and haemodynamic changes induced by quinidine are proportional to the plasma levels. Sokolow and Ball (1956) found that the majority of their patients with cardiac dysrhythmias were treated successfully with doses of quinidine which gave rise to a plasma concentration of approximately 6 µg/ml. In dogs, this level was attained when doses of about 3.2 mg/kg of quinidine were administered intravenously (Nakano and McCloy 1967). The latter workers also observed that intravenous administrations of quinidine (6.4 mg/kg or less) depressed neither myocardial contractility nor ventricular performance. The authors therefore concluded that doses of quinidine sufficient to treat majority

of the patients with cardiac dysrhythmias would not cause deleterious effects on the cardiovascular system. The blood levels of the other antimalarial drugs examined were not determined in the present study and there are no documented reports in the literature concerning plasma level reached after oral dosages.

Several investigators have advocated the use of certain antimalarial agents in the management and treatment of some cardiovascular disorders in man (for references, see Introduction to the present section). From the observations made and the results obtained in the present studies, it is evident that, even in therapeutic doses, the antimalarial compounds are toxic to the cardiovascular system, especially to the heart. In most of the animals used, cats and guinea-pigs, doses of the compounds above 10 mg/kg were usually lethal, the animal dying of cardiovascular arrest. On the basis of this acute cardiovascular toxicity, the idea of employing the agents in the management and treatment of cardiovascular disorders in man does not appear to be valid; there are far more effective and less toxic drugs available. The results of the present studies also indicate that most of the deaths usually attributed to antimalarial drugs in man are likely to be due to the chronic or acute toxic effects of the compounds on the cardiovascular system, especially on the heart.

SECTION 5

GENERAL CONCLUSIONS

5 GENERAL CONCLUSIONS

The present studies have shown that all the five anti-malarial drugs investigated (chloroquine, primaquine, quinine, proguanil and pyrimethamine), although structurally different, have a wide spectrum of pharmacological activity in common. These pharmacological actions explain some of their common clinical side-effects and toxicity.

Among the toxic side-effects often attributed to the compounds are diarrhoea, gastro-intestinal cramps and pain, skeletal muscle weakness, depression of the cardiovascular system, weak pulse, dizziness, syncope, convulsions and coma. The diarrhoea might be due to the relaxation of the intestinal smooth muscles by the compounds, while the gastro-intestinal muscle cramps are probably caused by contractions of the gastro-intestinal muscles induced by higher doses of the drugs. These contractions, which may be pronounced, may give rise to the gastro-intestinal pain observed clinically with these drugs. The skeletal muscle weakness induced by the drugs might be attributed to the weak curare-like action of the agents coupled with their local anaesthetic activity. As local anaesthetics, the compounds may be paralysing the motor nerves to skeletal muscles and thereby affect neuromuscular transmission.

The cardiovascular impairment caused by the drugs is most likely to be due to their direct effects on the heart and/

or their general depressant action on the cardiovascular system. Their anti-hypertensive action may be associated with their ability to antagonize the cardiovascular effect of noradrenaline, whilst their anti-dysrhythmic effects could be related to their local anaesthetic activity, and/or their ability to antagonize calcium, catecholamines, and other drugs on the heart. The weak pulse is probably associated with their depressant effects on the heart and cardiovascular system, resulting in bradycardia and hypotension.

Both the dizziness and syncope may result from the bradycardia and hypotensive mechanisms, resulting in pooling of blood in the lower part of the body. The convulsions often attributed to antimalarial agents may also be due to their hypotensive effects leading to a reduction in cerebral blood flow.

The results of the studies reported in this thesis indicate that the antimalarial drugs possess some anti-asthmatic activity (especially the quinoline compounds), anti-dysrhythmic and marked hypotensive effects, and also have relaxant effects on smooth and skeletal muscles. The results also suggest that the drugs are non-abortive and that therapeutic doses of the compounds are safe (non-toxic). However, since similar investigations were not carried out in a parallel manner in human subjects, it would be difficult to ascertain the extent of the above-named effects and safety of the agents at therapeutic

dose levels (used in the present studies) in man. Moreover, although the present studies were performed on essentially normal, healthy laboratory animals, it would not be valid to draw conclusions from these experiments and extrapolate them to man. In the light of the various pharmacological actions of the compounds shown in these studies, especially their actions on the heart and cardiovascular system in general, one would suggest that the drugs should be administered to humans, especially infants and children, under very strict medical supervision only. Furthermore, therapy should be stopped, or dosage drastically reduced, when the signs and symptoms of toxicity or side-effects appear. Where possible, pregnant women should be encouraged to refrain from the use of the compounds because of their abortifacient and teratogenic effects reported by Sollmann 1957; JAMA Editorial 1964; Whisnant, Espinosa, Kierland and Lambert 1963; Hughes, Esiri, Oxbury and Whitty 1971; Bosson, Lough Jr. and Hansen 1973; and Rollo 1975.

DRUGS USED

Drugs used were primaquine phosphate, proguanil hydrochloride, (±)-propranolol hydrochloride (Imperial Chemical Industries Limited); quinidine sulphate, quinine sulphate, chloroquine diphosphate, (-)-noradrenaline hydrochloride, acetylcholine chloride, (-)-phenylephrine hydrochloride (Sigma); pyrimethamine, bretylium tosylate, bethanidine sulphate (Burroughs Wellcome); atropine sulphate reserpine, tyramine hydrochloride, acetylcholine iodide, (-)-adrenaline hydrogen tartrate, carbachol chloride, 5,5-dithiobis-2-nitrobenzoic acid, histamine dihydrochloride, nicotine hydrogen tartrate, papaverine hydrochloride, physostigmine sulphate, strophanthin-g ("ouabain"), barium chloride, lactic acid, potassium chloride (British Drug Houses); (-)-isoprenaline bitartrate (Wyeth); lignocaine hydrochloride, cocaine hydrochloride (Macfarlane Smith); sodium pentobarbitone (Abbot Laboratories); salbutamol (Allen and Hanburys); guanethidine sulphate, phentolamine mesylate (Ciba); (+)-tubocurarine chloride (Duncan Flockhart); heparin (Evans Medical); alprenolol hydrochloride (Hassle); 5-hydroxy-tryptamine (Koch-Light); hexamethonium bromide, mepyramine maleate, suxamethomium bromide, dexamphetamine sulphate (May and Baker); bradykinin, methysergide bimaleate (Sandoz); indomethacin (Merck, Sharp and Dohme); oxytocin (Parke-Davis), dipyridamole ("persantin") (Boehringer Ingelheim).

Except where indicated, all drugs were dissolved on the day of the experiment in 0.9% $^{w}/_{v}$ sodium chloride solution. Pyrimethamine was dissolved in saline acidified with equimolar lactic acid. Indomethacin was dissolved in ethanol and the solution diluted 10 - 20 times with normal saline before use. All doses were expressed as molar concentrations except where otherwise stated.

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