INVITRO EVALUATION OF THE ACTION OF CHLOROQUINE AND RELATED COMPOUNDS ON CONTRACTILE PROCESSES IN SMOOTH MUSCLE

BY

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DEDICATION

I cannot repay the Lord for his goodness to me. I will raise the Cup of Salvation, I will call on God's name.

> "Praise the Father, the Son and Holy Spirit, Both now and for ever. The God who is, who was and is to come at the end of ages".

With gratitude to God, this thesis is lovingly dedicated to my teachers for unceasing inspiration on the subject, and my wife and children for their love and patience.

ABSTRACT

In this study, the effect of chloroquine on the contractions of isolated guinea-pig ileum (GPI) rat stomach strip (RSS), and endothelium free-rat aortic strip (RAS) were investigated. These muscles exhibit contractions suggesting differences in sources of activator calcium. The main agonists used were histamine (HIS), Acetylcholine (ACH), Noradrenaline (NA) and Potassium (K^+). A wide range of doses of CQ were used. The following drugs were also investigated: Amodiaquine (AMDQ), Mepacrine (MPC), Quinine (QUIN), Halofantrine (HFT) and Mefloquine (MFQ), in order to examine the pattern, if any, of structure-action relationships.

The effect of CQ on Ach induced contractions ranged from a potentiation in low CQ concentrations (10^{-12} -10^{-7}M), to inhibition at concentrations > 10^{-5}M. In this regard, drugs were classified into groups:

(I) CQ, AMDQ, MPC, QUIN, which consistently potentiated and inhibited at low and high concentrations respectively.

(II) HFT and MFQ, which showed neither marked potentiation nor inhibition. Potassium induced contractions were less affected by CQ than receptor mediated contractions.

Varying the concentrations of Ca^{2+} in the physiological salt solution (PSS) bathing the muscle, greatly affected the action

of CQ. In the RSS, Group I compounds at (10^{-7}M) potentiated ACH-induced contractions in PSS containing 1.8mM Ca^{2+} while the inhibitory effect of concentrations > 10^{-5}M was enhanced. Similar effects were observed in GPI and RAS. Thus potentiation of agonist contraction decreased in parallel with decrease in external Ca^{2+}, whereas inhibitory effects increased.

In PSS containing zero calcium and 0.5mM EGTA, contractions in GPI rapidly disappeared whereas in RSS and RAS residual contractions persisted. Low doses of antimalarial failed to potentiate the residual response but high doses completely abolished it.

The action of CQ in RAS, pre-contracted with NA or K^+ was studied. The relaxant effect of CQ was more marked on NA- than K^+ induced contractions. This effect was unaffected by methylene blue (10^{-3}M). Thus the mechanism of the relaxant action of CQ in RAS was unlike those of Ca^{2+} channel blockers or sodium nitroprusside.

The possibility of the relaxant effect being due to K^+ channel opening was investigated. The results suggested that K^+ channel opening is not an adequate explanation for this action of CQ in RAS.

These results show that:

(a) Two sites of action for CQ in smooth muscle. One site is the muscle-cell membrane where it may facilitate Ca^{2+} influx: this effect is more marked on receptor mediated than K^{+}-induced responses. The other site is intracellular, where the effect is observed at higher concentrations of antimalarial and manifests as inhibition of agonist contractions.

(b) The relaxant effect of CQ in arteries was most likely not due to calcium channel blockade nor opening of K^{+} channels: CQ probably interacted with intracellular contractile mechanisms;

(c) The effect of CQ on muscle contraction depends on muscle type, agonist used, and the concentration of Ca^{2+} in the bathing fluid. These findings suggest that vascular relaxation by CQ may contribute to the cardiovascular collapse encountered with chloroquine therapy.

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This is to express my immense gratitude to all those who have contributed towards the successful completion of this study.

I would in particular like to express my gratitude to my able supervisor, Professor D.T. Okpako, whose keen interest, unceasing inspiration, commitment has made this study a reality. For me, it has been a priviledge to draw from his vast resources having been a Fellow of local and international bodies. His suggestions, criticisms and constant on the spot assessment of the work, coupled with the use of his personal library and drugs is greatly appreciated.

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supplying me with animals. I wish to thank Dr. Joe Woods of the Chemistry Department, University of Ibadan for his interest and support, twice he took me to Lagos to purchase animals for this study.

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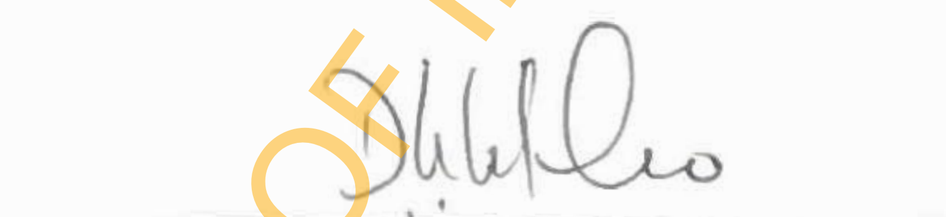
It is pertinent here to express my heartful thanks to my loving wife, Margaret - the "Jewel of inestimable value" for her steadfastness, prayers, whose commitment to the domestic and home keeping gave me an uninterrupted atmosphere to carry out this work; and to my children, Ehimhe, Osose, Eboseremhe and generations yet unborn, "who are fond to ask Daddy when shall you complete your Ph.D?" I thank them all for their concern and love.

To my God, the creator, in whom all things exist, through His love - by revelation of Christ and through His inspiration by the Holy Spirit for His enlightenment on the subject matter. I cannot fully repay the Lord for His goodness to me. To all and sundry I say thank you and God bless.

CERTIFICATION

This is to certify that the work recorded here was carried out by PETER I. AZIBA in the Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria under my supervision.



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CONTENTS

ABBREVIATIONS

Ach	-	Acetylcholine
AmDQ	-	Amodiaquine
Ca^{2+}	-	Calcium ion
$[Ca^{2+}]$	-	Calcium ion concentration
CaM	-	Calmodulin
C.Kinase	-	Protein Kinase C
CQ	-	Chloroquine
°c	-	Degree Centigrade
EGTA	-	Ethylene glycol bis (B-amino aethylene-N tetra acetic acid)
EC_{50}	-	Effective concentration of a drug that gave 50% of maximum response.
EDRF	-	Endothelium Derived Relaxing Factor
G-6-PD	-	Glucose-6-Phosphate Dehydrogenase
GPI	-	Guinea Pig Ileum
HFT	-	Halofantrine
H_2O	-	Water
K^+	-	Potassium ion
kg	-	Kilogram
[M]	-	Molar concentration
mM	-	milli Molar
ml	-	milli litre

mg	-	milligram
MPC	-	Mepacrine
MFQ	-	Mefloquine
mM	-	Micromolar
MB	-	Methylene blue
MLCK	-	Myosin Light Chain Kinase
NA	-	Noradrenaline
POC	-	Potential Operated Calcium Channel
pH	-	Hydrogen ion concentration
Quin	-	Quinine
RAS	-	Rat Aortic Strip
RSS	-	Rat Stomach Strip
SR	-	Sarcoplasmic Reticulum

CHAPTER ONE

INTRODUCTION

1.1 ANTIMALARIAL DRUGS: HISTORICAL BACKGROUND

The development of the antimalarial drugs forms one of the most important contributions to the history of Medicine. Most of the currently used antimalarial drugs have evolved from knowledge of the chemistry and pharmacology of the active substances of cinchona bark, a Peruvian medicinal plant used for treatment of fevers in the early 17th Century.

The therapeutic value of Crude Cinchona bark became known in the late 17th Century and was widely used for two centuries before its active constituents quinine and other related alkaloids were isolated in 1820. The salts of quinine soon became available commercially for use and cinchona remained the only source of the drug until quinine was synthesized, other cinchona alkaloids are still extracted from cinchona bark.

1.2 SYNTHETIC ANTIMALARIAL DRUGS USED

Later development of antimalarial drugs were based on the quinine structure particularly the quinoline moiety. The first synthetic antimalarial pamaquine, emanated from efforts by Schulemann and his colleagues in 1928 to combine 6-methoxy quinoline structure which was previously found to exhibit

additional antimalarial activity in congeners of methylene blue (James and Giles, 1985).

Later studies which were based on Ehrlich's work gave an invaluable lead to a host of other new antimalarial compounds. Pamaquine the first compound to emanate from these researches was very useful against avian malaria, but it could not replace quinine as it had little effect against P. falciparum infections. The search for more potent and less toxic antimalarial drugs began in 1943 during the Second World War by French workers who synthesized a large number of aminoquinoline compounds.

AMINOQUINOLINES

These compounds were based on the quinine structure and also according to the spectrum of their antimalarial activity. They have been divided into: 4-aminoquinoline derivatives: this group includes Chloroquine, hydroxychloroquine and amodiaquine. Chloroquine is the most extensively used of the 4-aminoquinolines. It contains the same alkyl side chain as quinacrine, it differs from the latter in having a quinoline instead of an acridine nucleus and in lacking the methoxy radical.

Chloroquine bears close resemblance to pamaquine and pentaquine. It is a very potent blood schizonticidal drug effective against the erythrocytic form of all four plasmodial species. It is also an effective prophylactic drug in sensitive strains of P. falciparum. Among its other major pharmacological actions, chloroquine is a fairly potent anti-inflammatory agent and also shows some quinidine like action on the heart. It is used in the therapy of Amoebic dysentery,

Amodiaquine:

Is a congener of chloroquine. Amodiaquine is a very effective agent for suppressing and alleviating sporozoite induced vivax malaria in human volunteers. When compared to chloroquine in potency, it is active only against erythrocytic forms of the parasite; relapses occur when medication is stopped (Coatney et. al., 1950. See 1973 WHO scientific group). The drug is adequately used in controls of acute clinical attack of vivax or falciparum malaria. Trials of amodiaquine in various parts of the world indicate that it is an active and relatively non-toxic antimalarial. It has been given to patients with liver, cardiac and renal disease without untoward reactions.

Preliminary investigation indicates that amodiaquine like chloroquine is effective in amoebic hepatitis, it is not useful in intestinal Amoebiasis.

8-Aminoquinolines:

Primaquine was one of the most effective drugs in the 8-aminoquinoline series prepared by Elder Field and associate (1946) in connection with the Second World War antimalarial search programme. Primaquine has the advantage of being effective against the erythrocytic stage of the plasmodium infection as in P. vivax infection where the liver stage is dominant. Primaquine is not used much in West Africa because of (i) toxicity due to G6PD deficiency; and (ii) P. falciparum is the dominant parasite of West Africa.

Another example of 8-aminoquinoline is Mepacrine which was the outcome of introducing the side chain into acridine structure within which is contained the 6-methoxy-quinoline. It has long been displaced clinically by chloroquine. It was found useful in 1930 and evaluated in time to be of use in the Second World War thereby becoming the main substitute for quinine. It is primarily blood schizontocides. It is active in vitro against G. lamblia. Mepacrine administration is accompanied initially by adverse effects such as dizziness,

headache, and gastric upset but these subside as treatment continues more serious toxic effects develop later; these include toxic psychosis, bone marrow depression and dermatitis but these are not common. The drugs also stains the tissues bright yellow, but this has no toxic but cosmetic implication.

QUININE

It is an alkaloid derived from Cinchona bark (Quinidine, the D-isomer of quinine also has some antimalarial activity) but is used mainly for its antidysrhytmic effects on the heart. Its mechanism of action as an antimalarial agent is not understood, but it is known to intercalate the DNA.

Quinine was relegated to a drug of second choice when chloroquine was introduced, but with the emergence and spread of chloroquine resistance, quinine has again assumed therapeutic importance. it is effective against the erythrocytic forms of all four species of plasmodia, but has no effect on exoerythrocytic forms or on the gametocytes of P. falciparum resistant to chloroquine.

1.3 NEW COMPOUNDS WITH ANTIMALARIAL ACTIVITY

The currently used antimalarials have been in clinical use for some considerable time for more than 40 years but

increasing awareness of the hazard of drug resistance has stimulated further research towards the development of alternative drugs. Advances in biological, biochemical and chemical knowledge of the disease and its methodology have provided the basis for the development of many interesting new compounds. Out of the many compounds that have been synthesized and tested for antimalarial activity, many promising substances have emerged.

Mefloquine and quinine are two main agents belonging to the quinoline-methanols. The former is a new drug which shows great promise but quinine is the main quinoline methanol currently in use. Mefloquine has a long plasma half life and a very large apparent volume of distribution. It is a potent rapidly acting schizonticide in all human malaria but is without effect on gametocytes and hypnozoites. A single oral dose of mefloquine (1000-1500mg) is completely effective in curing patients with drug sensitive to multi-drug resistant falciparum malaria; side effects which are fairly common are dizziness and gastrointestinal upset but these are mild and self limiting.

HALOFANTRINE

It is a new antimalarial drug used in this study. It belongs to the Phenanthrene methanol group. It is a highly

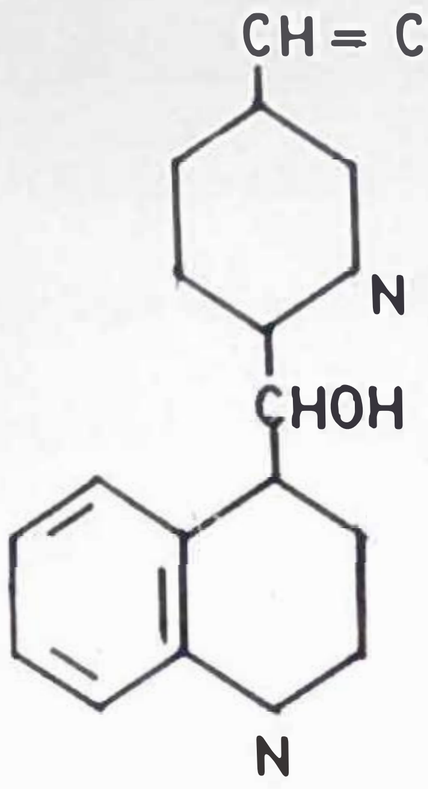
effective blood schizonticide, active in erythrocytic stage of the parasite life cycle. Halofantrine is effective in all types of acute malaria.

Figure 1:

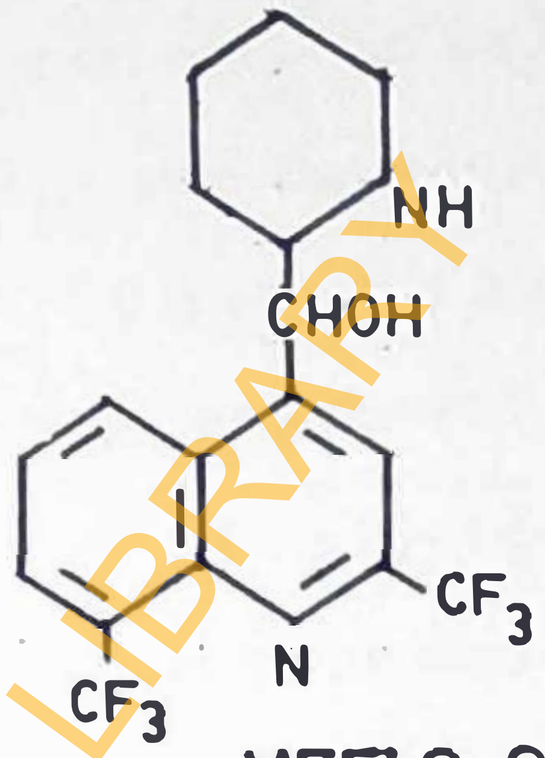
Structures of some antimalarial drugs.

STRUCTURES OF SOME ANTIMALARIAL DRUGS

4 – QUINOLINE METHANOLS

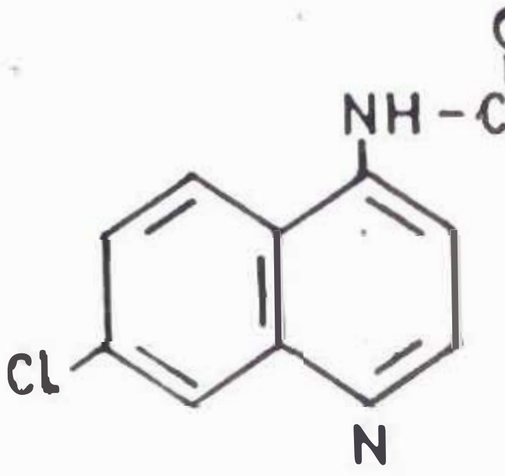


QUININE

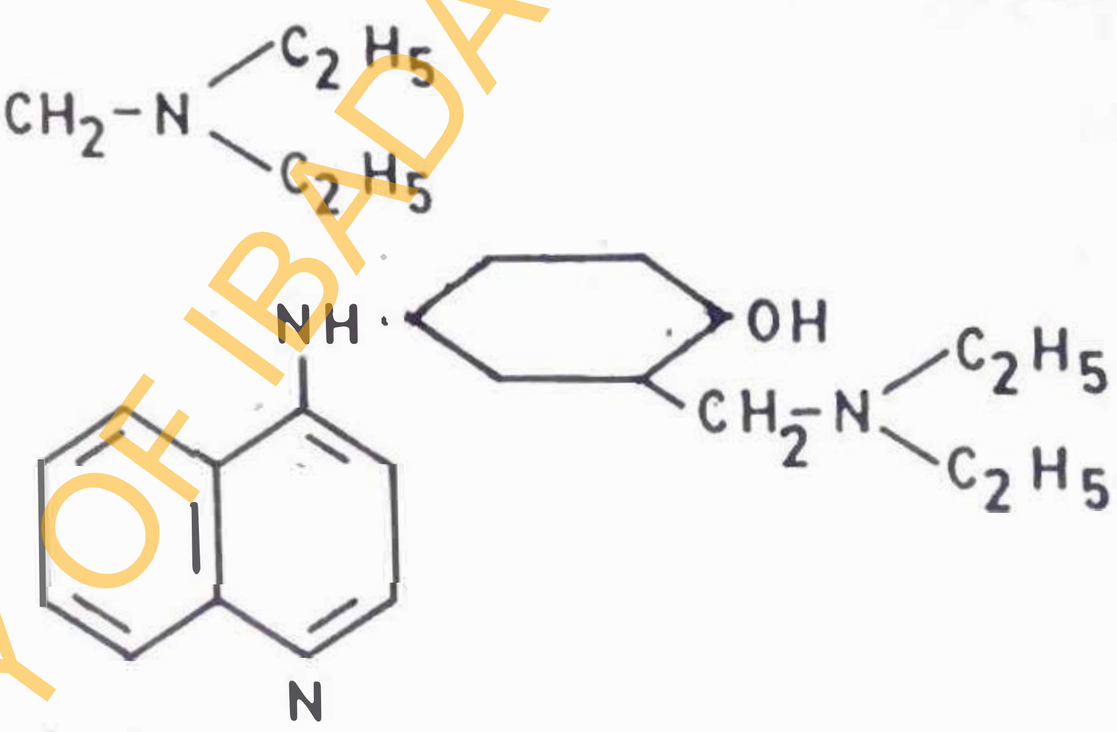


MEFLO QUINE

4 – AMINO QUINOLINES

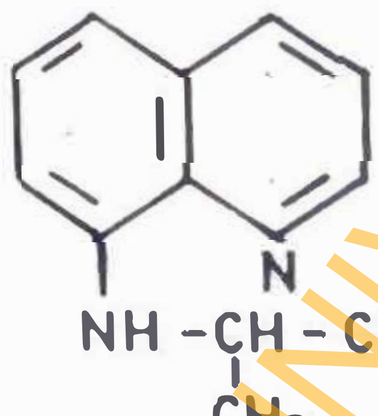


CHLOROQUINE

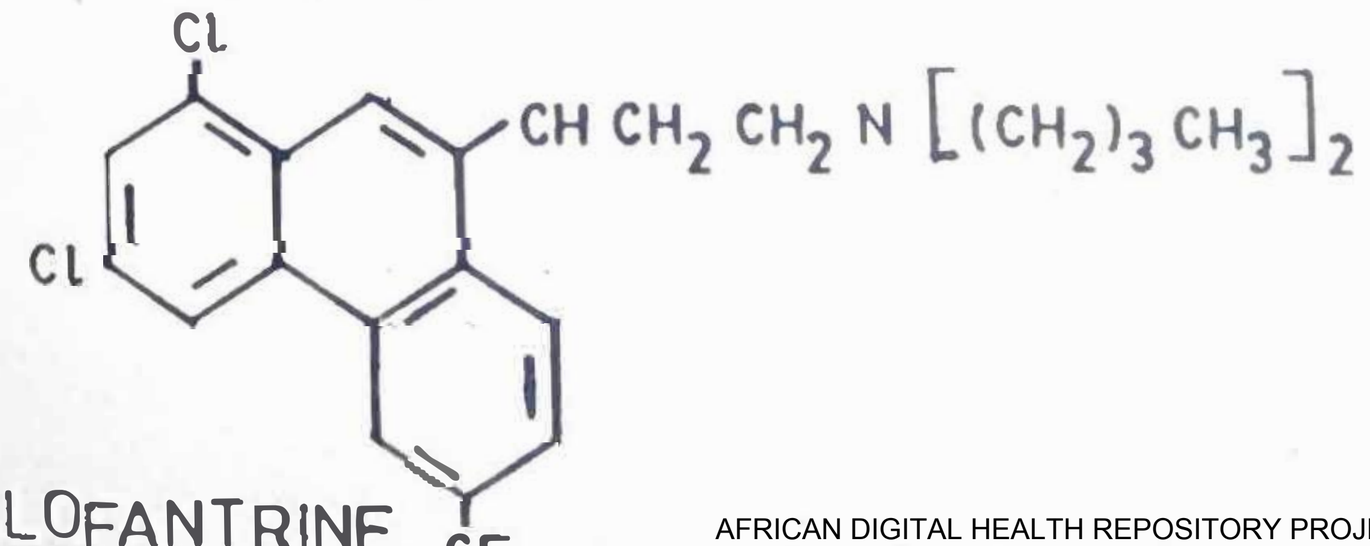


AMODIAQUINE

8 – AMINO QUINOLINE



PHENANTHRENE METHANOL



HALOFANTRINE

1.4 DRUG RESISTANCE IN MALARIA.

Drug resistance has become a common occurrence in malaria chemotherapy. Chloroquine a 4-aminoquinoline was developed as a substitute for quinine in the treatment of malaria during the Second World War. It is effective in the eradication of malaria parasites but since 1960 reports of chloroquine resistant malaria have appeared from Thailand and Colombia (D.V. Moore and J.E. Lanler, 1961), see (James & Giles, 1985). Chloroquine resistant plasmodium had spread to 15 countries in Easter Asia and Oceania, 10 in South America and 15 in Africa South of the Sahara, see (James & Giles, 1985). Because of this development, various drugs have been introduced whether for prophylactic or curative purposes in varying responses to the specie or strain of parasites.

The World Health Organization (WHO) Technical Report Series, Volume 529, 1973, graded this phenomenon into classes I, II, III as defined below.

SENSITIVITY

Where clearance of asexual parasitaemia within 7 days of initiation of treatment without subsequent recrudescence.

Resistance (R_I): This is an evidence of asexual parasitaemia as in sensitivity but no clearance followed by recrudescence.

Resistance (RII): This is categorised with marked reduction of asexual parasitaemia but no clearance; while resistance (RIII) is categorised as where no marked reduction of asexual parasitaemia, with these development in drug resistance, recent studies by Salako et. al. (1988) showed that P. falciparum showed full sensitivity in vitro to quinine with a mean fever clearance time of 1.4d using the in vitro microculture technique. In the Nigerian isolates of P. falciparum, the minimum inhibitory concentration for quinine was 1.28μmol/litre in vitro. The IC_{50} and IC_{99} were 0.25 and 0.8μmol/litre respectively. When compared to chloroquine the authors concluded that quinine and chloroquine in a population where chloroquine resistance was not a problem, the result showed that slower parasitological and clinical response to quinine than chloroquine.

1.5 EXCITATION-CONTRACTION COUPLING IN SMOOTH MUSCLE

Contraction of smooth muscle is triggered by depolarisation of the fibre surface membrane. Depolarisation of the smooth muscle fibre of most of the gastro-intestinal tract leads to initiation of the action potential discharge. In certain types of smooth muscle, the depolarisation spread inwardly along the membranes of the transverse tubules and

cause the release of Ca^{2+} from the terminal cisternae of the sarcoplasmic reticulum. Sufficient evidence favours the idea that during depolarisation, the surface membrane, charge movement occurs from extracellular medium into the muscle. Ca^{2+} and Na^{+} influx during depolarisation have been conclusively demonstrated in mammalian gastro-intestinal smooth muscle (Perry and Grand, 1977; Brading, 1979; Bolton, 1979, 1981). it seems likely that the key factor in the activation of the contractile protein is as in skeletal muscle, a rise in the free Ca^{2+} concentration. This has been estimated at about $10^{-7}M$ calcium so that below this concentration the muscle is relaxed. The control of intracellular Ca^{2+} concentration $[Ca^{2+}]$ involves many factors.

The increase in intracellular Ca^{2+} concentration can be brought about in several ways. Ca^{2+} can enter from extracellular fluid down the concentration gradient, especially when the membrane is depolarised and Ca^{2+} permeability increased. Ca^{2+} is the main ion carrying the inward current for the action potential in most gastro-intestinal smooth muscle, though Na^{+} participates to varying degrees in different tissues. The amount of calcium entering during an action potential could raise Ca^{2+} threshold for contraction (Goodford, 1970). However, it is likely that Ca^{2+}

entering from outside the cell is readily taken up into intracellular stores.

Calcium can be released from binding sites at the inner surface of the plasma membrane or from the sarcoplasmic reticulum or other intracellular structures such as Mitochondria, and it is believed that Ca^{2+} release and Ca^{2+} sequestration play a major role in the sequence of contraction and relaxation (Perry and Grand, 1979).

Ca^{2+} bound at the outer surface of the plasma membrane plays an important role as a stabiliser controlling mainly Na^{+} conductance (Na^{+} as well as Ca^{2+} and other divalent cations, compete on those membrane sites).

Calcium may enter smooth muscle cells via Ca^{2+} channels coupled to pharmacological receptors the so-called receptor-operated Ca^{2+} channel (Bolton, 1979). The reduction of Ca^{2+} necessary for the muscle to relax, can be achieved by (i) intracellular Ca^{2+} sequestration and (ii) by Ca^{2+} extrusion across cell membrane. The evidence suggests that both Na^{+} - Ca^{2+} exchange mechanism (Reuter et. al., 1973; Blaustein, 1974; Van Breemen et. al., 1979) and Ca^{2+} transport mechanism (Schatmann, 1973) operate in smooth muscle and their relative importance for each muscle may depend on the individual capacity of the internal storage space.

Recently two Ca^{2+} pathways through which extracellular Ca^{2+} may pass into the cell membrane were described (Bolton, 1977; Van breemen et. al., 1979; Meisheri et. al., 1981; Cauvin et. al., 1984). The idea was brought about following the observation that when high K^{+} was employed to produce depolarisation and contraction of smooth muscle other agonists such as Acetylcholine or Noradrenaline which activate smooth muscle by stimulating discrete pharmacological receptor could still elicit contractile responses, suggesting that the contractile response and the Ca^{2+} movement associated with K^{+} depolarisation on one hand and those that occurred via receptor activation on the other occurred by different mechanisms. More direct evidence was obtained for this hypothesis (Mesheri, et. al., 1979) when 45 Ca^{+} fluxes were correlated with contractile responses. The Ca^{2+} pathways i.e. Ca^{2+} channels stimulated by changes in membrane potential have been designated potential sensitive Ca^{2+} channels or voltage-dependent Ca^{2+} channel, while Ca^{2+} channels that are opened by stimulation of pharmacological receptors are known as receptor-operated Ca^{2+} channels (Bolton, 1979; Van Breemen et. al., 1979; Meisheir et. al., 1981).

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Bolton and Kitamura (1983) have shown that extracellular Ca^{2+} influx does not occur via Ca^{2+} channels coupled to

muscarinic cholinoceptors or mammalian visceral smooth muscle providing further evidence in support of the existence of at least two Ca^{2+} entry pathways in mammalian smooth muscle Ca^{2+} not only triggers the contractile process, but also controls quantitatively the output of mechanical tension by regulating the amount of ATP that is metabolised during activity. The rapid rise in free intracellular Ca^{2+} resulting from increased transmembrane Ca^{2+} influx and a simultaneous liberation of Ca^{2+} from sarcoplasmic reticular stores, is thought to initiate the splitting of ATP by the Ca^{2+} dependent ATPASE of the myofibril, so that phosphate bond energy is transformed into mechanical work (Fleckenstein, 1977).

Contractility is therefore reversibly lost upon Ca^{2+} withdrawal. Alterations in the extracellular Ca^{2+} concentration generally leads to parallel changes in the amount of ATP consumed by the contractile system, the magnitude of the mechanical tension developed, and the extra uptake of oxygen related to the mechanical tension generated.

1.6 MECHANISM OF EXCITATION CONTRACTION COUPLING IN SMOOTH MUSCLE

The mechanical action responsible for smooth muscle contraction are associated with its contractile machine that

is the proteins. These proteins are arranged in an organized thick and thin filaments (Devine and Somlyo, 1971). The thin filaments are fibrous actin average 5-8nm in size and attach to dense bodies well connected to the cell membrane, while the thick filaments are bundles of myosin molecules about 15.5nm in size with lateral projection suggestive of cross bridges, extending toward the thin actin filament. Their functions in contraction can be compared to both the spark plug and piston of the contractile machine, they not only develop mechanical force responsible for contraction but also act as enzyme that quickens or aids the release of energy by which force is developed (Bohr, 1973).

When a powerful excitatory agent such as Acetylcholine or Histamine is placed in an environment of as smooth muscle, it will induce or accelerate a series of cellular reactions, usually the first step in the chain of reactions is the formation of a reversible complex between molecules of the agonist and specific receptors in the muscle plasma membrane. This will result in a change in tension or length of the smooth muscle fibres or both (Hurwitz and Suria, 1971). This interaction between the excitatory drug and the specific tissue receptors provides the stimulus which activates some sort of membrane transport or membrane releasing system or

both. This activated transport or release system promotes the flow of Ca^{2+} from extracellular storage sites. This in turn leads to an increase in the concentration of free Ca^{2+} in the vicinity of the contractile proteins. These ions bind reversibly to specific sites on the contractile protein and thereby initiate a mechanical response. As the excitatory drug is removed or withdrawn from the environment of the smooth muscle by washing the flow of Ca^{2+} into the cytoplasm is reduced, and the contracted cell begin to relax. Relaxation is initiated by the removal of free Ca^{2+} from the cytoplasm (Prosser, 1974). The removal of free cytoplasmic Ca^{2+} is probably accomplished by a metabolically dependent calcium pump located in the plasma membrane or the membrane of intracellular organelles (Batra, 1973). The pump transports these ions to sites where they are sequestered to the external medium (Hurwitz Fitzpatrick Debbas and London, 1973; Batra, 1973).

1.7 EVIDENCE FOR INVOLVEMENT OF CALCIUM IN EXCITATION CONTRACTION COUPLING

Calcium is involved in the process of membrane depolarisation (Sperelakis, 1962) and in excitation

contraction coupling (Shene and Wassermann. 1963; Daniel. 1964; Frank, 1964).

Evidence for Ca^{2+} involvement in smooth muscle contraction is the fact that relaxation or contraction failure occurs more rapidly in the absence of calcium in the physiological salt solution bathing the smooth muscle preparation (Daniel and Robinson, 1962). The lost contraction is regained rapidly when calcium is reintroduced into the environment (Hurwitz Joiner Von Hugen and Davenport, 1969), (Somlyo and Somlyo, 1971). Robertson (1960) observed that 10 - 12 minutes were required for a major reduction of responsiveness of the rabbit ileum whereas significant recovery occurred less that 20 seconds after return to calcium.

The guinea pig ileum under similar treatment lost responsiveness in less than 10 minutes. Offiah (1981) the present study; it suggests that Ach stimulation uses up Ca^{2+} (membrane or intracellular).

1.8 SOURCES OF CALCIUM IN SMOOTH MUSCLE

This is based on evidence obtained from numerous physiological studies on various types of smooth muscle fibres. Currently there is a consensus that the calcium ions

associated with mechanical activity in smooth muscle arise from different sources (Hudgens and Weiss, 1968; Van Breemen and Daniel, 1966). One is the pool of calcium that is present in the extracellular fluid which is loosely bound to superficial sites in the muscle fibres, the other is the tightly bound pool of calcium that is intracellular location in the fibre. Histological and Histochemical studies have shown two loci inside the cell where mobilisable calcium may be sequestered; they are the sarcoplasmic reticulum and the mitochondria of the smooth muscle fibre (Somlyo, 1972). These two sites that store Ca^{2+} ions needed for contraction vary for all types of smooth muscle fibres. This may be responsible for the rate at which some muscle fibre lose responsiveness to stimulation withdrawal of Ca^{2+} from the bathing fluid for example in the rat mesenteric artery contractile responses to Noradrenaline were sustained for a long period up to 1 hour after removal of Ca^{2+} from the external medium (Adeagbo and Okpako, 1980). It is then suggested that Ca^{2+} needed for certain arterial smooth muscle is intracellular in source. On the other hand, intestinal smooth muscle such as guinea pig ileum as mentioned earlier rapidly loses responsiveness to agonist action; this indicated that external calcium is crucial for responses in this type of muscle.

Preliminary studies in this laboratory have confirmed previous observations that guinea pig ileum loses responsiveness to Histamine very rapidly in the absence of external Ca^{2+}. By the use of different concentrations of Ca^{2+} in the external medium and the different time intervals of drug administrations, result were obtained which suggested that in this preparation the Ca^{2+} directly involved in smooth muscle contraction was cellular in origin (Okpako and Oladitan, 1979).

In vascular muscle however, it is now almost certain that two pools of Ca^{2+} are available for mobilization by agonist for the excitation contraction coupling process (Bevan et. al., 1973; Van Breemen et. al., 1979). Most vascular smooth muscle preparations respond to agonists like Noradrenaline (NA) in a biphasic manner. There is an initial rapid but transient phasic contraction followed by a second sustained vascular smooth muscle to K^+ are either markedly suppressed or completely abolished in a calcium free ($O-Ca^{2+}$) medium or in the presence of calcium channel blockers. The initial phase, on the other hand is resistant to both treatments (Godfraind and Kabba, 1972; Kalsner et. al., 1970). Thus response to K^+ as well as the second phase of NA response are said to be largely dependent on extracellular calcium.

The initial transient phase of NA response is said to be due to the mobilisation of intracellular calcium by the agonist (Bohr, 1963; Beva et. al., 1982). Consequently, calcium channels have been classified into two: Potential Operated Channel (POC) employed by K^+ and more susceptible to calcium withdrawal and blockade by calcium channel blockers while those employed by agonists like NA are said to be Receptor Operated Channels (ROC) and are less sensitive to calcium withdrawal and blockade by calcium channel blockers (Putney, 1978; Bolton, 1979). It is unlikely however, that these channels are totally specific for calcium (Blaustein, 1977; Triggle, 1981).

1.9 MECHANISM OF CALCIUM MOBILISATION BY AGONISTS: RECENT VIEWS

When an agonist such as Noradrenaline activates a smooth muscle by binding to its receptors on the plasma membrane, a specific phospholipase C is activated via a specific guanine nucleotide protein, phospholipase C catalyses the breakdown of phosphotadylinositol-4-phosphate (PIP) and phosphatidylinositol (Pl) with the generation of inositol-1-4-5-triphosphate (IP_3) and diacylglycerol (DAG). IP_3 has been shown to act as an intracellular messenger to induce the mobilisation of Ca^{2+} from intracellular non-mitochondrial pool, thought to be the

sarcoplasmic reticulum (SR), located just beneath and closely associated with the plasma membrane (Somlyo, 1985; Griendling et. al., 1986; Takuwa et. al., 1986; Eggermong et. al., 1989).

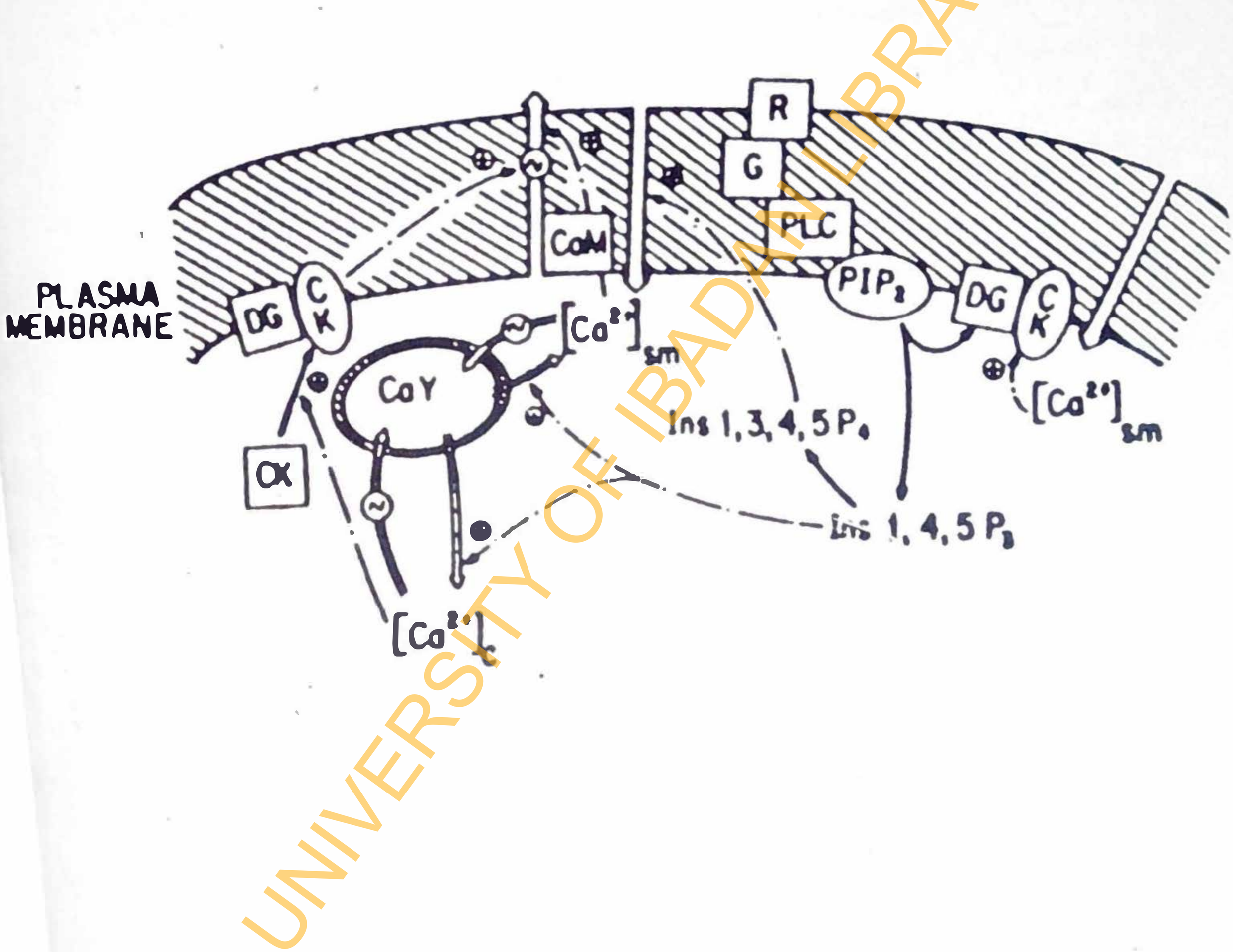
Consequently there is a transient increase in the concentration of calcium in the cytosol (Ca^{2+}) and sub-membrane (Ca^{+}) domain of the smooth muscle. This initial rise in Ca^{2+} leads to calmodulin-dependent activation of the plasma membrane Ca^{2+} pump. The transient nature of the rise in the concentration of free Ca^{2+} is caused by (i) a limited store of a Ca^{2+} in the sarcoplasmic reticulum (SR) that is soon depleted; (ii) a Ca^{2+} - calmodulin dependent activation of the plasma membrane Ca^{2+} pump, catalysing the efflux of Ca^{2+} from the cell. This Ca^{2+} - calmodulin dependent activation constitute a direct negative feedback loop which attenuate any rise in cystolic Ca^{2+} by stimulating Ca^{2+} extrusion across the plasma membrane and or (iii) the fact that increase in IP_3 is transient and rapidly falls back to basal values. IP_3 is thought to regulate calcium channel activity. Alternatively, the calcium channels are directly linked with the receptors and the latter regulate the activity of the former (Eggermont et. al., 1987; Colucci et. al., 1986).

Dillon and his colleagues (see Rasmussen et. al., 1987) have shown that smooth muscle is rich in Ca^{2+} receptor protein

calmodulin (CaM) and a specific CaM-dependent enzyme the myosin light chain kinase (MLCK), increase in (Ca^{2+}) results in phosphorylation of myosin light chains (MCLP) leading to actin-dependent activation of myosin ATPASE. Muscle contraction results presumably by the formation of cyclic actin myosin cross-bridges. This corresponds to the first transient phase of vascular muscle contraction. Recent evidence has shown that caldesmon, a protein is closely associated with the actin tropomyosin domain of smooth muscle.

Figure 1.2

A schematic representation of the mechanism of calc
mobilisation in Smooth Muscle (Rasmussen et al., 198
R: receptor, G: Guanine nucleotide binding protein, P
Phospholipase C; P_1P_2: Phosphatidylinositol 4,
biphosphate; DG: diaclyglycerol, Ins. 1,45 P_3: Inosi
1,4,5-tetrakis phosphate, CaY, Sacroplasmic reticu
calcium.
$(Ca^{2+})_{ci}$: Calcium concentration in the cytosol
$(Ca^{2+})_{sm}$: Calcium concentration in the submembrane
Ck: Protein kinase C, C_{1k}: Calcium sensitive pla
membrane associated protein kinase C.



In the absence of Ca^{2+} and CaM, Caldesmon, by binding to actin-tropomyosin, inhibits cross-bridge in a relaxed state. Addition of Ca^{2+} and CaM alters the interactions between caldesmon and actin-tropomyosin, so that actin forms cross-bridges with phosphorylated myosin light chains. The consequence of the increase in the concentration of DAG is the activation of protein kinase C, since DAG is the only well characterised natural activator of C-kinase (Rasmussen et. al., 1987). C-kinase is universally present in animal cells including smooth muscle. It exists in two forms, a Ca^{2+} insensitive form and a Ca^{2+} sensitive form. Conversion of the insensitive form to the sensitive form involves its binding to phospholipids and DAG.

The increase in DAG content of plasma membrane and the transient increase in intracellular free Ca^{2+} concentration are two major events that combine to activate C-kinase by bringing about the association of the C-kinase with the endoplasmic face of the plasma membrane (Wolf et. al., 1985). In this locations, the enzyme is sensitive to changes in calcium concentration in the range of 5×10^{-7} to 10^{-6}M. Changes in the rate of calcium influx, change in the activity of the membrane associated C-kinase. The sustained increase in DAG concentration also maintain C-kinase in its membrane

associated from leading directly or indirectly to the phosphorylation of a different subset of cellular proteins, including caldesmon and a number of intermediate filament protein (e.g. desmin synemin) and a few cytosolic protein. The rate of phosphorylation of caldesmon and other actin-binding filament proteins allows for actin myosin cross-bridge cycling even at relatively low calcium concentration. The cycling is necessary for tension maintenance and accounts for the second, sustained phase, there is a sustained increase in the rate of Ca^{2+} efflux via the plasma membrane Ca^{2+} pump due to a C-kinase dependent stimulation of the pump activity (Rasmussen et. al., 1987). Also, IP_3 only stimulates the efflux pathway from the SR and does not inhibit the influx pathway, there is an increased rate of cycling of Ca^{2+} across the sarcoplasmic reticulum (Rasmussen et. al., 1987).

1.10 ACTIONS OF ANTIMALARIALS ON MUSCLE CONTRACTIONS

Quinine and chloroquine produced a fall in the blood pressure of anaesthesised dog (Nelson, 1927 a,b; Hait, 1947-48; Sofola, 1980) as well as in man (Olatunde, 1970). The hypotensive effects of quinine and chloroquine have been attributed to peripheral vasodilatation. The plasma concentration that produced complete block of Adrenaline

induced vasoconstriction as well as the vasoconstriction response to splanchnic nerve stimulation in anaesthesised dog was (7µg/ml).

Ebeigbe and Aloamaka (1982) studied the action of chloroquine on the mechanical activity of the rat portal vein and reported that chloroquine depressed spontaneous mechanical activity and inhibited Noradrenalin induced contractions. They concluded that the vascular smooth muscle depressant effects of chloroquine was attributable to an inhibitory action by chloroquine on extracellular Ca^{2+} influx.

Huddart and Saad (1977, 1978) studied the effects of quinine on rat ileal smooth muscle and reported that quinine caused relaxation of the rat ileum. The relaxant effect of quinine as well as the time course of the response was mimicked by Lanthanum, an inorganic Ca^{2+} channel blocker which strongly competes for plasma membrane binding sites, effectively sealing membranes against Ca^{2+} influx (Godfraind, 1976).

Huddat and Saad (1977) also measured 45 Ca^{2+} movement and found that quinine prevented the entry of Ca^{2+} in much the same fashion as La^{3+}. In the rat ileal strips contracted with KCl and acetylcholine in which myoplasmic Ca^{2+} levels are raised quinine induced relaxation of the contraction and this was

was accompanied by efflux of $^{45}Ca^{2+}$. Similar results were obtained with La^{3+}. Huddat and Saad (1977) therefore concluded that the site of action of quinine in the rat ileum on the plasma membrane was by direct action, the compound prevents the entry of Ca^{2+}. Rather, similar results were obtained by Savage and Akinlalu (1985) on rat rectum. These authors found that quinine enhanced rat rectal contractility at low concentrations while it relaxed the rat rectum at higher concentrations by a Ca^{2+} antagonistic action.

In the isolated lizard rectum, Savage and Lawal (1986) observed that quinine produced powerful contractions of the lizard rectum which were inhibited by Ca^{2+} withdrawal and by low concentrations of Nifedipine (a calcium channel blocker) suggesting that quinine induced contractions in this tissue preparations were due to extracellular Ca^{2+} influx into the muscle. In a recent study on rat isolated ileum, Ebeigbe et. al. (1986) showed that chloroquine depressed spontaneous as well as acetylcholine induced contractions of the rat ileum, which the authors attributed to inhibition of Ca^{2+} influx as well as to impaired mobilization of Ca^{2+} from acetylcholine sensitive membrane bound pool. Myocardial depression produced by quinine and chloroquine is well documented. Shine (1973) found that quinine in millimolar concentrations depressed

tension development in the perfused rabbit intraventricular septum; while Ikhinwin et. al. (1981) reported that quinine and chloroquine induced myocardial depression in pithed turtles.

The depression of cardiac contractility in the turtle was fully reversible by excess $CaCl_2$. Thus ample evidence has been provided from several independent studies to suggest that quinine and chloroquine interfere with Ca^{2+} translocation in intestinal, vascular and cardiac muscles and indeed, other muscle types. It is also noteworthy that Akodu (1985) found that quinine exerts antimuscarinic, antiadrenergic as well as potent Ca^{2+} antagonistic action on the rat anoccoccygeus muscle; in the study it was concluded that the multiple actions of quinine might be explicable on the basis of Ca^{2+} influx stimulated via various pathways. Olatunde (1970) studied the effects of chloroquine on histamine and acetylcholine and 5-hydroxytryptamine (5-HT) contractions in guinea pig ileum and concluded that chloroquine antagonism of smooth muscle contraction was due to its direct spasmolytic action rather than by a specific antagonism of receptors. On the other hand, Akubue (1975), studied the effects of chloroquine on Histamine induced contractions of the isolated guinea pig ileum and concluded that chloroquine at a

concentration of 0.75µg/ml^{-1} was a specific antagonist of Histamine at H_1-receptors. Famaey et. al. (1975) also studied the inhibitory effects of chloroquine among other various structurally unrelated compounds such as narcotics, non-steroidal anti-inflammatory drugs (NSAID) steroidal anti-inflammatory drugs (SAID) on electrically induced contractions of isolated guinea pig ileum and found that chloroquine (2.5µg/ml and 10µg/ml) inhibited electrically induced contractions in the same manner as the narcotics, NSAID and that this inhibition was dose related and was reversed by low concentrations of prostaglandin (see also Famaey et. al., 1977a,c). These authors also concluded that the antagonism of PGE_2, Histamine and 5-HT was non-specific and non-competitive. Okpako (1978) studied the interaction of the aminoquinolines, Amodiaquine and Mepacrine with PGE and Histamine in various mammalian tissues, he found that in the rat stomach strip low concentrations of the antimalarial drugs potentiated contractions to PGE_2. All drugs inhibited PGE_2 induced contractions in guinea pig ileum and there was no evidence of specific interaction with receptors. Makinde (1977) studied the mechanisms of cardiotoxic effect of chloroquine on isolated atria. The spontaneous contractile tension and rate of isolated atria were recorded and found that low

concentration of antimalarial produced a reversible dose dependent negative chronotropic and negative inotropic effects on the muscle, while higher doses of chloroquine caused complete cessation of spontaneous action shortly after adding the drugs. Studies on antimalarial effect on skeletal muscle have also been reported. Okwuasaba et. al. (1990) determined the effects of acute and chronic administration of chloroquine on the responsiveness of rat diaphragm to electrical stimulation of phrenic nerve. These authors concluded that acute and chronic chloroquine administration resulted in reduction in response of the diaphragm to electrical stimulation and drug action. The authors suggested that these effects were dependent on its direct inhibitory action on skeletal muscle probably due to interference with Ca^{2+} mobilisation within the muscle.

1.11 OBJECTIVES OF THE PRESENT STUDY

In view of the several reports of the interaction of quinine and chloroquine with Ca^{2+} in different muscle preparation from different species in the literature, the present study was designed to study the probable sites of actions of antimalarial in smooth muscle. In order to broaden the scope of the study and to be able to infer any structure-

action relationships, the following antimalarial drugs were studied: Chloroquine and Amodiaquine (4-aminoquinoline), Mepacrine (8-aminoquinolines), Quinine and Mefloquine (Quinoline methanol), and Halofantrine (Phenathrene methanol).

It is generally accepted that contractions evoked in different smooth muscles may involve calcium mobilised from extracellular or intracellular stores (Bolton, 1979) depending on muscle preparation and agonist used. Therefore, the following smooth muscle preparations have been used in this study: the rat stomach strip (RSS), guinea pig ileum (longitudinal muscle strip), and rat aortic strips.

Previous studies (Offiah, 1981) and preliminary experiments in the present series suggested that the guinea pig ileum which has very little tone under these experimental conditions has a small intracellular calcium storage capacity compared to the rat stomach strip preparation (see also Sparrow and Simmonds, 1965; Hurwitz and Joiner, 1969).

The rat aortic strip preparation was used as an example of a vascular smooth muscle preparation. The main drugs used to excite the smooth muscles were acetylcholine (Ach), Histamaine (Hist), Noradrenaline (NA), Potassium (KCl) and Calcium. The aim was to see whether the type of receptor activated had any effect on the action of the antimalarial or

whether the latter had different effects on receptor and potential operated calcium channels.

CHAPTER TWO

MATERIALS AND METHOD

2.1 ANIMALS

All animals used in this study - albino rats (Sprague Dawley Strain); Guinea pigs (bred from a strain developed in Vom Nigeria) were brought from the National Institute of Medical Research, Yaba, Lagos and locally bred in the Department animal house. The animal house was adequately ventilated. Rats were fed on standard livestock pellets (Pfizer Nigeria Limited) supplemented with green grass. All animals had free access to water.

2.2 PHYSIOLOGICAL SOLUTIONS

The following physiological solutions were used:

Tyrode Solution mM/l

Sodium Chloride 138

Sodium bicarbonate II

Potassium Chloride 3.4

Magnesium Sulphate (H_2O) 6.5

Sodium hydrogen phosphate ($2H_2O$) 2.4

Calcium Chloride 1.8

Glucose 5.0

The temperature of the solution bathing the tissue was maintained at 37°C and was aerated. In Ca^{2+} free solution

$CaCl_2$ was omitted while in some experiments, Ca^{2+} was varied from 1.8mM, 0.9mM and 0.45mM.

2.2a Ca^{2+} EGTA CONTAINING TYRODE

In this solution $CaCl_2$ was omitted and 0.5mM EGTA was added. The addition of EGTA - a chelator was to make sure no interference of external Ca^{2+} from the bathing physiological solution.

2.2b PHYSIOLOGICAL SOLUTIONS

Krebs solution in mM /l

This was a modified krebs solution as used by Kosterlitz, Lydon and Watt (1970).

Sodium Chloride	118
Sodium Bicarbonate	25
Glucose	10
Potassium Chloride	1.2
Calcium Chloride	1.6
Potassium Dihydrogen Phosphate	1.2
Magnesium Phosphate	1.2

2.2c DEPOLARISING KREBS 55mM-g/L

Sodium Chloride	1.8
Potassium Chloride	4

Potassium Dihydrogen Phosphate 1.2

Sodium Bicarbonate 2.0

Glucose 1.0

(Calcium chloride was omitted)

2.3 DRUGS

The drugs used in this study include Acetylcholine Bromide (Sigma), Amodiaquine (Sigma), Chloroquine Diphosphate Anhydrous (Sigma), Ethylene-Diaminetetra Acetic Acid EGTA (Sigma), Halofantrine and Mefloquine were kindly provided by Dr. A.M.J. Oduola from supplies obtained through the World Health Organisation (WHO). Noradrenaline, Histamine (Sigma), Diltiazem (Sandoz), Methylene blue (Laboratory HBL reagent).

2.4 DRUG SOLUTIONS

Stock solutions of Halofantrine, Mefloquine, were dissolved in 30% ethanol, acetylcholine was dissolved in 0.1M hydrochloric acid. The rest of the drugs were dissolved in distilled water and these stock solutions kept at -20°C until required for use.

2.5 STATISTICAL ANALYSIS

Results are expressed as mean standard error of the mean, where n represents the number of observations in the group. Where comparison between groups were made using student 't' test, the difference between the groups was taken to be significant when $P < 0.05$.

2.6a WHOLE ILEUM PREPARATIONS

Guinea pigs of either sex weighing 250-300g were used. They were stunned by a blow on the head and exsanguinated. The ileum was removed, the region 8-12cm nearest the ileocecal junction was discarded. The lumen of the ileum was flushed with 10ml of physiological salt solution from a 10ml pipette. The mesentery was then trimmed away. Pieces of ileum 2-3cm long were then set up in 20ml organ bath. Both ends of the ileum were open allowing mucosal secretion and debris to be voided. The tissue was bathed in Tyrode physiological solution, gassed with air and thermostatically maintained at a temperature of 37°C. Contractions were monitored by a force displacement transducer (FT. 03) connected to a Grass Polygraph Model 7D. Before beginning the experiment, the ileum was left in the bath for 45 minutes to equilibrate during which time the bath fluid was repeatedly changed. Contractions were evoked by Acetylcholine, Histamine Potassium

Chloride, the drugs were allowed to remain in contact with tissue until the maximum response for each contraction had been recorded. Drugs were added in randomised manner, the drugs were then washed out and the tissue allowed to rest before another stimulus was applied. During resting time the bath fluid was changed at intervals.

2.6b LONGITUDINAL MUSCLE STRIP PREPARATION

The ileum having been removed, was flushed as in the whole ileum preparation with a pipette containing Tyrode solution. A piece of ileum approximately 10cm long was stretched on a glass rod and the mesentery trimmed with curved scissors as close to the gut as possible. Sheets of the longitudinal muscle were obtained by the method of Ambache (1954). The detachment of longitudinal sheet at one end of the segment before the sheet could be pulled was easier by tangential stroking (Rang, 1964) starting at the mesenteric border with a damp wisp of cotton wool until about 3-4cm of longitudinal muscle were completely free at one end. The free end was now pulled vertically to obtain the sheet, strands of circular muscle adhered to the starting end but were left behind as the rest of the sheet was detached by pulling. In order to obtain robust preparations, large guinea pigs were

used. The preparation just described yields a strip with auerbach plexus attached.

2.6c MOUNTING OF THE LMS

The longitudinal muscle strip of the guinea pig ileum was mounted in the organ bath, the tension on the muscle was 0.5g. The temperature of the organ bath was kept constant at 37°C by circulating warm water around the jacketed organ bath. Muscle contractions were monitored on a force displacement transducer, model FT. 03, and recorded on polygraph model 7D.

2.6d PREPARATION OF RAT STOMACH STRIP

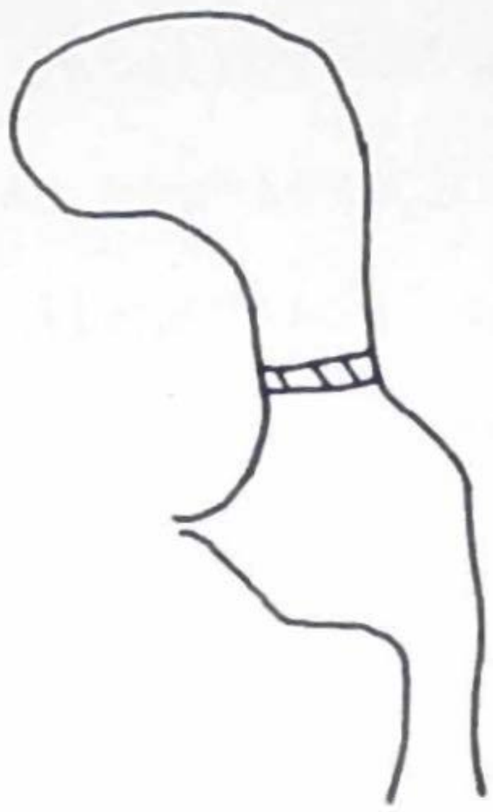
Rats of either sex were starved overnight but had access to water. The animals were killed by a blow on the head and bled out. The stomach was dissected free from abdomen and placed in Tyrode solution at 37°C aerated with air. The fundus is recognised by its grey colour, translucent balloon-like tissue. The fundic strip was prepared essentially as described by Vane (1957). The fundus was cut open into a strip by cutting spirally. One end of the strip was tied to the tissue holder and lowered into the organ bath, while the other end was attached to a force displacement transducer, model FT. 03, under a resting tension of 1g.

Fig 3: Diagram showing the method of cutting fundic strip.

Reproduced from Vane J.R., 1957.

DIAGRAM SHOWING THE METHOD OF CUTTING FUNDUS STRIP

(a)

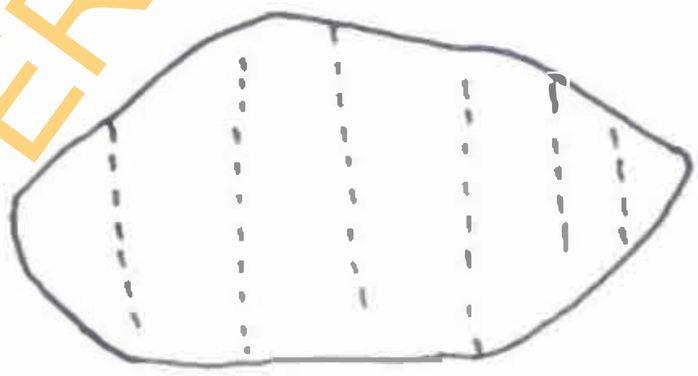


WHOLE STOMACH

(b)

FUNDUS

(c)



FUNDUS OPENED INTO A PLATE OF TISSUE CUT ALONG DOTTED LINES.

(d)

STRIP PULLED AND TIED EACH END.

2.6e PREPARATION OF RAT AORTIC STRIP

Adult albino rats 150-200g were killed by a blow on the head and bled out as previously described. The thoracic aorta was removed by dissection and placed in a petri dish containing physiological salt solution buffered at pH 7.2-7.4. The vessel was carefully cleaned of adhering connective tissues, and a piece of probe was inserted through the lumen to rub off the endothelium. A spiral cut was made according to Furchgott and Bhadrakom (1953). The tissue was equilibrated for one hour in a 20ml organ bath. During this period, the bathing fluid was changed every twenty minutes. The tissue was connected as previously described for the rat stomach strip and guinea pig longitudinal muscle.

CHAPTER THREE

RESULTS

3.1 EFFECT OF SELECTED ANTIMALARIAL DRUGS ON HISTAMINE INDUCED CONTRACTIONS OF LONGITUDINAL MUSCLE STRIP (LMS) OF GUINEA PIG ILEUM

The aim of this experiment was to compare the effect 10^{-7} concentration of different antimalarial drugs on a submaximal contraction caused by 5 x 10^{-8} M Histamine. Submaximal contractions were elicited to histamine. The dose of histamine was then repeated in te presence of 10^{-7} M concentration of antimalarial. The responses in the presence of the antimalarial were expressed as a percentage of the mean response before the addition of the antimalarial. The tables (1 and 2) show that CQ, MPC and AMDQ enhanced histamine response by 75, 88 and 90% respectively.

The antimalarials showed a dual effect in their interaction with acetylcholine and Histamine. In low doses (10^{-12} M - 10^{-7} M) the predominant and most consistent effect was potentiation of agonist contraction. Doses higher than 10^{-5} M caused inhibition of Hist contraction. In most of the work that followed low (10^{-7} M) and high (10^{-4} M) concentrations of antimalarial were used where necessary as potentiating and inhibitory concentrations.

TABLE 1:

A comparative effect of 10^{-7}M concentration of the drugs is shown in Table 1. In this experiment, the effect of this concentration of aminoquinoline on submaximal contraction caused by 5×10^{-8}M Histamine were determined. The table shows that CQ, MPC and AMDQ enhanced the Histamine by 75, 88 and 90% respectively, whereas MFQ and HFT produced enhancement of 14 and 8% respectively.

TABLE 1: THE EFFECT OF 10^{-7}M CONCENTRATION OF CHLOROQUINE AND RELATED DRUGS ON CONTRACTIONS OF GUINEA PIG ILEUM TO HISTAMINE IN NORMAL TYRODE

DRUG	MEAN CONTROL RESPONSE TO HISTAMINE (MM)	MEAN RESPONSE TO HISTAMINE IN THE PRESENCE OF DRUG	PF	% POTENTIATION OF EACH RESPONSE
Chloroquine	3.6±0.60 (12)	6.3±0.25(12)	0.62	75
Mepacrine	5.5±0.25 (10)	7.5±0.15 (10)	0.55	88
Amodiaquine	5.5±0.25 (12)	10.5±0.25 (12)	0.51	90
Mefloquine	3.5±0.81 (8)	4.0±0.15 (8)	0.92	14
Halofantrine	3.5±0.35 (8)	3.8±0.25 (8)	0.99	8

Figures in parenthesis represent number of observations.

Some parameters in the table were significantly different ($p < 0.05$) except MFQ and HFT as shown in the PF column.

TABLE 2:

Relative potencies of antimalarials in inhibiting Histamine-induced contractions are shown as % inhibition of contraction induced by a standard dose of Histamine ($8x10^{-8}M$).

Note: CQ and MPC are highly potent where MFQ and HFT are not. Amodiaquine was not tested in this concentration due to insolubility.

Some parameters within the table were significantly different* ($p < 0.05$).

TABLE 2: THE EFFECT OF 10^{-4}M CONCENTRATION OF CHLOROQUINE AND RELATED DRUGS ON CONTRACTIONS OF GUINEA PIG ILEUM TO HISTAMINE $5X10^{-8}$M IN NORMAL TYRODE SOLUTION

DRUG	MEAN CONTROL RESPONSE TO HISTAMINE (MM)	MEAN RESPONSE FOR HISTAMINE IN THE PRESENCE OF DRUG	% INHIBITION OF HISTAMINE
Chloroquine	3.6±0.60 (12)	* 1.2±0.52 (12)	66
Hepacrine	5.5±0.25 (10)	* 0.8±0.21 (10)	86
Amodiaquine	5.5±0.02 (12)	Not Tested	Not Tested
Mefloquine	3.5±0.08 (8)	3.2±0.10 (8)	8
Halofantrine	3.5±0.03 (8)	3.3±0.03 (8)	6

Figures in parenthesis represent number of observations.

3.2 EFFECTS OF SELECTED ANTIMALARIAL DRUGS ON CONTRACTIONS OF GUINEA PIG ILEUM TO ACETYLCHOLINE

The following antimalarial drugs were studied; Chloroquine (CQ), Amodiaquine (AMDQ), Mepacrine (MPC), Halofantrine (HFT) and Mefloquine (MFQ). Control response curves to acetylcholine were obtained by random addition of the agonist. Thereafter the tissue was incubated in various concentrations of antimalarial for 30 minutes. In some experiments time control preparation were included to ensure that changes in contraction induced by drug were independent of time-dependent changes in tissue sensitivity. A dose response curve was constructed. Each response to Ach was expressed as a percentage of the maximum obtained before the addition of chloroquine. The antimalarials showed a dual effect in their interaction with acetylcholine.

In low doses (10^{-12} - 10^{-7}), the predominant and most consistent effect was potentiation of agonist contraction. Doses higher than ($> 10^{-6}$) caused inhibition of Ach contraction. These results are shown in (figure 4). It can be seen that the effects of MPC and AMDQ on Ach contraction in guinea pig ileum were similar to those of chloroquine. On the other hand, MFQ and HFT showed neither distinct potentiation at low doses nor inhibition of the higher dose of 10^{-4}M. The

nature of the inhibition by CQ, MPC, and AMDQ was consistent with non-competitive antogonism with maximum response depressed but there was no increase in threshold dose.

Note: In this experiment, percentage maximum response was used in order to normalise responses from different experiments. In some other experiments, absolute unit of the height of contraction (mm) were used.

Figure 4: Dual effect of CQ on Ach induced contraction of isolated guinea pig ileum.

O----O CQ 10^{-7}M.

●----● CONTROL.

□----□ CQ 10^{-4}M

Bars represent S.E.M. where (n=9 exp.)

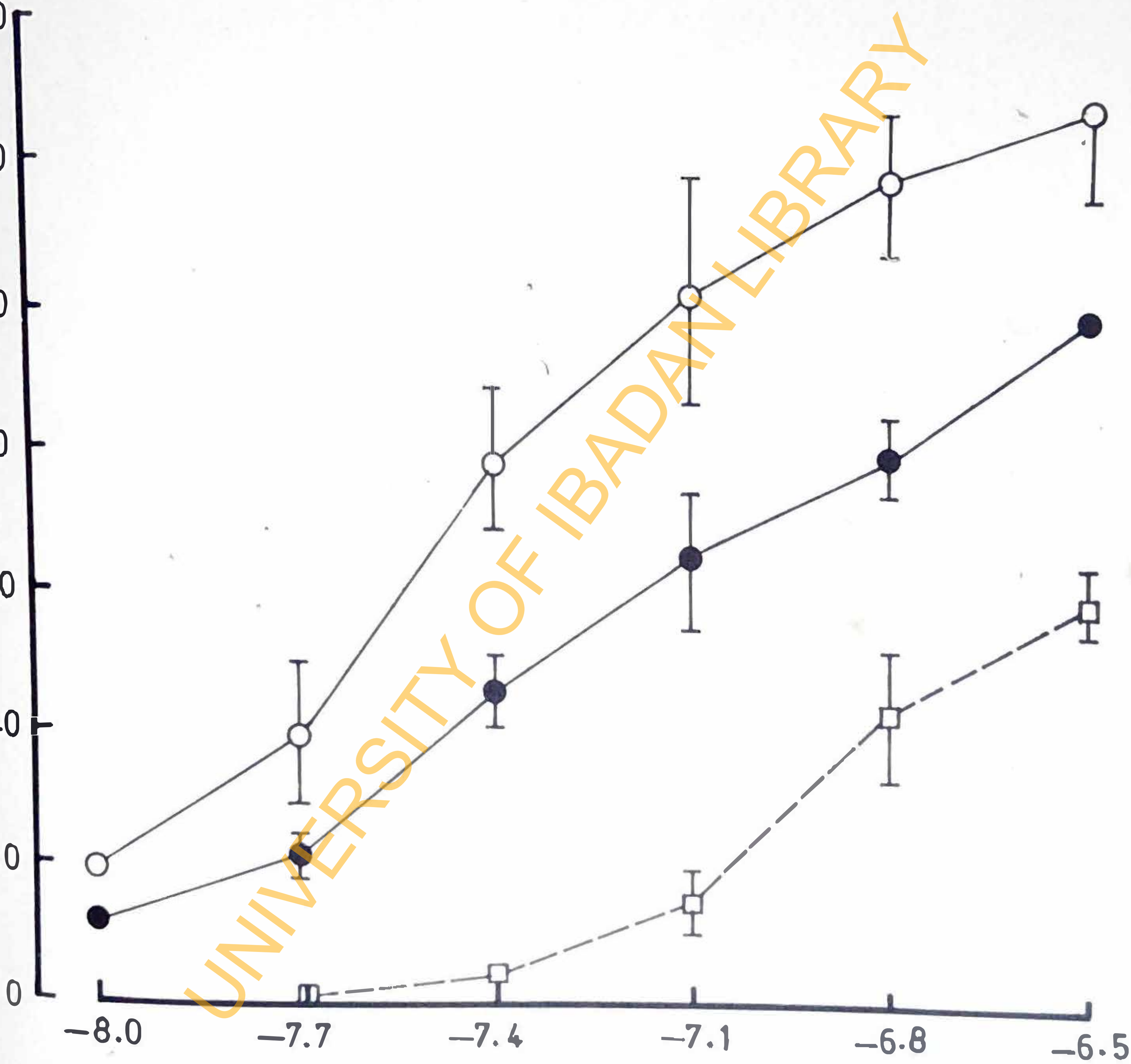


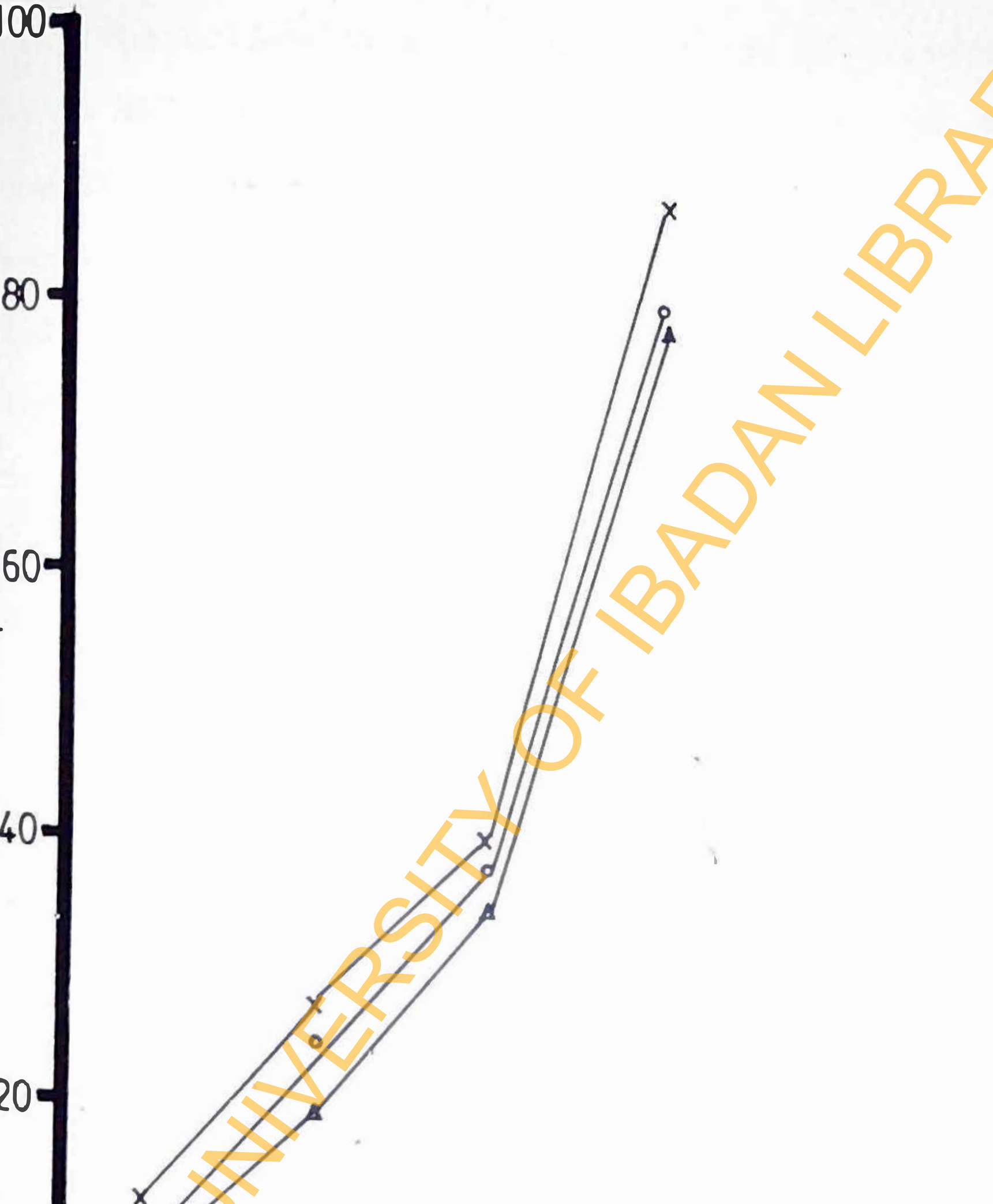
Figure 5: Effect of Halofantrine on Histamine induc
contraction of isolated guinea pig ileum.

x——x HFT 10^{-7}M,

o——o Control,

Δ——Δ HFT 10^{-4}M.

Each point represents the mean and standard error of s
experiments.

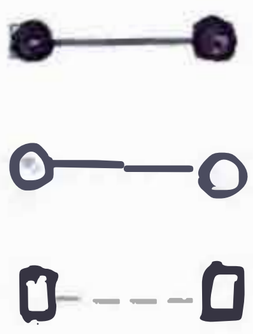


3.3 INTERACTION OF ANTIMALARIALS ON CONTRACTIONS INDUCED BY POTASSIUM CHLORIDE

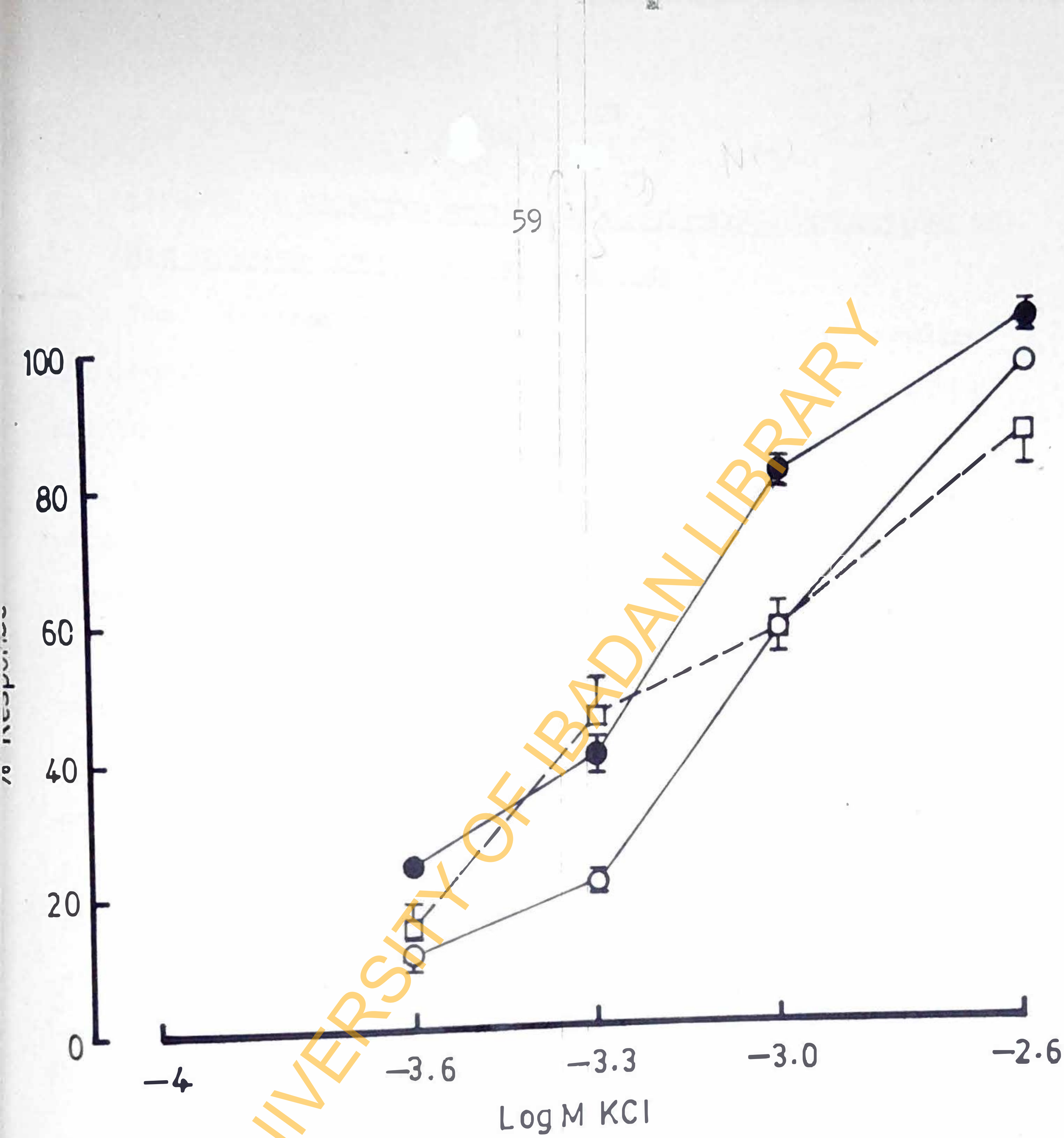
The following antimalarial drugs were studied: CQ, AMDQ, MPC, HFT and MFQ. It is known that potassium-induced contractions of smooth muscle may not involve specific receptor occupation but a mobilisation of external calcium following membrane depolarisation. It was therefore of interest to test the effect of selected antimalarials on K^+ induced contractions. The procedure for eliciting contractions as earlier mentioned was similar except that high K^+ (mM) was used before and after incubating the tissue in antimalarial.

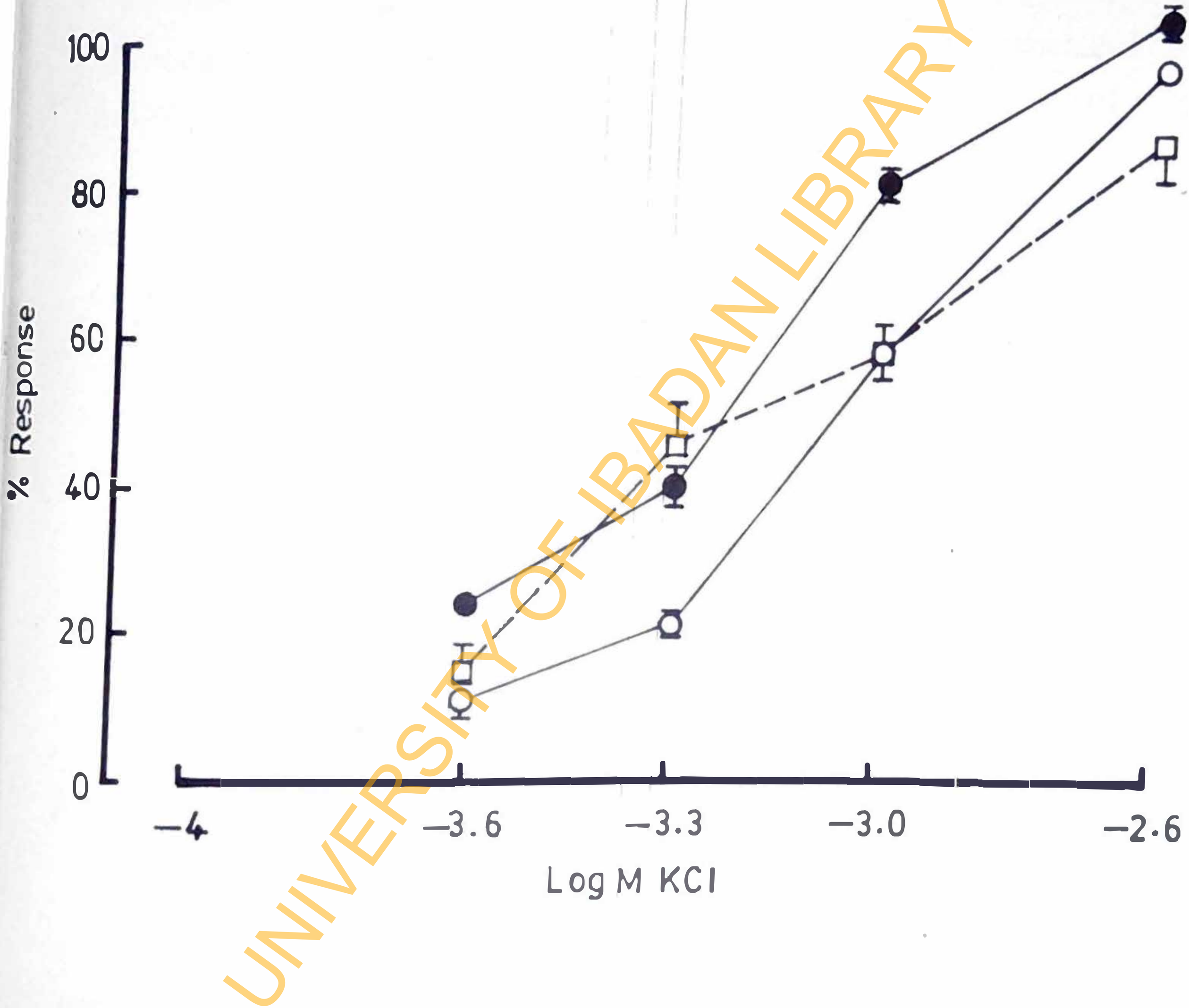
It can be seen that low dose CQ potentiated K^+, but doses of antimalarials that produced marked inhibition of Acetylcholine and Histamine contractions in rat stomach strip and guinea pig ileum respectively had very little inhibitory effect on KCl induced contractions in rat stomach strip. Contractions induced by high potassium were thus less affected by antimalarials than receptor mediated contractions. (Figure 6)

Figure 6: Effect of CQ on K+ induced contraction of the isolated guinea pig ileum



Each point represents the mean and standard error of six experiments.





34 EFFECTS OF SELECTED ANTIMALARIAL DRUGS ON CONTRACTIONS OF RAT STOMACH STRIP TO ACETYLCHOLINE

The following antimalarial drugs were studied, Chloroquine (CQ), Amodiaquine (AMDQ), Mepacrine (MPC), Halofantrine (HFT) and Mefloquine (MFQ).

Control Dose response curves to acetylcholine were obtained by random addition of the agonist. Thereafter, the tissue was incubated in various concentrations of antimalarial for 30 minutes. Time control preparation in some experiment were included to ensure that changes in contraction induced by drug were independent of time dependent changes in tissue sensitivity. Dose response curves were constructed by adding the dose of agonist in a random manner until a maximum response was obtained. Each response to Ach was then expressed as a percentage of the maximum response obtained before the addition of chloroquine.

In general, the antimalarials showed a dual effect in their interaction with acetylcholine. In low doses 10^{-12} - 10^{-7}M the predominant and most consistent effect was Potentiation of agonist contraction. Doses higher than (>10^{-6}M) caused inhibition of Ach contraction (Fig. 4).

The effects of MPC and AMDQ on Ach contraction in rat stomach strip were similar to those of chloroquine. On the

3 4 EFFECTS OF SELECTED ANTIMALARIAL DRUGS ON CONTRACTIONS OF RAT STOMACH STRIP TO ACETYLCHOLINE

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In general, the antimalarials showed a dual effect in their interaction with acetylcholine. In low doses 10^{-12} - 10^{-7}M the predominant and most consistent effect was potentiation of agonist contraction. Doses higher than (>10^{-5}M) caused inhibition of Ach contraction (Fig. 4).

The effects of MPC and AMDQ on Ach contraction in rat stomach strip were similar to those of chloroquine. On the

other hand, MFQ and HFT showed neither distinct potentiation at low doses nor inhibition of the higher dose of 10^{-4}M (See Fig. 5). The nature of the inhibition by CQ, MPC and AMDQ was consistent with non-competitive antagonism, with maximum response depressed but there was no increase in threshold dose (See Fig. 4).

Fig. 7 : Dual effect of antimalarial (CQ) on Ach induced contraction of the isolated rat stomach strip.

O———O, control Ach alone.

In the presence of CQ 10^{-7}M ●———● .

and 10^{-4}M □———□ ;

Each point represents the mean and standard error of six experiments.

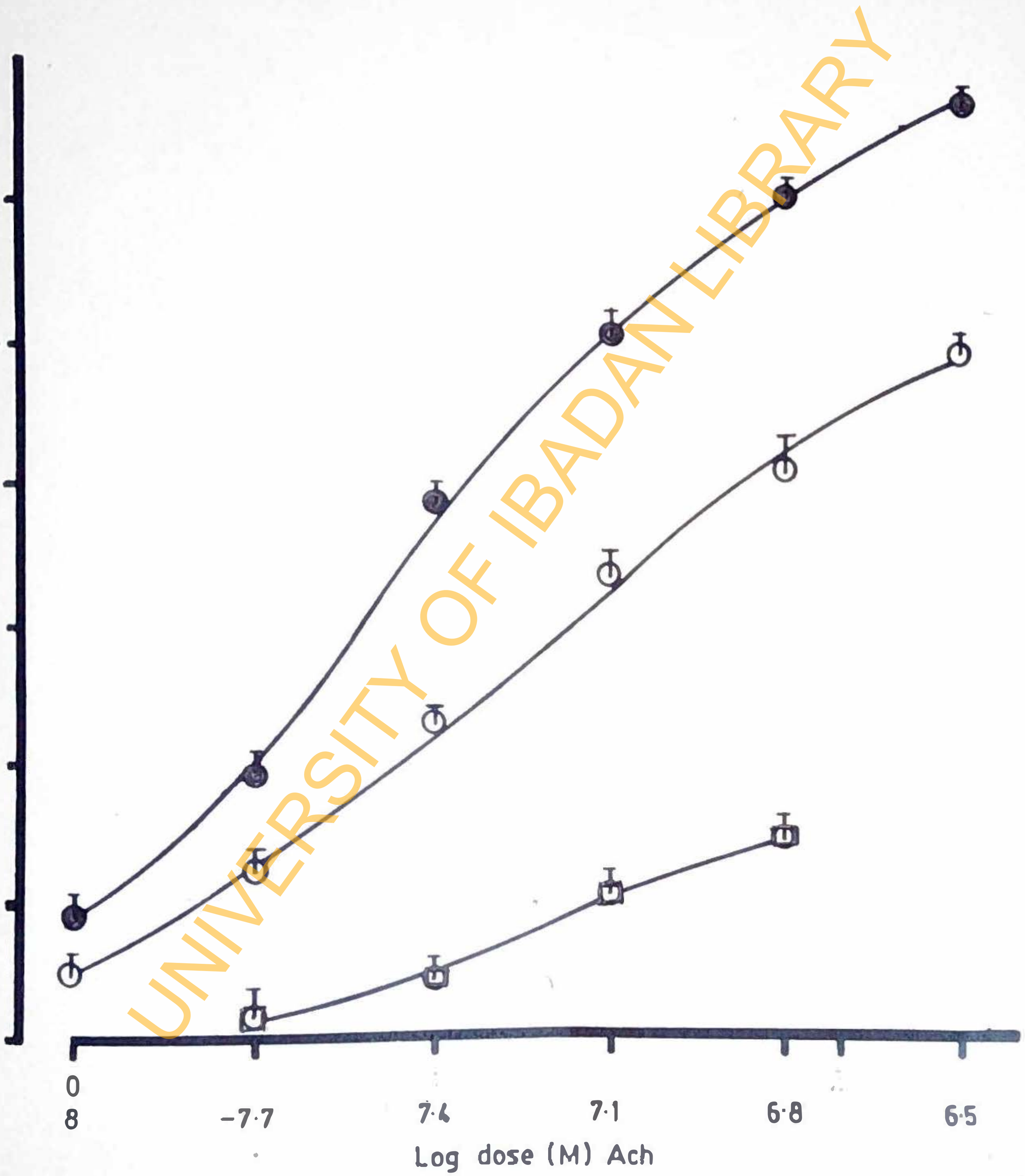


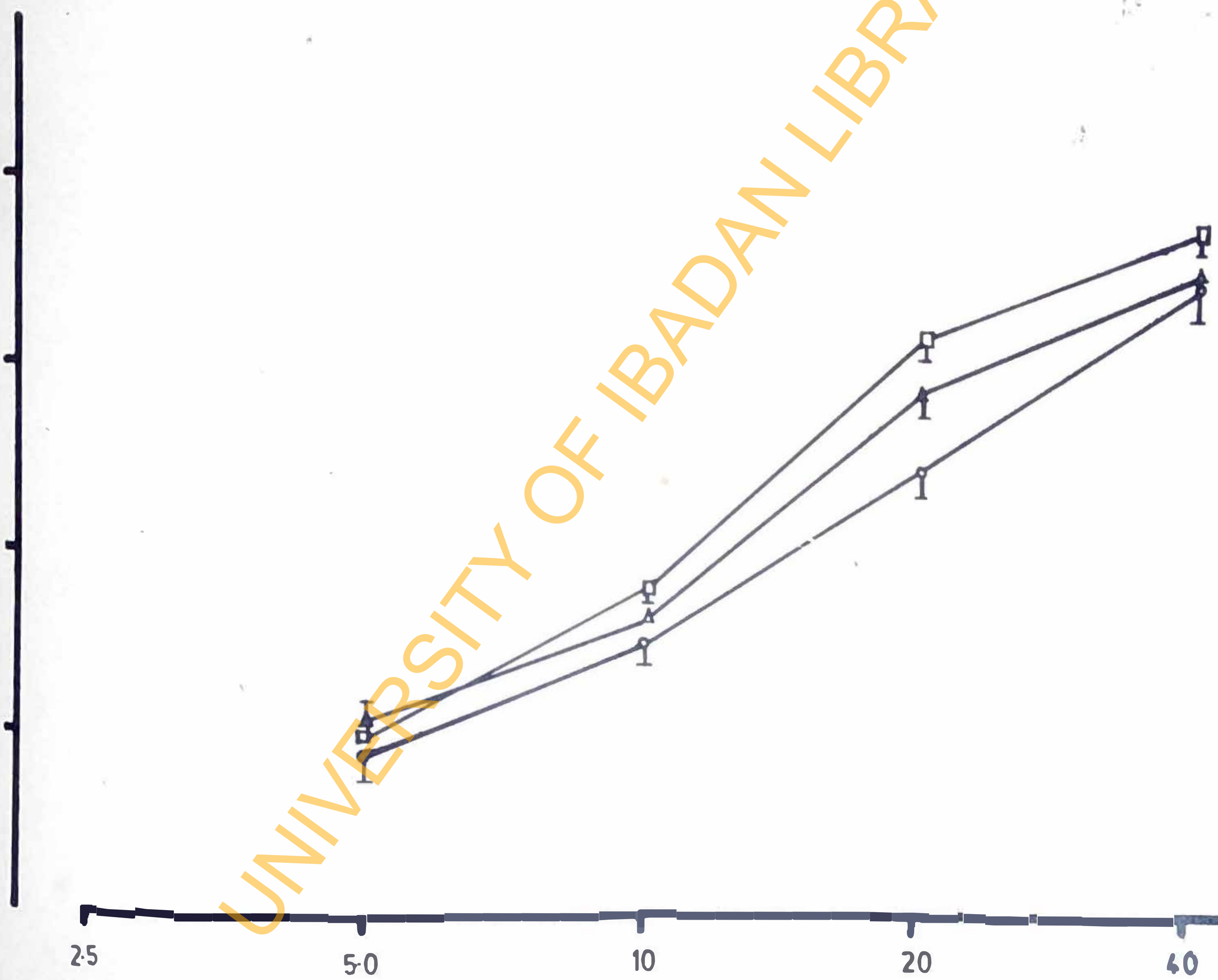
Fig. 8: Effect of different concentrations of Mefloquin (Mfq) on Ach induced contraction of the isolate rat stomach strip.

□----□ MFQ 10^{-7}M,

▲----▲ Control, Ach alone.

○---○ MFQ 10^{-4}M

It can be seen that potentiation and inhibition o antimalarial were not markedly distinct. Eac point represents the mean and standard error of si experiments. Some bars omitted to avoid overlap.



3.5 COMPARATIVE EFFECTS OF LOW (10^{-7}M) AND HIGH (10^{-4}M) CONCENTRATIONS OF THE ANTIMALARIALS IN RAT STOMACH STRIP

A comparison of the effects of 10^{-7}M concentration of the drugs is shown in Table 3.

In this experiment, the effect of this concentration of the antimalarial on a submaximal contraction caused by $5x10^{-8}$M acetylcholine were determined. The table shows that CQ, MPC, and AMDQ enhanced the acetylcholine response by 71, 92 and 89% respectively, whereas MFQ and HFT produced enhancements of 29 and 20% respectively.

The result of a similar experiment using the higher concentration (10^{-4}M) of the drug on Ach-induced contractions as shown in Table 4. It can be seen that CQ, MPC and AMDQ are potent inhibitors of Ach at this concentration. In contrast MFQ and HFT are virtually without effect being only 12% and 1%.

Table 3 : RAT STOMACH STRIP

A comparative effect of low (10^{-7}) concentration of the drugs is shown in Table 3. In this experiment, the effect of this concentration of aminoquinoline on submaxial contraction caused by $5x10^{-8}$M Ach were determined. The table shows that CQ, MPC and AMDQ enhanced Ach responses by 71, 92 and 89% respectively whereas MFQ and HFT produced enhancements of 29 and 20% respectively.

Some parameters within the table were significantly different ($p < 0.05$) except MFQ and HFT effect on agonist induced contraction (see PF).

Potentiator factor (PF) was estimated by calculating the ratio of the dose of Ach for a given effect before and in the presence of antimalarial.

TABLE 3: THE EFFECT OF 10^{-7}M CONCENTRATION OF CHLOROQUINE AND RELATED DRUGS ON CONTRACTIONS OF RAT STOMACH STRIP TO ACETYLCHOLINE $5X10^{-8}$M IN NORMAL TYRODE

DRUG	MEAN CONTROL RESPONSE TO ACH (MM)	MEAN RESPONSE TO ACH IN THE PRESENCE OF DRUG	PF	% POTENTIATION OF EACH RES RESPONSE
Chloroquine	8.5±0.32 (12)	14.8±0.52 (12)	0.63	71
Mepacrine	6.0±0.24 (12)	11.5±0.64 (10)	0.50	92
Amodiaquine	9.0±0.52 (10)	17.0±0.43 (10)	0.55	89
Mefloquine	7.8±0.45 (8)	10.0±0.71 (8)	0.95	29
Halofantrine	15±0.32 (8)	17.9±0.81 (8)	0.98	20

Figures in parenthesis represent number of observations.

TABLE 3: THE EFFECT OF 10^{-7}M CONCENTRATION OF CHLOROQUINE AND RELATED DRUGS ON CONTRACTIONS OF RAT STOMACH STRIP TO ACETYLCHOLINE $5X10^{-8}$M IN NORMAL TYRODE

DRUG	MEAN CONTROL RESPONSE TO ACH (MM)	MEAN RESPONSE TO ACH IN THE PRESENCE OF DRUG	PF	% POTENTIATION OF EACH RES RESPONSE
Chloroquine	8.5±0.32 (12)	14.8±0.52 (12)	0.63	71
Hepacrine	6.0±0.24 (12)	11.5±0.64 (10)	0.50	92
Amodiaquine	9.0±0.52 (10)	17.0±0.43 (10)	0.55	89
Mefloquine	7.8±0.45 (8)	10.0±0.71 (8)	0.95	29
Halofantrine	15±0.32 (8)	17.9±0.81 (8)	0.98	20

Figures in parenthesis represent number of observations.

TABLE 4 :

Relative potencies of antimalarials in inhibiting A
induced contractions are shown as % inhibition of contract
induced by a standard dose of Ach ($5x10^{-8}M$).

Note: CQ and MPC are highly potent compared to MFQ and H
Amodiaquine was not tested in this concentration due
insolubility.

Some parameters within the table were significan
different ($p < 0.05$) except MFQ and HFT; while Amodiaquine
not tested, because it precipitated out of the physiologi
solution.

TABLE 4: THE EFFECT OF 10^{-4}M CONCENTRATION OF CHLOROQUINE AND RELATED DRUGS ON CONTRACTIONS OF RAT STOMACH STRIP TO ACETYLCHOLINE $5X10^{-8}$M IN NORMAL TYRODE SOLUTION

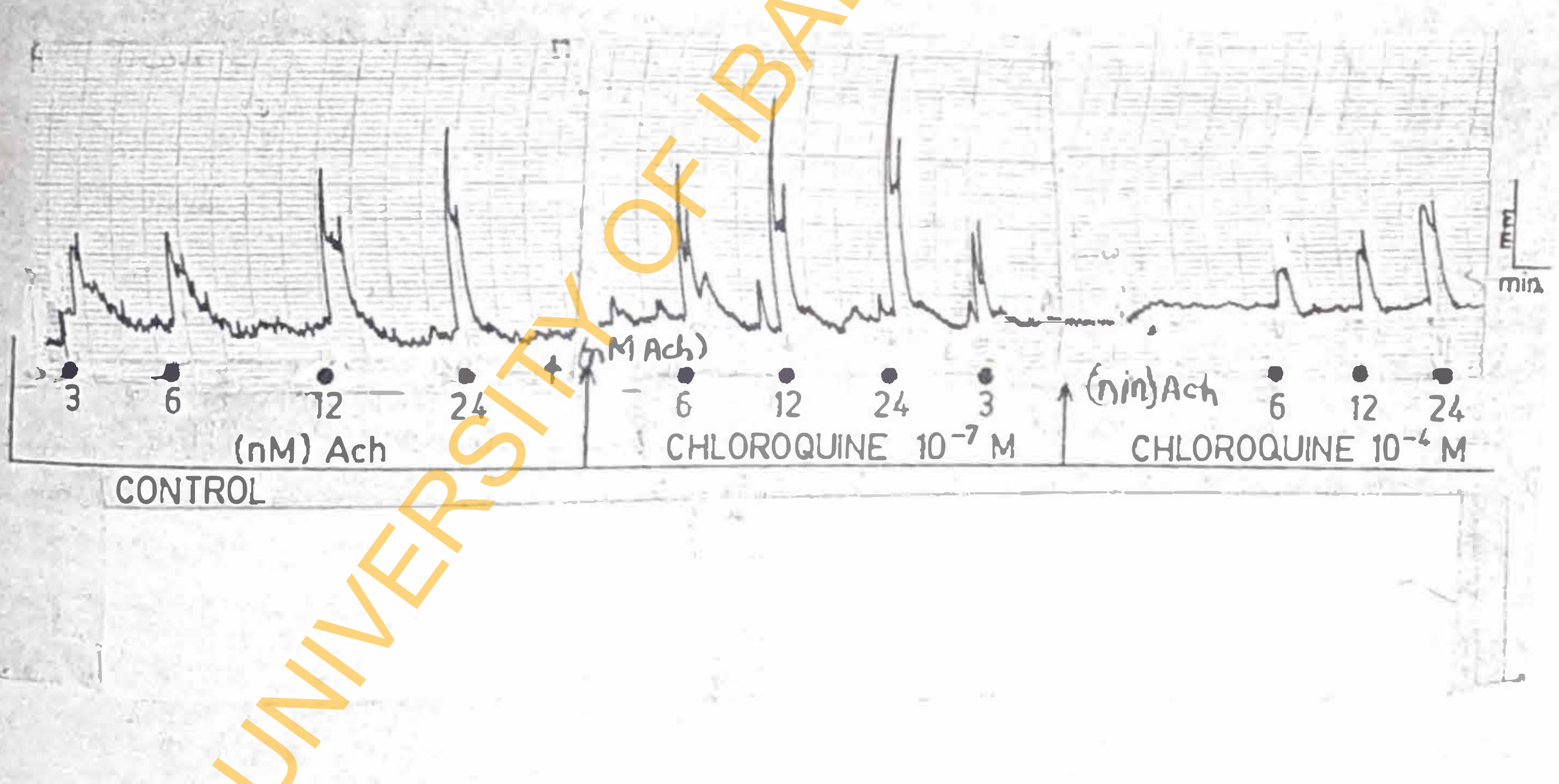
DRUG	MEAN CONTROL RESPONSE TO ACH (MM)	MEAN RESPONSE TO ACH IN THE PRESENCE OF DRUG	% INHIBITION OF ACH RESPONSE
Chloroquine	8.5±0.35 (12)	3.7±0.62 (12)	63
Mepacrine	6.0±0.22 (10)	-	100
Amodiaquine	9.0±0.50 (12)	Not Tested	Not Tested
Mefloquine	7.8±0.45 (8)	6.9±0.15 (8)	12
Halofantrine	15±0.32 (8)	14.9±0.81 (8)	1

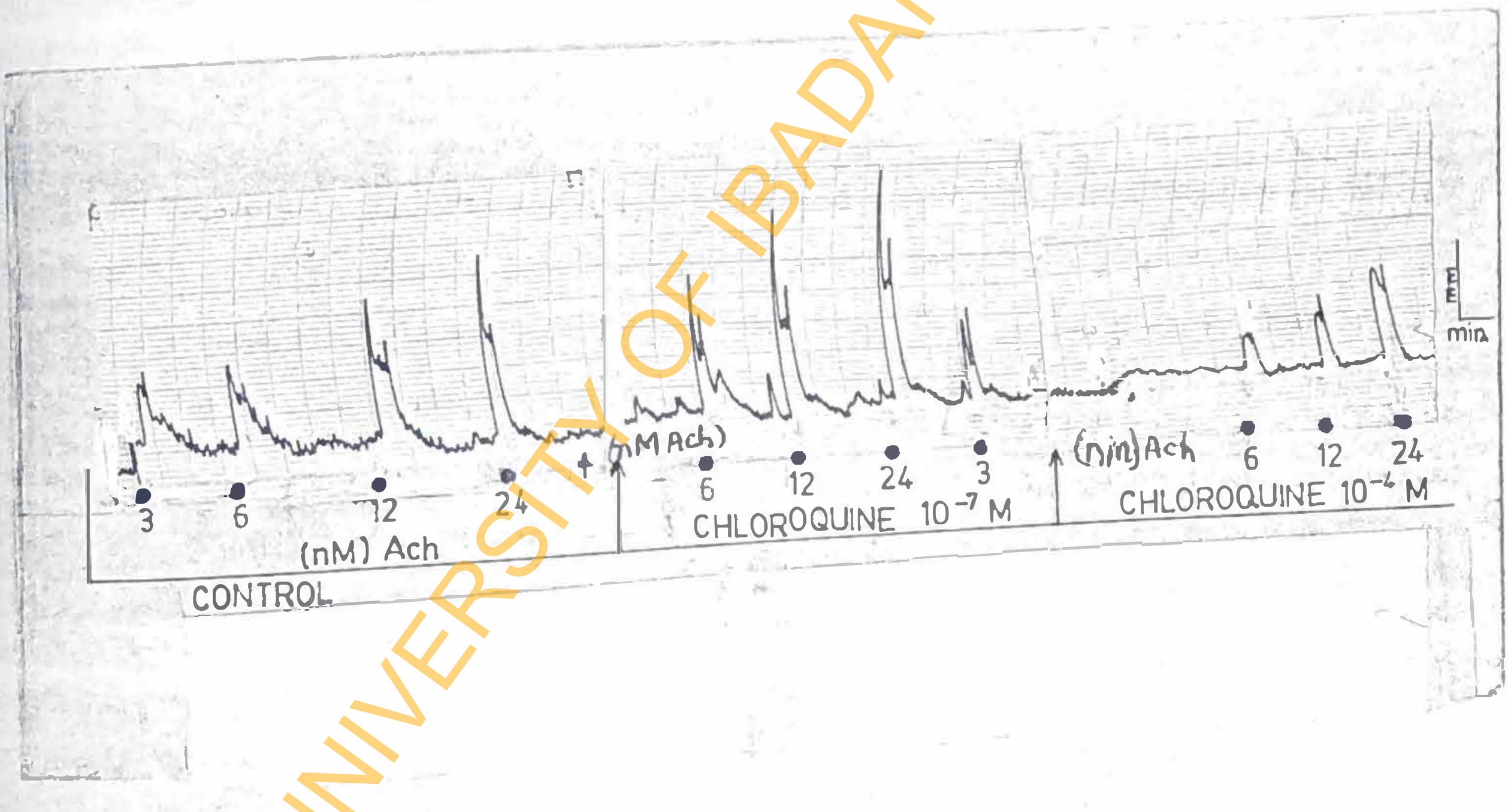
Figures in parenthesis represent number of observations.

Figure 9: Representative tracings of the effect of Chloroquine pre-incubation on Ach induced Contractions.

Calibration: Horizontal 2.5mm and vertical 1g.

Arrows indicate point of chloroquine addition.





3.6 EFFECT OF VARYING THE CONCENTRATION OF CALCIUM IN PHYSIOLOGICAL SALT SOLUTION ON ANTIMALARIAL ACTION

The aim of this experiment was to see how changes in the concentration of Ca^{2+} in the external medium affected the responses to Ach in the presence of low and high concentration of the drugs studied. The aim was also to investigate the possibility that the antimalarial drugs might interact with Ca^{2+} at the cell membrane. The same procedure for determining agonist effect was used in these experiments.

(a) Rat Stomach Strip

The concentration of calcium in the Tyrode solution was varied from 1.8mM to 0.45mM. At each Ca^{++} concentration, the effect of low ($10^{-7}M$) and high ($10^{-4}M$) concentrations of antimalrial on Ach. DRC was determined. The results are shown in Figures 10-13. It can be seen that as extracellular calcium decreased the potentiating effect of low dose antimalarial decreased, while the inhibitory effect of high dose antimalarial increased. The degree of leftward shift of agonist dose response curve was in inverse relation to the concentration of calcium in the bathing medium.

At 0.45mM Ca^{2+}, low dose antimalarial rather than potentiate, inhibited agonist contraction (see Figure 11)

Figure 10: GUINEA PIG ILEUM

Effect of two different concentrations of CQ on Ach contractions of guinea pig ileum in 0.9mM Ca^{2+} and 1.8mM Ca^{2+}.

Control responses

○——○ in 1.8mM Ca^{2+} (Ach alone)

●——● in 0.9mM Ca (Ach alone)

Responses in

□——□ 10^{-7} MCQ + 0.9mM Ca^{2+}

■- - -■ 10^{-4} MCQ + 0.9mM Ca^{2+}

Each point is a mean of 10 experiments. Vertical bars are ±SEM.

Level of significance ($p < 0.05$).

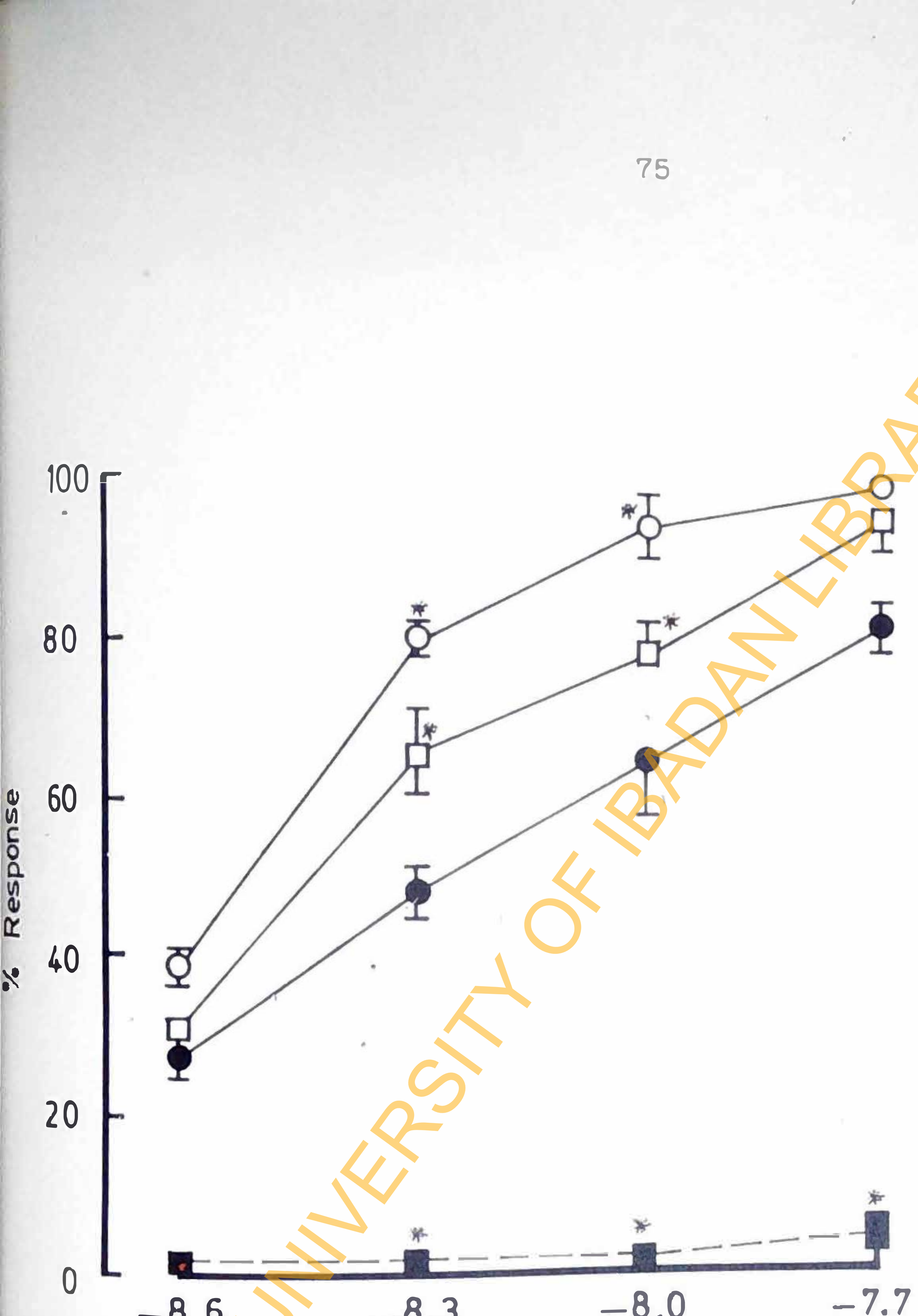


Figure 11: GUINEA PIG ILEUM

Effect of two different doses of CQ on Ach induced contractions in guinea pig ileum in 0.45mM Ca^{2+}.

Control in 1.8mM Ca^{2+} (Ach alone)

0.45mM Ca^{2+} (Ach alone)

Responses in 10^{-7} MCQ + 0.45mM Ca^{2+}

10^{-4} MCQ + 0.45mM Ca^{2+}.

Each point is a mean of 10 experiments, vertical bars indicate ± SEM.

* Level of significance ($p < 0.05$).

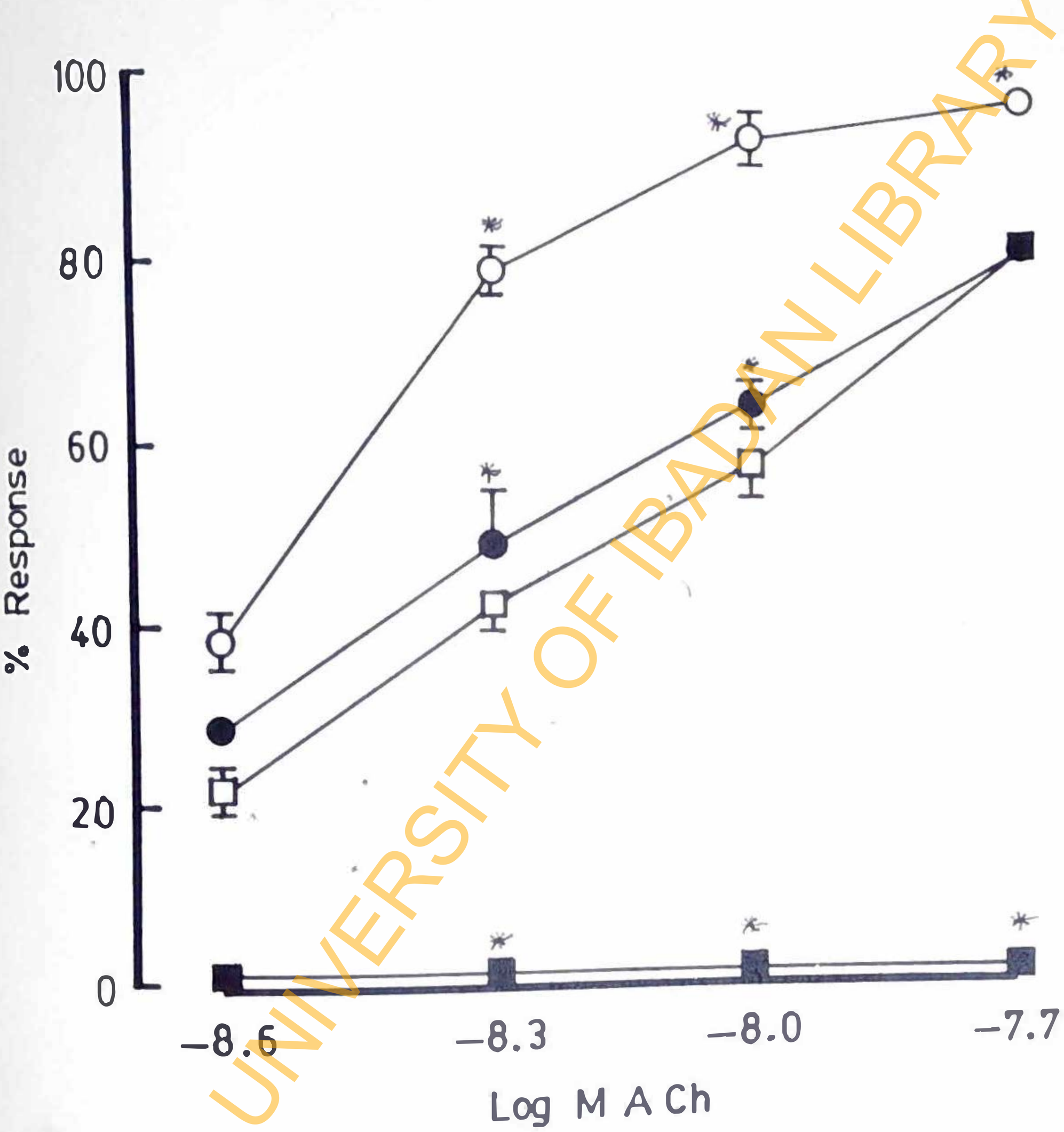


Figure 12: RAT STOMACH STRIP

Effect of AMDQ on Ach induced contraction in rat stomach strip in 0.9mM Ca^{2+}.

Control in ⊂—⊃ 1.8mM Ca^{2+} (Ach alone)

●—● 0.9mM Ca^{2+} (Ach alone)

Responses in 10^{-7}M AMDQ + 0.9mM Ca^{2+} ■—■

Each point is a mean of 8 experiments, vertical bars indicate ± SEM.

* Level of significance ($p < 0.05$).

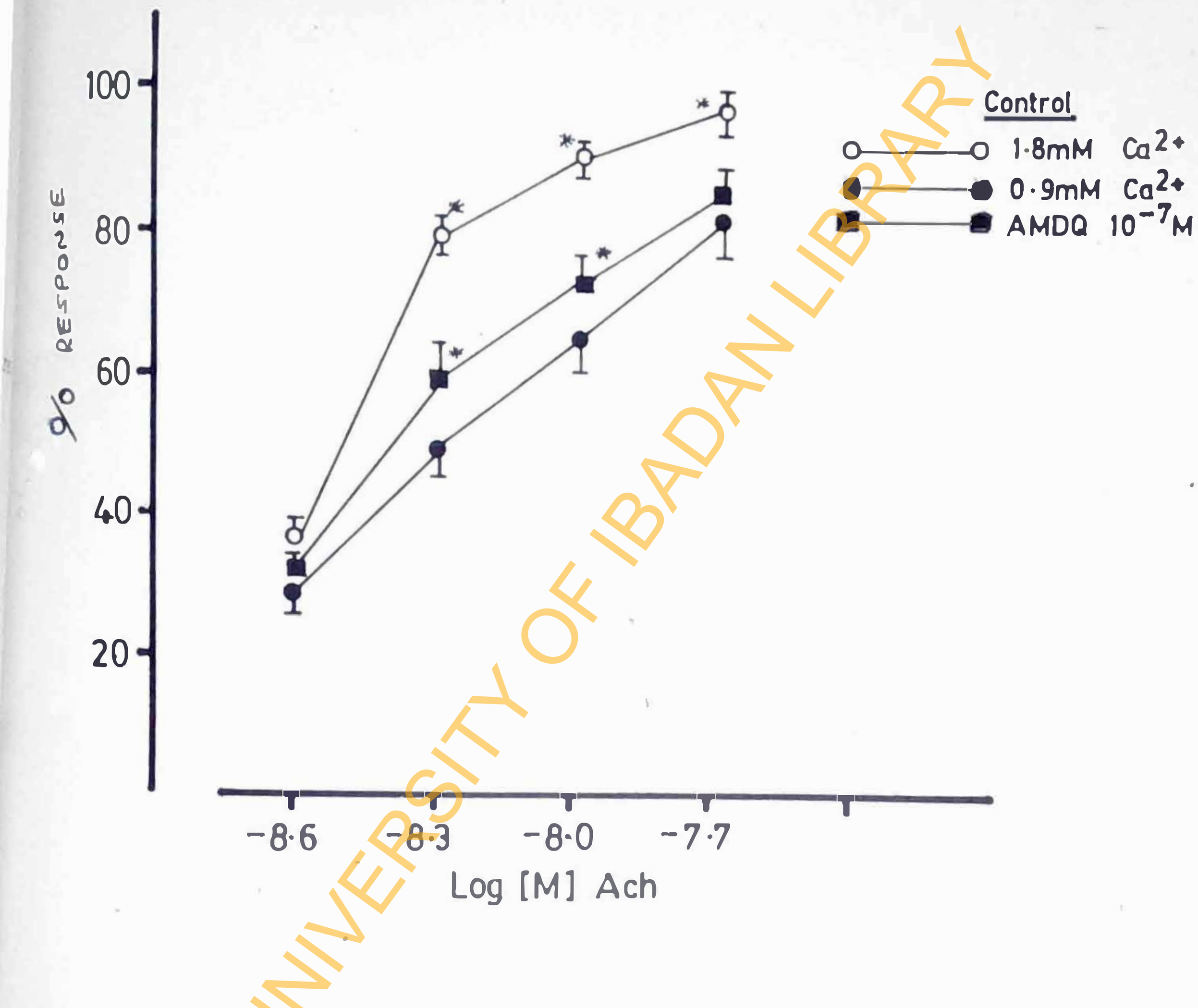


Figure 13: RAT STOMACH STRIP

Effect of AMDQ on Ach induced contraction in rat stomach strip in 0.45mM Ca^{2+}.

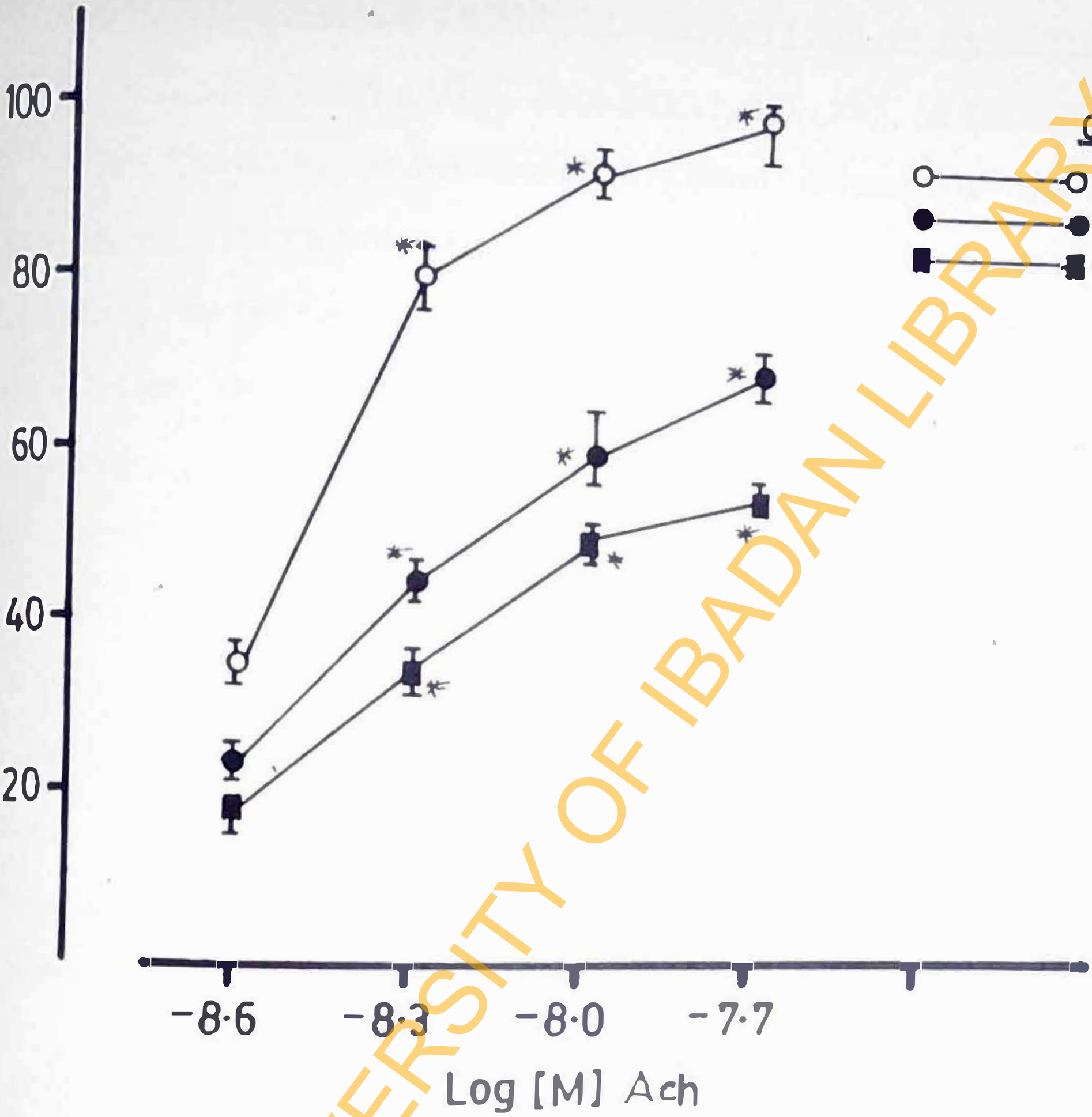
Control in 1.8mM Ca^{2+}

0.45mM Ca^{2+}

Response in 10^{-7}M AMDQ + 0.45mM Ca^{2+}

Each point is a mean of 8 experiments, vertical bars indicate standard error.

* Level of significance ($p < 0.05$).



3.7 EFFECT OF CALCIUM DEPRIVATION IN PHYSIOLOGICAL SALT SOLUTION ON ACH INDUCED CONTRACTIONS IN RAT STOMACH STRIP (RSS), GUINEA PIG ILEUM (GPI) AND RAT AORTIC STRIP (RAS)

In this experiment, different doses of agonist Ach, Histamine, Noradrenaline (NA), were applied until a dose response relationship was established. The dose of agonist producing about 75% of maximum response was selected and repeatedly applied to the tissue at regular intervals, until a steady response was obtained. The normal physiological solution was then replaced with one from which Ca^{2+} had been omitted. Within 10 minutes of changing to $O\text{-}Ca^{2+}$ solution, contraction of the guinea pig ileum virtually declined to zero, while responses were still elicited by agonist in Rat Stomach Strip (See Figure 14). The response following calcium deprivation in the medium is referred to as residual response.

3.7 EFFECT OF CALCIUM DEPRIVATION IN PHYSIOLOGICAL SALT SOLUTION ON ACH INDUCED CONTRACTIONS IN RAT STOMACH STRIP (RSS), GUINEA PIG ILEUM (GPI) AND RAT AORTIC STRIP (RAS)

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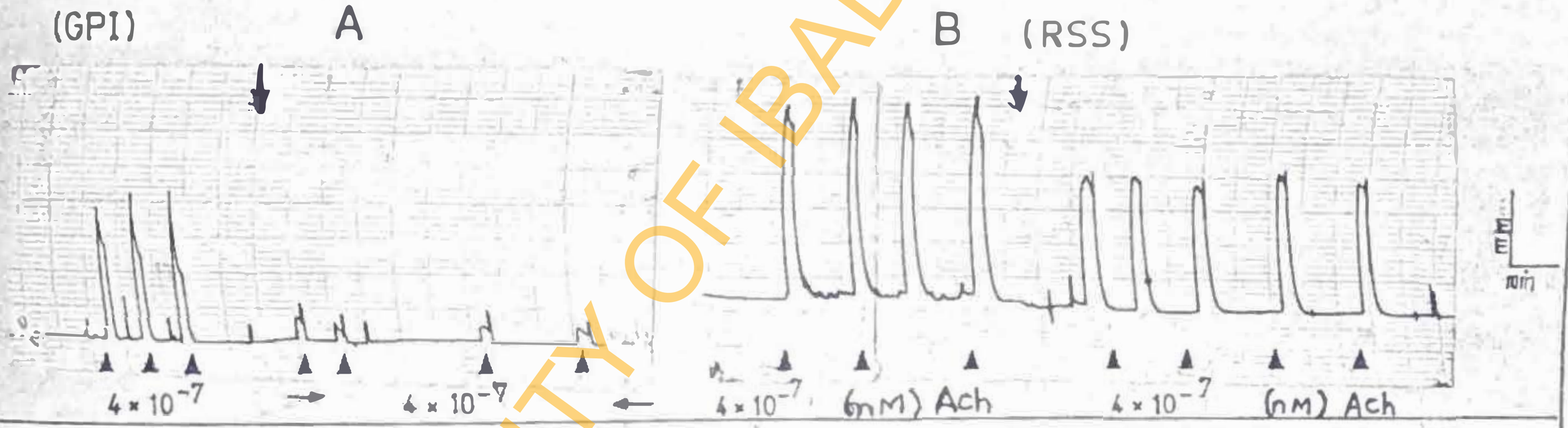
Figure 14: RESIDUAL RESPONSES IN GPI AND RAT STOMACH STRIP

Representative tracings of residual responses in O-Ca 0.5mM EGTA physiological salt solution (PSS) in guine pig ileum, GPI, (A) and rat stomach strip, RSS, B Arrows indicate incubation in O-Ca^{2+}, 0.5mM EGTA.



SUBMAXIMAL DOSE OF HISTAMINE (GPI).

SUBMAXIMAL DOSE ACETYLCHOLINE (RSS).

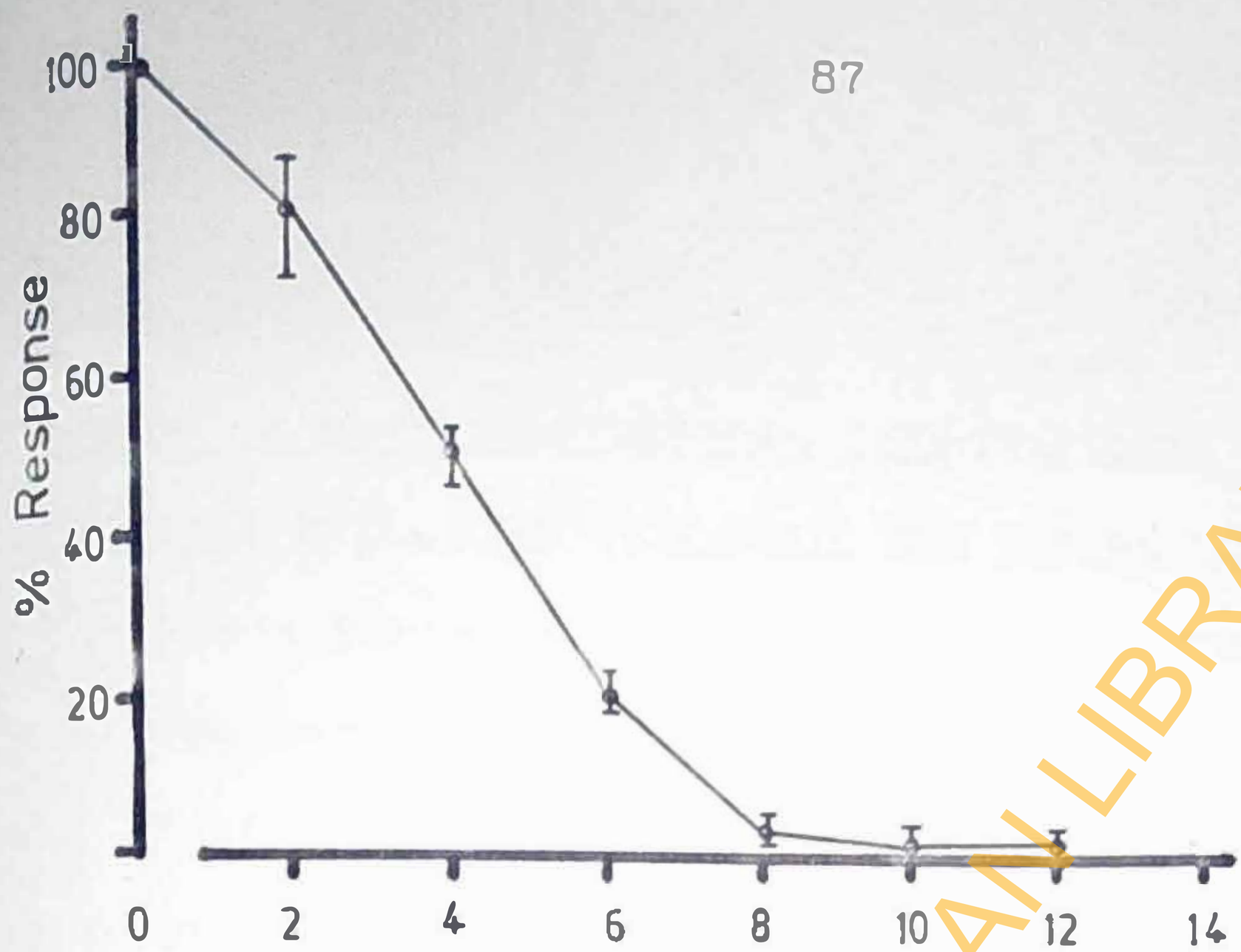
3.8 EFFECT OF ZERO EXTERNAL CALCIUM (Ca^{2+}) ON RESPONSES OF THE GUINEA PIG ILEUM AND RAT STOMACH STRIP TO SUBMAXIMAL DOSE OF HISTAMINE AND ACETYLCHOLINE

In this experiment, different doses of agonist were applied until a dose-response relationship was established for Histamine and Acetylcholine. The submaximal dose i.e. a dose producing about 75% of the maximum response was selected and repeatedly applied to the tissue at regular intervals of 2 minutes, until the response was steady. Histamine was used as agonist in the guine pig ileum and Ach in the Rat stomach strip. The physiological salt solution (Tyrode) was replaced with one in which $CaCl_2$ was omitted i.e. (Ca^{2+} free Tyrode solution). Contractions were elicited every two minutes after Ca^{2+} withdrawal. In the rat stomach strip responses were elicited every five minutes after Ca^{2+} withdrawal. The decline in responses to the agonist after the removal of external Ca^{2+} was rapid and less than ten minutes totally abolished in guinea pig iluem; the decline appeared to follow an exponential pattern (see fig 4 pg 87); whereas the responses elecited in Rat stomach strip were sustained for much longer under this experimental condition. A typical trace showing the pattern of responses for guinea pig ileum (Histamine) and Rat stomach strip (Ach) is shown in figure .

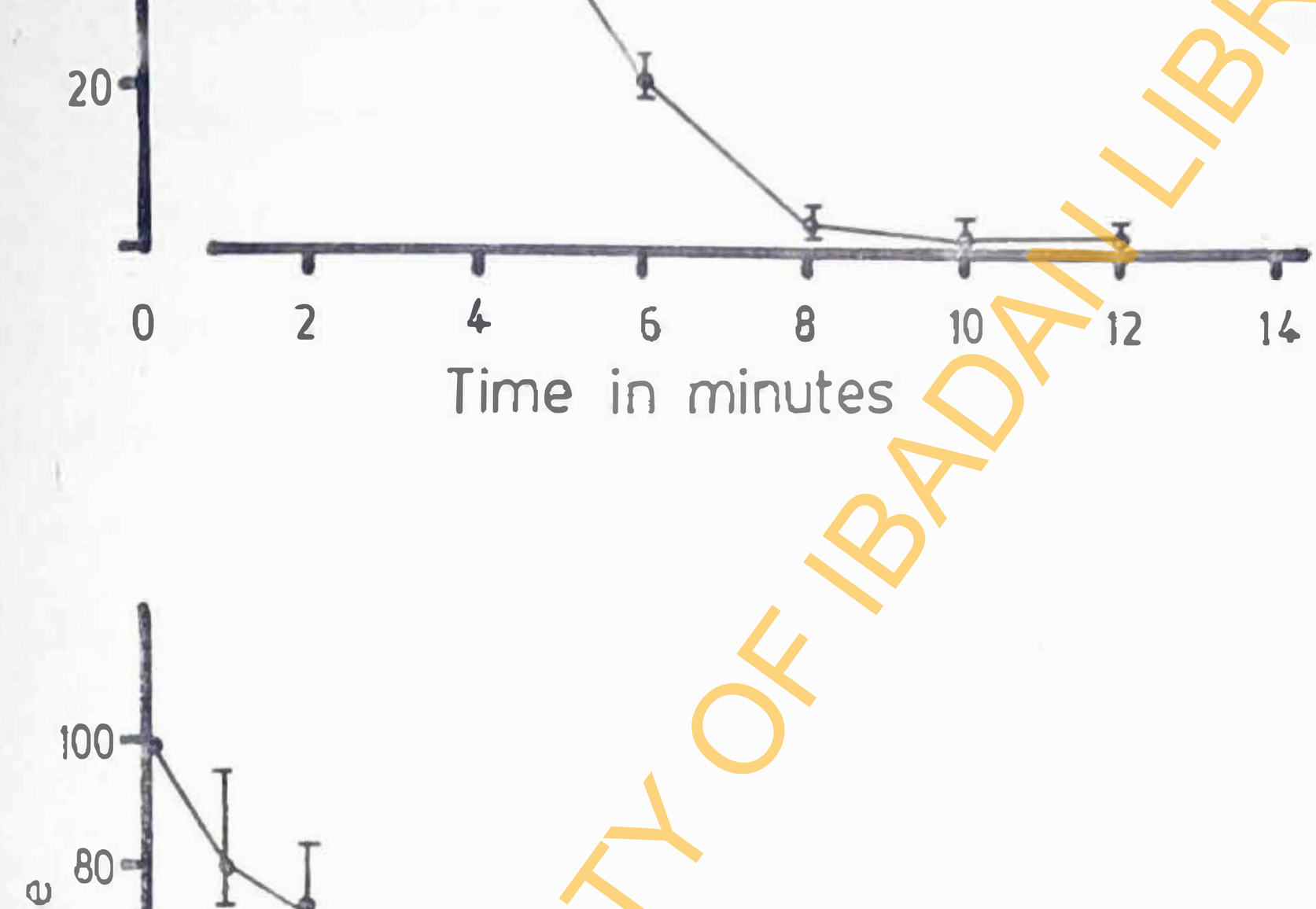
Figure 14a: RAT STOMACH STRIP

Time-related decline in responses of the isolated guinea pig ileum (A), and rat stomach strip (B) in Ca^{2+} free tyrode, each point is a mean of 8 experiments. Vertical bar represents standard error of the mean. In the guinea pig ilem responses were elicited every two minutes after Ca^{2+} withdrawal. In the RSS responses were elicited every five minutes after calcium withdrawal. Each response was expressed as a percentage of the mean of 8 responses before calcium withdrawal.

(a)



(b)



3.9 EFFECT OF Ca^{2+} DEPRIVATION ON CONTRACTIONS TO POTASSIUM CHLORIDE IN RAT STOMACH STRIP AND GUINEA PIG ILEUM

In this experiment different doses of K^+ were applied until a dose response curve relationship was established. A dose of K^+ producing 75% of maximum contraction was selected and repeatedly administered thereafter the normal physiological solution was replaced with Tyrode in which Ca^{2+} was omitted containing 0.5mM EGTA.

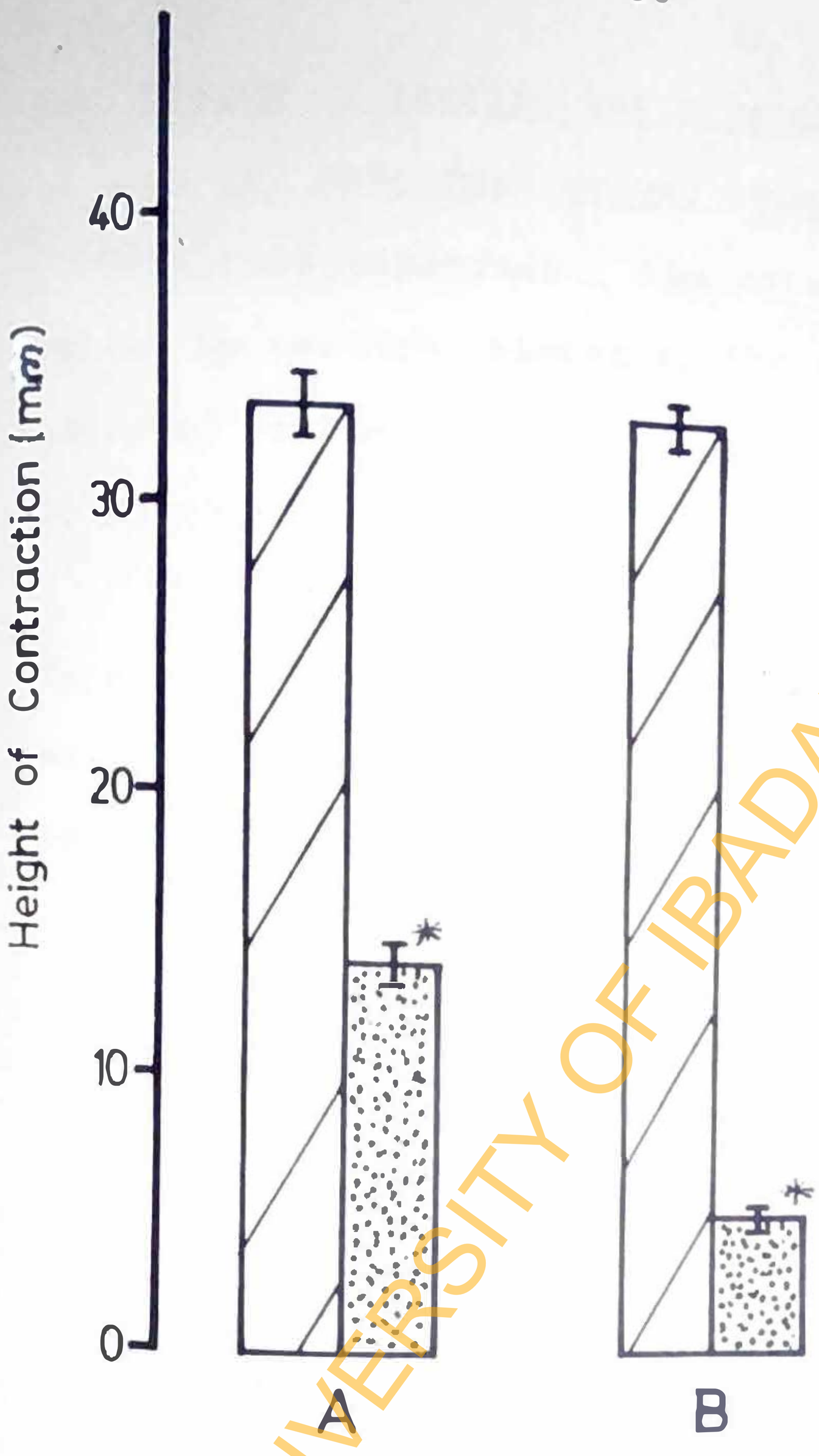
Each response was expressed in millimetre height of contraction (A) rat stomach strip: (B) guinea pig ileum.

It can be seen that after Ca^{2+} removal, responses to KCl though reduced persisted. The percentage reduction in response following Ca^{2+} removal was computed for each tissue. This result is in line with the result obtained with Ach in Ca^{2+} free medium where the decline in agonist response was more rapid in guinea pig ilem than Rat stomach strip, which suggests that there is a greater store of intracellular Ca^{2+} in Rat stomach strip than guinea pig ileum. (See figure 15, p90).

Figure 15a: RAT STOMACH STRIP AND GUINEA PIG ILEUM

Effect of Ca^{2+} deprivation on contraction to K^+ in (A) rat stomach strip (8mM) and in (B) guinea pig ileum (8mM). Stripped columns are control responses in normal tyrode. The dotted columns are responses in Tyrode solution in which Ca^{2+} was omitted and containing 0.5mM EGTA. Vertical bars are S.E.M. (n=6).

* Significantly different from control ($P<0.05$).



3.10 EFFECT OF VARYING THE EXTERNAL Ca^{2+} IN THE BATHING FLUID ON THE RESPONSES OF RAT STOMACH STRIP TO ACH.

In this experiment, the external Ca^{2+} concentration was varied to see how changes in the concentration of Ca^{2+} in the external medium affected the response to submaximal dose ($4 \times 10^{-7}M$) of acetylcholine.

The first sets of experiment were carried out in normal Tyrode solution, i.e. 1.8mM Ca^{2+} which was thereafter replaced with Tyrode containing 0.45mM Ca^{2+}; after which the solution was again changed to a Ca^{2+} free Tyrode solution contaning 0.5mM EGTA. Height of contractions were expressed in millimetre (See fig.15b pg.93).

Figure 15b: RAT STOMACH STRIP

Influence of varying the Ca^{2+} concentration in the bathing fluid on the effect of CQ on Ach ($4x10^{-7}M$)-induced contraction.

Open Column: Control contractions in (1.8mM Ca^{2+})

Striped Column: Contractions in (0.45mM Ca^{2+})

Dotted Column: Contraction in O-Ca^{2+}

Filled Column: Contraction in (O-Ca^{2+} + 0.5mM EGTA)

Vertical bar S.E.M. (n-16) from 4 preparations.

* Significantly different from control ($P<0.05$).

Figure 15b: RAT STOMACH STRIP

Influence of varying the Ca^{2+} concentration in the bathing fluid on the effect of CQ on Ach ($4x10^{-7}M$)-induced contraction.

Open Column: Control contractions in (1.8mM Ca^{2+})

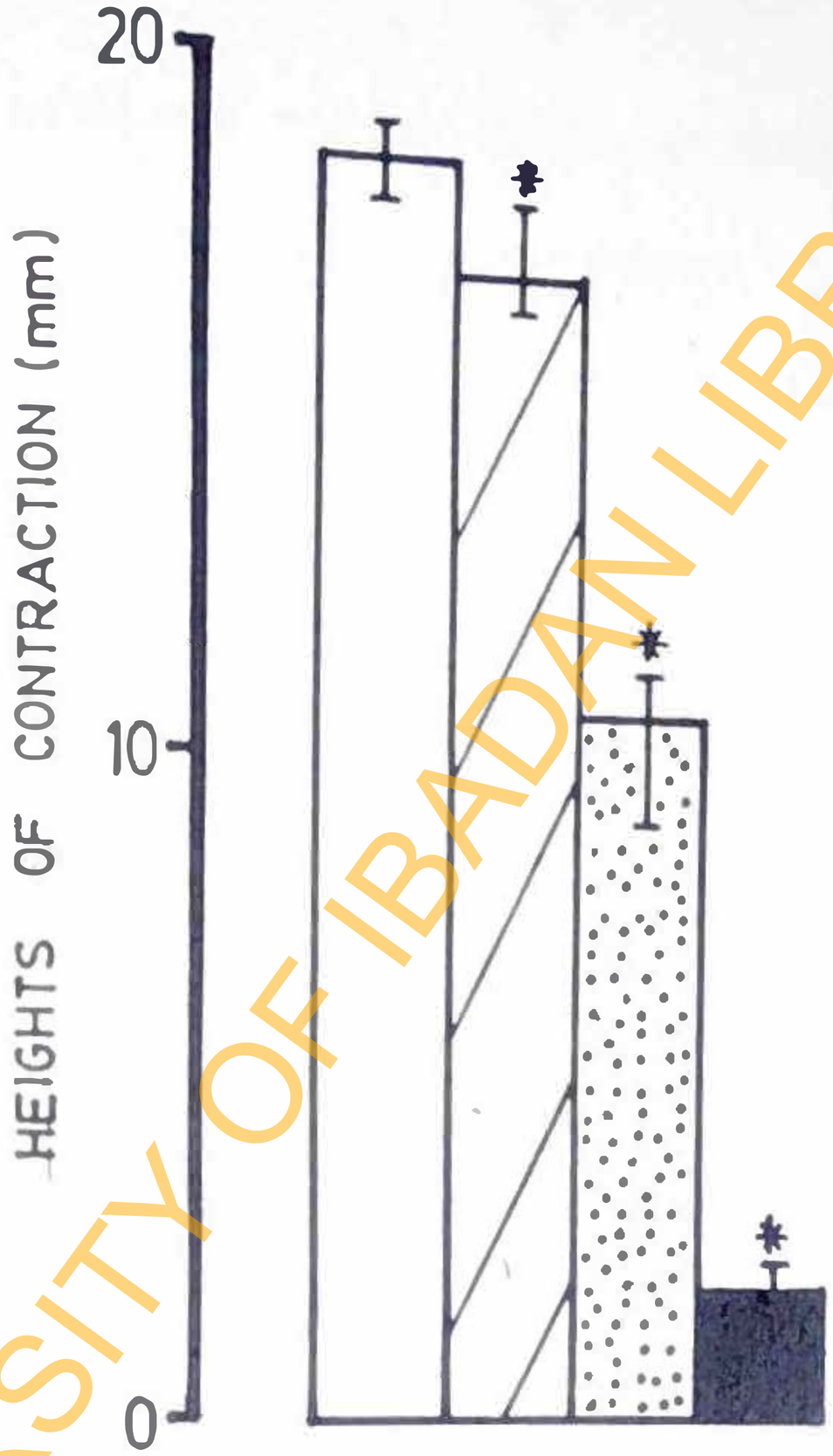
Striped Column: Contractions in (0.45mM Ca^{2+})

Dotted Column: Contraction in O-Ca^{2+}

Filled Column: Contraction in (O-Ca^{2+} + 0.5mM EGTA)

Vertical bar S.E.M. (n-16) from 4 preparations.

* Significantly different from control ($P<0.05$).



3.11 EFFECT OF VARYING EXTERNAL Ca^{2+} IN THE PHYSIOLOGICAL SALT SOLUTION ON THE ACTION OF ANTIMALARIAL (QUININE) ON RAT AORTIC STRIP

As in the rat stomach strip, this experiment was designed to show the effect of Quinine on responses to Noradrenaline in normal Kreb solution containing 1.6mM Ca^{2+} and in Kreb solution containing 0.4mM Ca^{2+}. The procedure was to establish control responses, and a submaximal dose of Noradrenaline (10^{-6}M). The artery was then incubated in Krebs solution containing 10^{-7}M Quinine. the submaximal doses of Noradrenaline were then repeated and again in presence of 10^{-4}M Quinine. The same kind of experiment was repeated in a fresh pareparation suspended in Kreb solution containing 0.4mM Ca^{2+}. The results are presented in (fig 15c p97) It can be seen from the figure that 10^{-7}M Quinine had no effect on the NA-induced responses in normal Kreb solution, but 10^{-4}M Quinine reduced this response by 70% and this effect was statistically different from control at ($p < 0.05$).

On the other hand 10^{-7}M Quinine significantly reduced the effect of Noradrenaline in Kreb solution containing 0.4mM Ca^{2+} and 10^{-4}M Quinine abolished the response to Noradrenaline in this medium. These results are similar to those earlier reported in RSS and gpI using Ach and Histamine (see fig 14 p84).

The results confirm that the inhibitory effect of antimalarial increases as concentration of Ca^{2+} in external medium decreases.

Figure 15c: RAT AORTIC STRIP

Effect of Ca^{2+} on the action of quinine on NA-induced (10^{-6}M) contraction in (A) 1.6mM and in (B), 0.4mM Ca^{++}

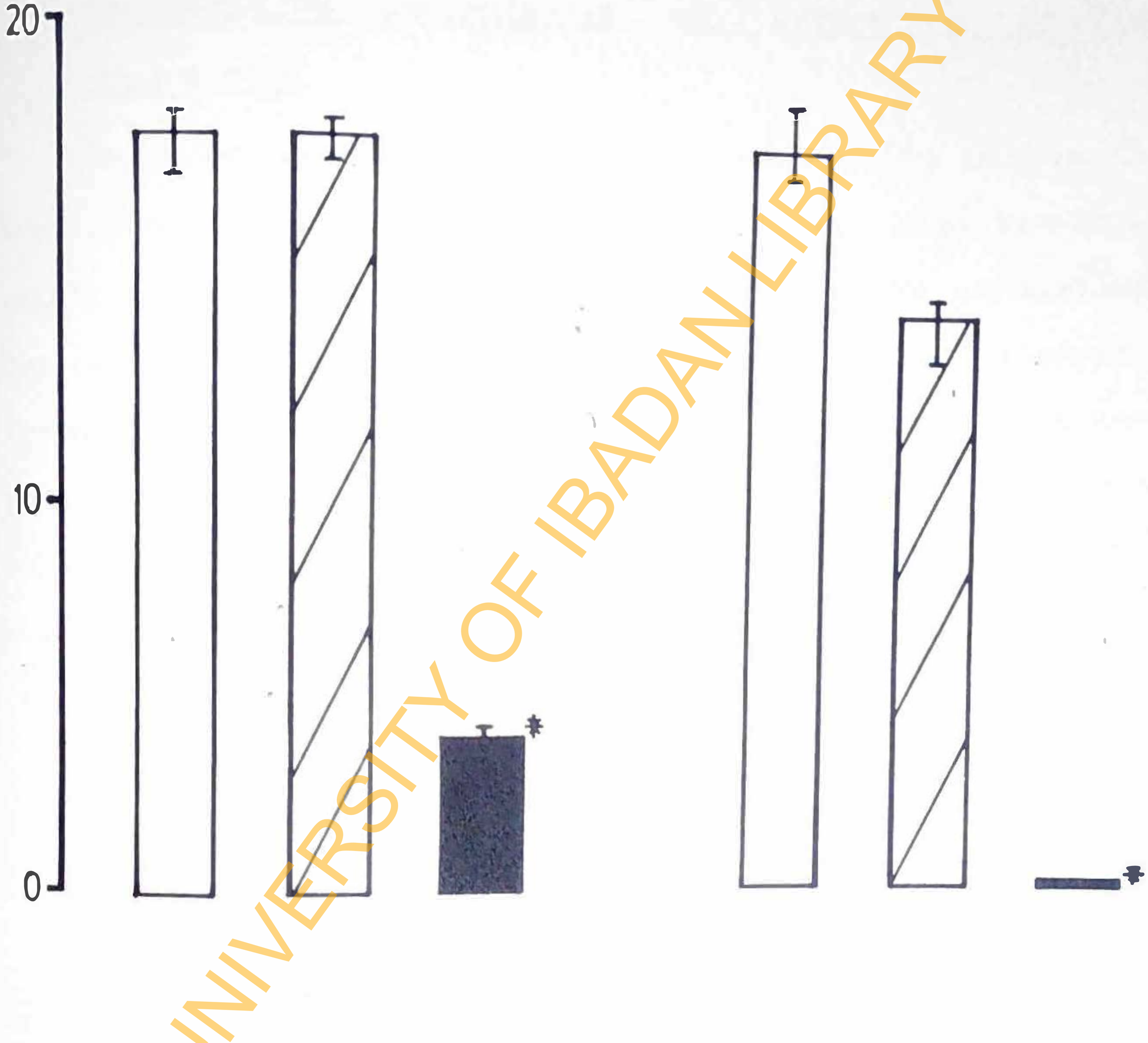
Open columns: Control contractions.

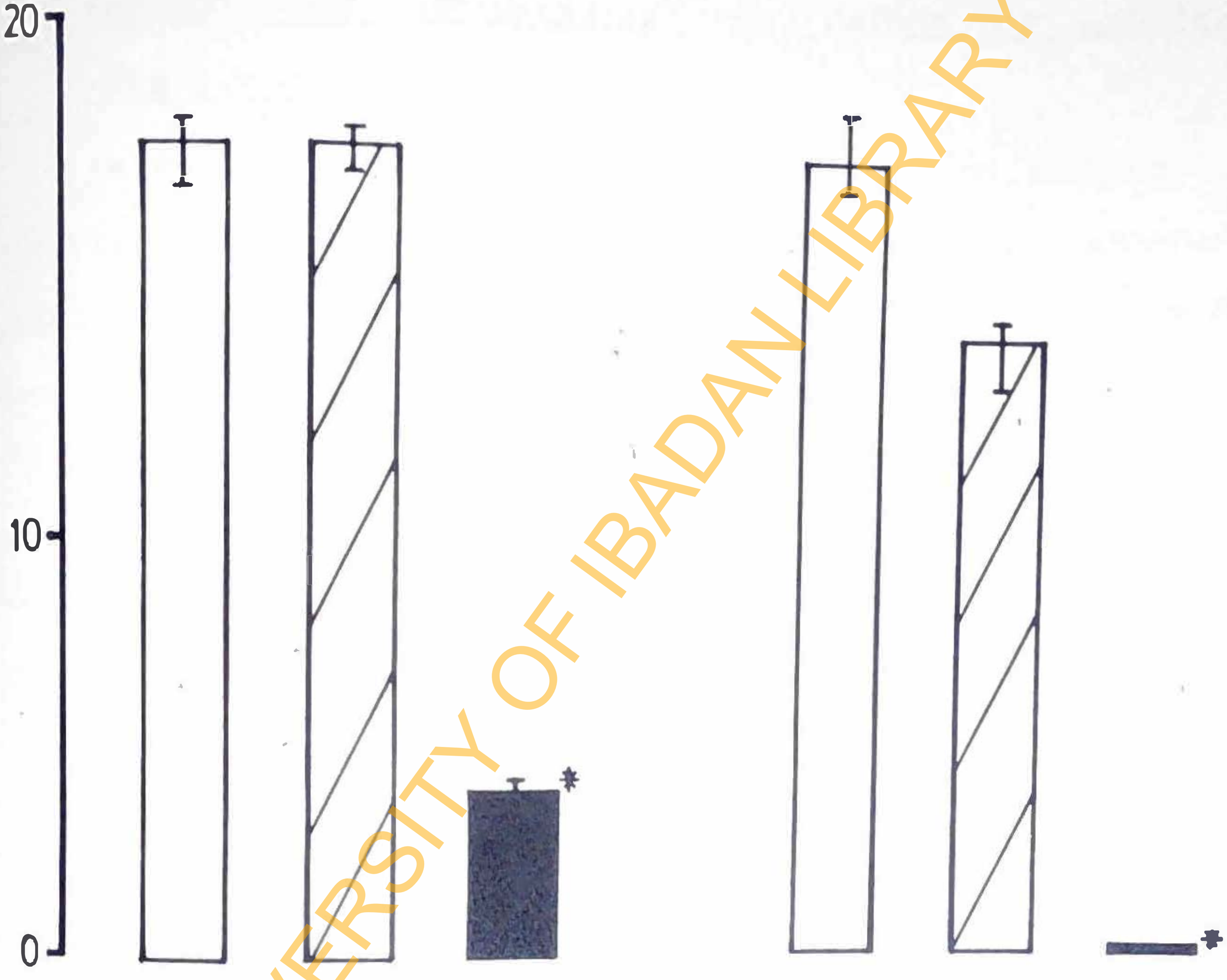
Striped column: Contraction in the presence of 10^{-7}M Quinine.

Filled Column: Contractions in the presence of 10^{-4}M

Vertical bars: S.E.M. (n 6-9 exp.) from three aortic preparations.

* Significantly different from control ($P<0.05$)





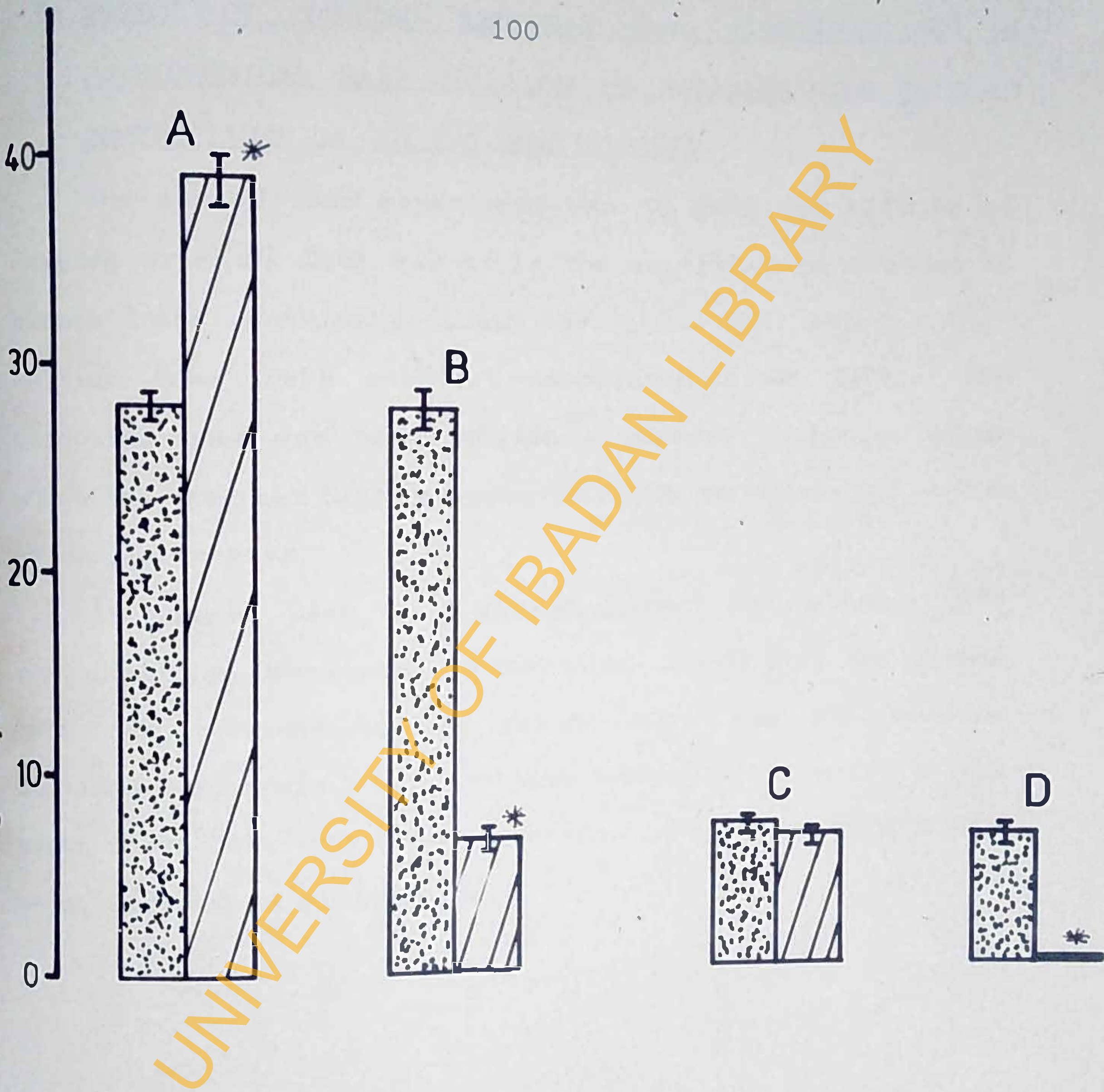
3.12 EFFECT OF CHLOROQUINE ON ACETYLCHOLINE INDUCED CONTRACTIONS OF ISOLATED RAT STOMACH STRIP IN THE PRESENCE OF ANTIMALARIAL AND EFFECT OF CALCIUM DEPRIVATION

In these series of experiments, contraction induced by acetylcholine was recorded, after obtaining, dose response relationship. A submaximal dose producing 75% of maximum contraction was selected. This dose was recorded in normal Tyrode solution in the absence of chloroquine (A) and in the presence of low and high doses (B). Similar investigation was carried out in Tyrode solution in which Ca^{2+} was omitted. (See C and D fig 15 ps 100)

Figure 15d: RAT STOMACH STRIP

Effect of CQ on Ach (400nm) evoked contraction of isolated r stomach strip in presence (A) 10^{-7}M enhanced ACH responses normal Tyrode. In (B) 10^{-4}M CQ significantly reduced AC responses in normal Tyrode. In (C) where the Tyrode solutio lacked Ca^{2+} and contained EGTA, 10^{-7}M CQ had no effect on AC residual effect, whereas 10^{-4}M CQ in (D) completely abolished the residual ACH response. (A) 10^{-7}M and (B) 10-4M CQ in strped column in normal tyrode solution; while dotted columns responses in control. Effect of Ca2+ deprivation on contraction to Ach (C and D). Dotted columns represents responses in control and striped column represents responses in the presence of CQ. (C) 10^{-7}M (D) 10^{-4}M respectively. Vertical bars are S.E.M. (n=6).

* Significantly different from control ($P<0.05$).



3.13 EFFECT OF VARYING EXTERNAL Ca^{2+} CONCENTRATION IN PHYSIOLOGICAL SALT SOLUTION ON NORADRENALINE INDUCED CONTRACTIONS OF THE RAT AORTIC STRIP

The aim of this experiment was to show the effects of varying external Ca^{2+} vis-a-vis the contractions induced in normal Krebs containing (1.6mM Ca^{2+}); (0.4mM); and O - Ca^{2+} calcium free Krebs solution containing 0.5mM EGTA. The procedure used was to establish a control response, after which the external Ca^{2+} concentration was varied in the medium as earlier stated.

It can be seen in (figure 15 e p 103) that as external Ca^{2+} concentration decreases, contraction diminished to stable responses, whereas in the guinea pig ileum contractions declined completely. Contractions were still elicited in rat aorta under Ca^{2+} free Krebs solution. Heights of contraction were recorded in millimetres.

Figure 15e: RAT AORTIC STRIP

Influence of varying the calcium concentration in the bathing fluid on the effect of quinine on NA (10^{-6}M)-induced contractions.

Open column: Control contractions (1.6mM Ca^{2+})

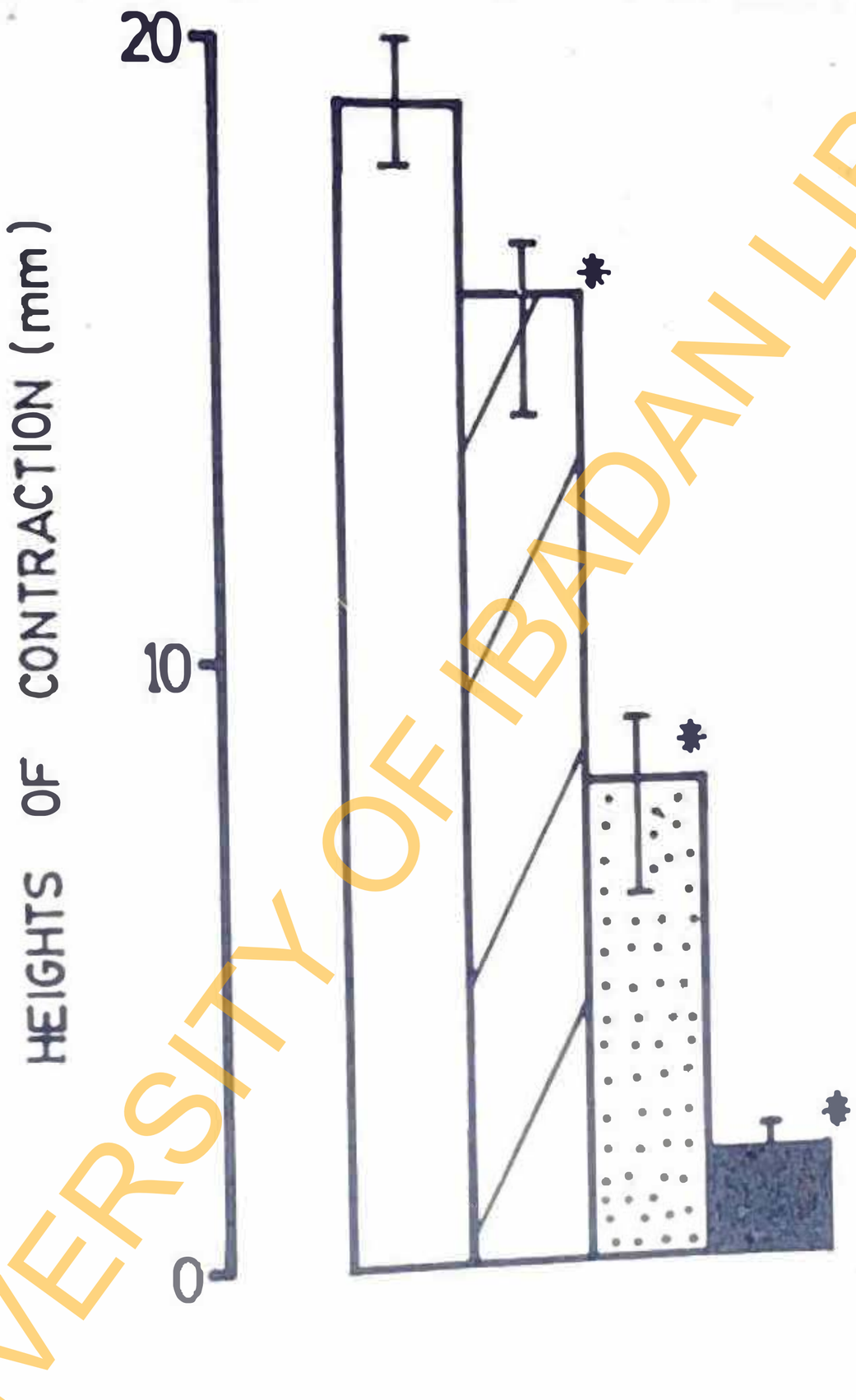
Striped column: Contraction (0.4mM Ca^{2+})

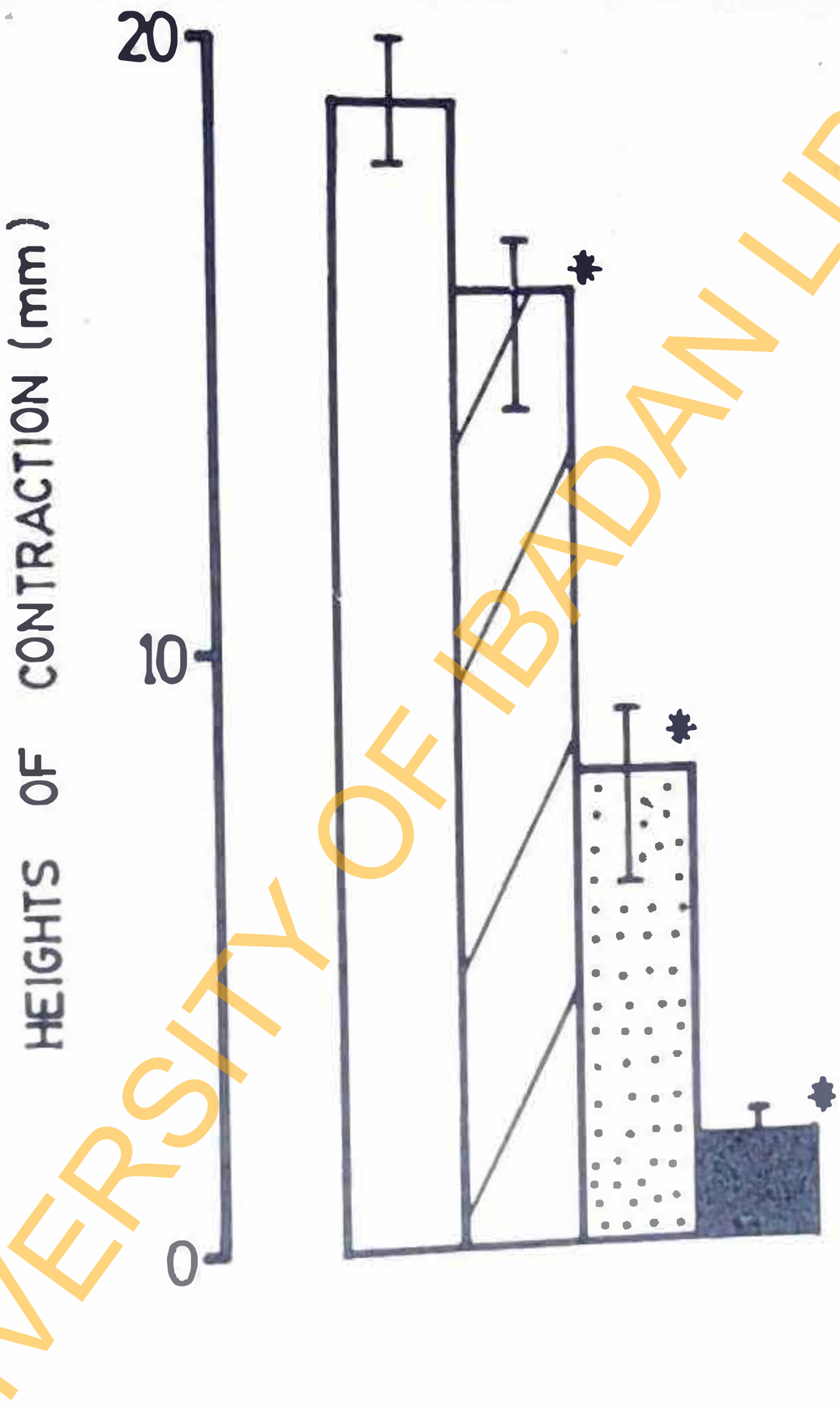
Dotted column: Contraction (0. Ca^{2+})

Filled column: Contraction in O-Ca^{2+} + 0.5mM EGTA

Vertical bar S.E.M. (n=8) from 3 aortic preparations.

* Significantly different from control ($P<0.05$).





3.14 EFFECT OF ANTIMALARIAL (CQ) ON RESIDUAL RESPONSES TO ACETYLCHOLINE IN ISOLATED RAT STOMACH STRIP

In these series of experiments, after constructing a dose response curve to Acetylcholine, a submaximal dose (4×10^{-7}M) was selected. This dose was repeatedly used to elicit responses, until stable response were obtained; thereafter, the muscle was incubated in low dose antimalarial as previously described under method. ACH contraction were potentiated. In panels C and D the tissue was incubated in Tyrode solution from which Ca^{2+} had been omitted. In this medium low dose antimalarial did not potentiate responses elicited with submaximal dose of ACH whereas high dose antimalarial 10^{-4}M completely abolished responses elicited by acetylcholine (See figure 16).

Figure 16 RAT STOMACH STRIP PREPARATION

The effect of calcium deprivation on the interaction between CQ (10^{-7}M) and Ach responses. At the dots, Ach $4x10^{-7}$M was added and the maximum response recorded. In panels A and B the tissue was incubated in normal Tyrode solution. 10^{-7}M CQ potentiated the Ach induced contractions. In panels C and D, the tissue was incubated in Tyrode solution from which calcium was omitted and also contained 0.5mM EGTA. 10^{-7}M CQ had no effect on the Ach induced contractions (see text).

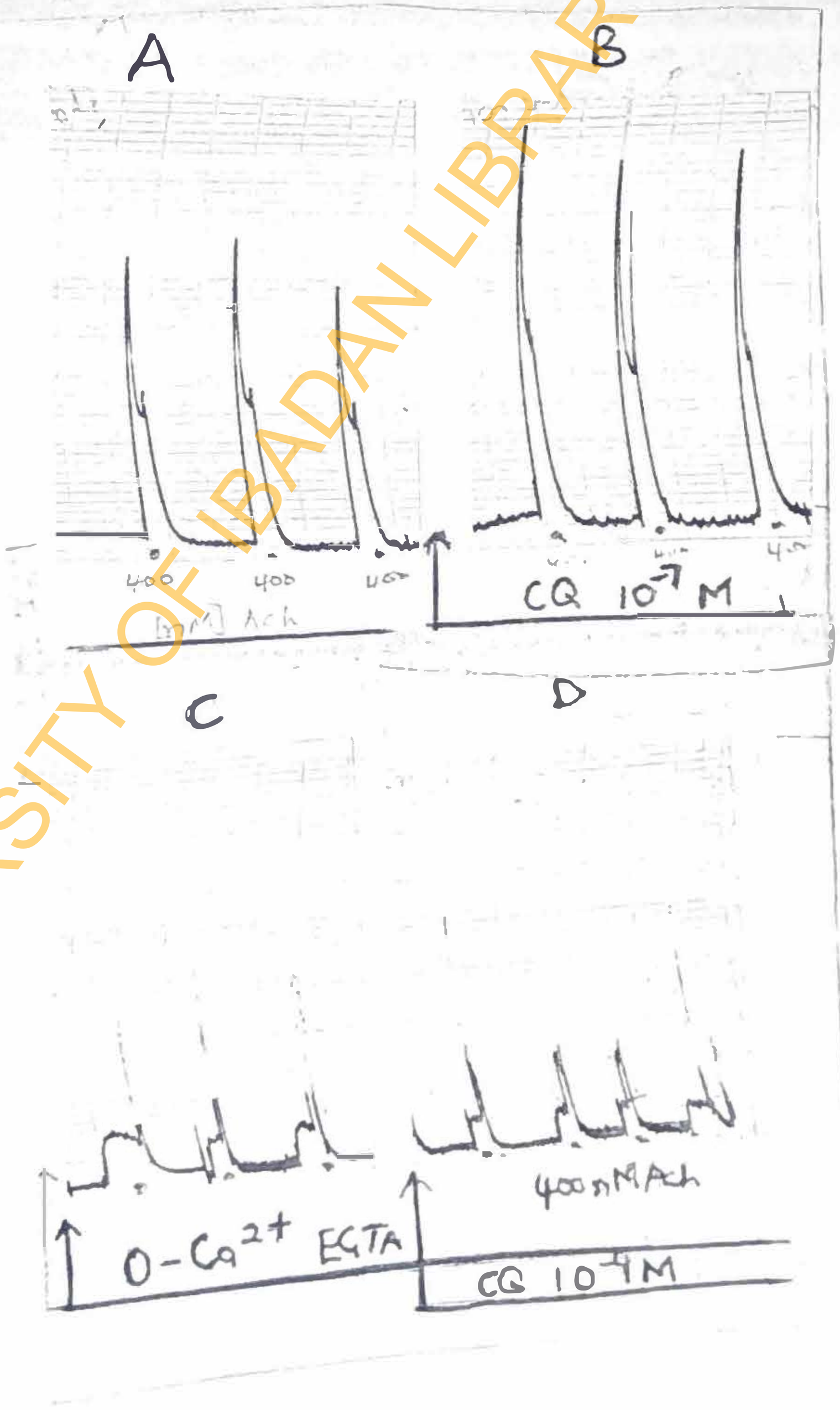
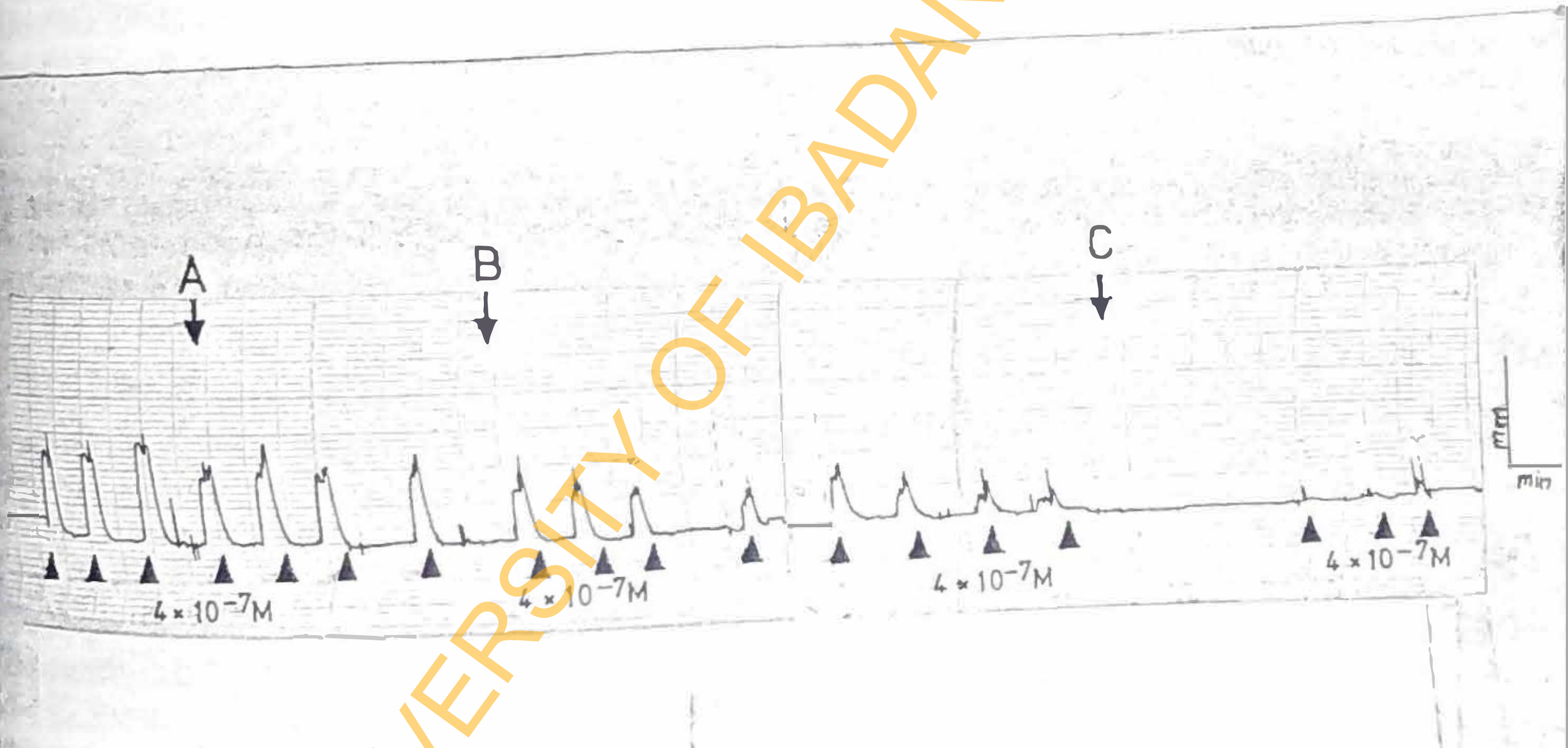


Figure 17:

Representative tracings of the effect of chloroquine on isolated rat stomach strip contraction in Ca^{2+} free solution containing 0.5mM EGTA before (A), and after chloroquine low dose at point (B), high dose 10^{-4} at C. Arrows indicate point of addition.



3.15 EFFECT OF CHLOROQUINE, MEPACRINE ON CONTRACTILE RESPONSES TO ACETYLCHOLINE

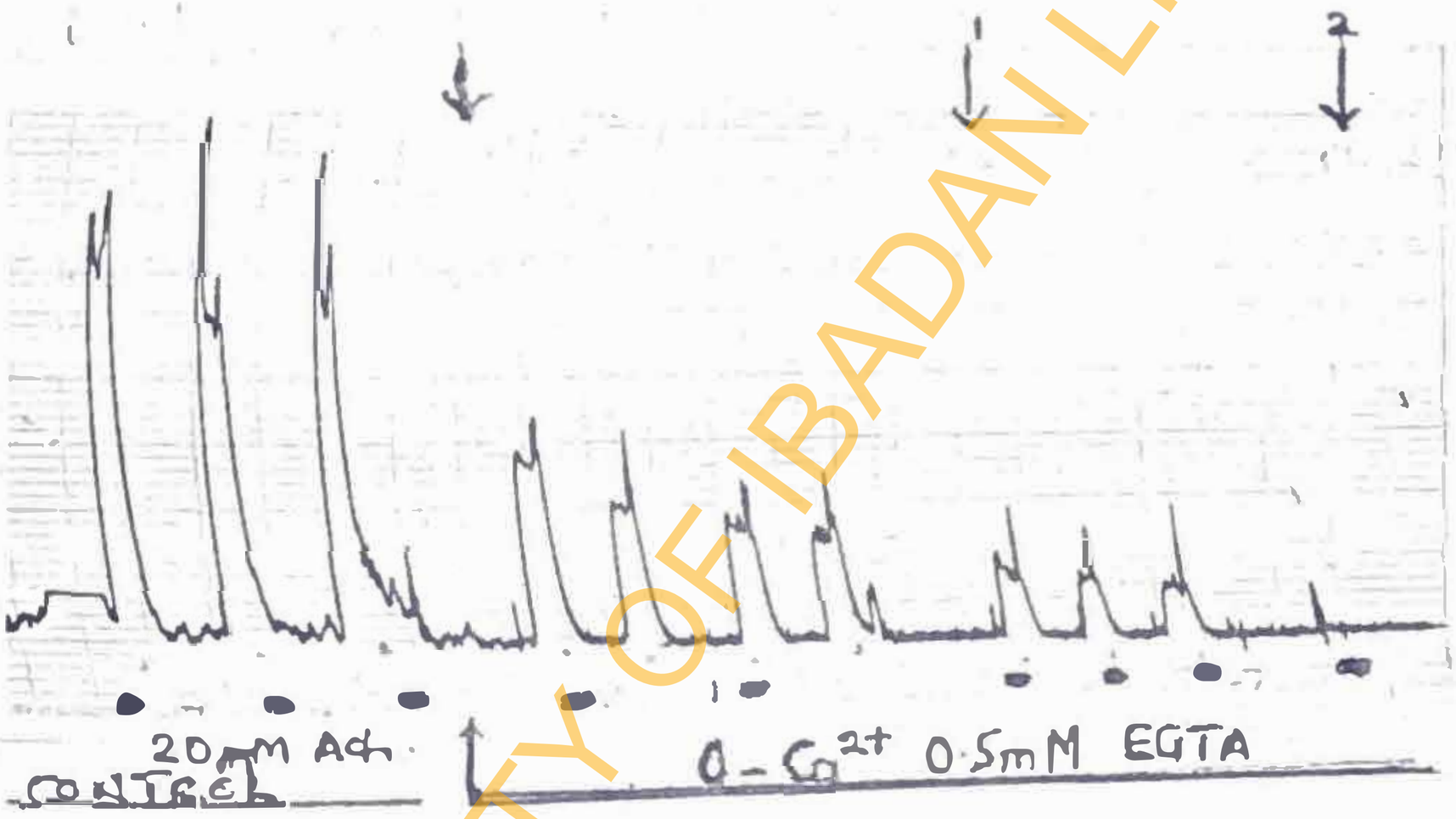
The aim of this experiment was to see the action of two different antimalarial CQ - (4 aminoquinoline) and Mepacrine (8 aminoquinoline) on Acetylcholine induced contractions in isolated rat Stomach Strip.

Dose response relationship was established after which a submaximal dose ($2 \times 10^{-8}M$) was selected. This dose of acetylcholine was used to stimulate the muscle in normal physiological Tyrode solution, thereafter the solution was changed to zero Ca^{2+} containing 0.5mM EGTA medium; the procedure was again repeated. From the tracing displayed in (figure 18, p111) it can be seen that both antimalarials showed similar actions in both low ($10^{-7}M$), and high $10^{-4}M$ concentration of antimalarial, despite structural disimilarity.

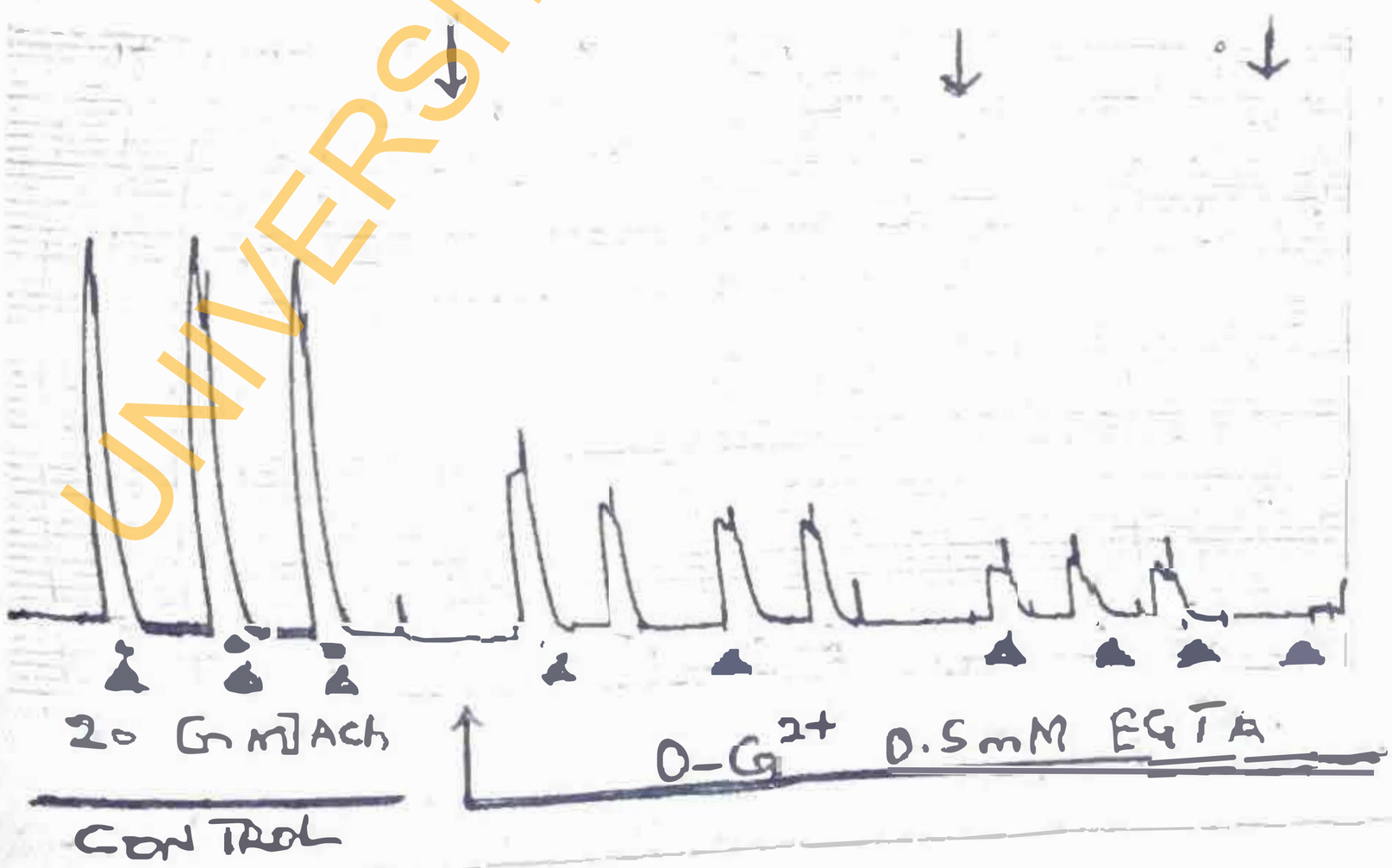
Figure 18: RAT STOMACH STRIP.

Representative tracings of the effect of low dose CQ (A
and Mepacrine (MPC) B; on contraction induced by Ac
submaximal dose $2x10^{-8}$M before and after O-Ca^{2+} 0.5mM BGT
medium. Arrows indicated point of antimalarial additio
(1 & 2, 10^{-7}M and 10^{-4}M).

A



B



3.16 RESULTS ON STUDIES WITH THE RAT AORTIC STRIP

The rat aortic strip preparation as described in the method was free of endothelium. It is known that the endothelium produces a relaxing factor (EDRF) now proposed to be nitric oxide (Palmer, Ferridge & Moncada, 1987) which is known to modify the response of the vascular smooth muscle to agonists (Furchgott, 1983; Tayo and Bevan, 1987). The absence of endothelium was established in the preliminary experiments (Fig19 p114) by observing that Ach consistently failed to relax NA-induced contraction of the muscle (Furchgott and Zawadki, 1980). This preparation responded to NA (10^{-6}M) and high KCl (15-60mM) dose dependently and dose response curves were constructed by cumulative addition of contractile agents (see Fig20 p116). Again in some experiments, a time control preparation was included to ensure that changes in contraction induced by drug was independent of time dependent changes in tissue sensitivity.

Figure 19: RAT AORTIC STRIP PREPARATION.

Loss of relaxing response of preparation of rabbit aor
after rubbing of the intimal surface. The record sho
the point at which each drug (Ach) concentration addi ti
was made.

Figure 19: RAT AORTIC STRIP PREPARATION.

Loss of relaxing response of preparation of rabbit aor
after rubbing of the intimal surface. The record sho
the point at which each drug (Ach) concentration additi
was made.

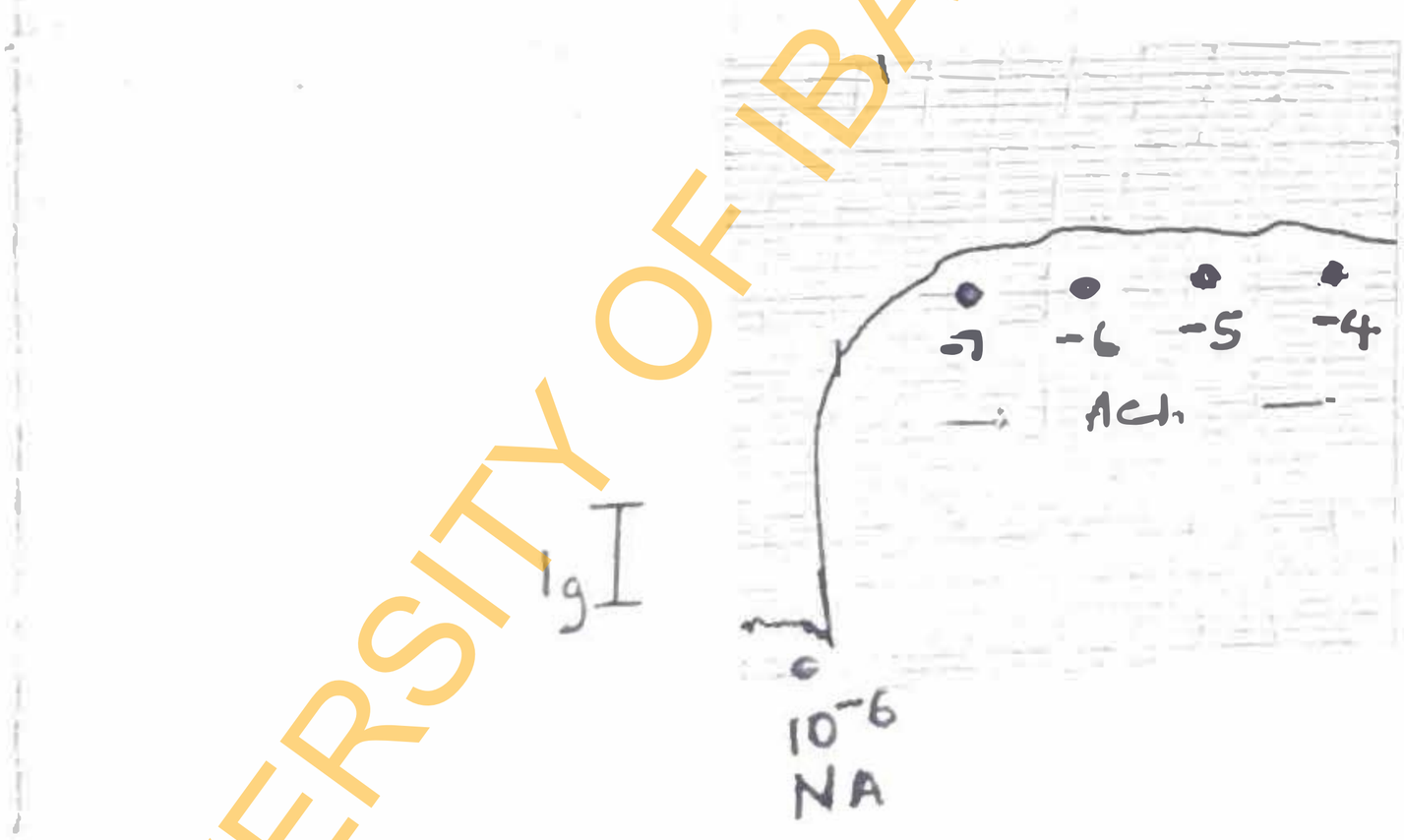
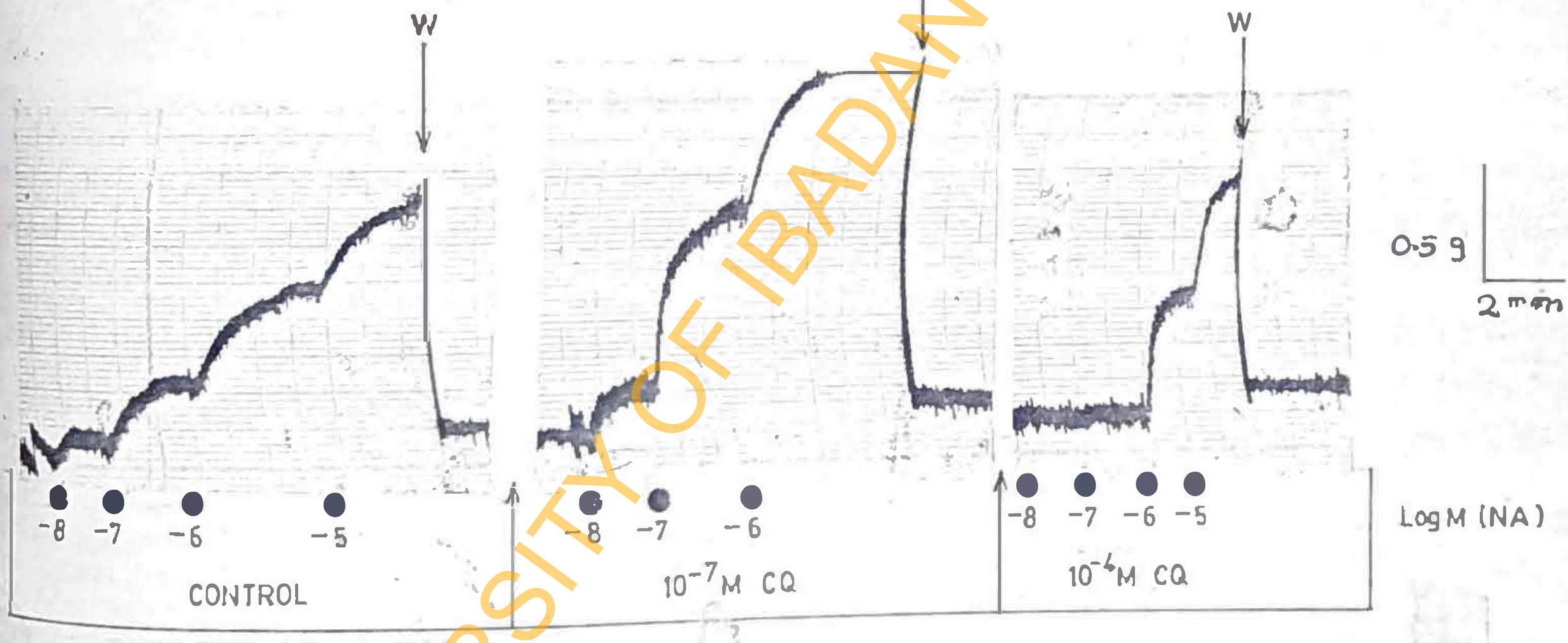


Figure 20: RAT AORTIC STRIP PREPARATION

Representative tracing of the effect of chloroquine CQ pre-incubation on NA induced contractions.

Calibration: horizontal 2 mins, vertical 1gm.

Arrows indicate point of CQ additions, W indicates wash.



3.17 EFFECT OF SELECTED ANTIMALARIALS ON NORADRENALINE INDUCED CONTRACTIONS OF THE AORTIC STRIP

Control dose response curves were obtained by cumulative and single dose addition of Noradrenaline. Thereafter the tissue was incubated in low dose antimalarials for 30 minutes. NA contractions were then repeated in the presence of antimalarials. Low dose CQ potentiated NA induced contractions, (see Fig 21 p 119) The tissue was again incubated in high dose antimalarial (>10^{-5}M) for same period of equilibration. High dose antimalarial inhibited NA induced contractions. The nature of inhibition was consistent with non-competitive antagonism since maximum response was surpressed and no parallel shift of the curve to the right was observed (Fig. 21).

Results for other antimalarials AMDQ, MPC were again similar, but MFQ and HFT again did not significantly potentiate nor inhibit muscle contractions.

Figure 21 RAT AORTIC STRIP

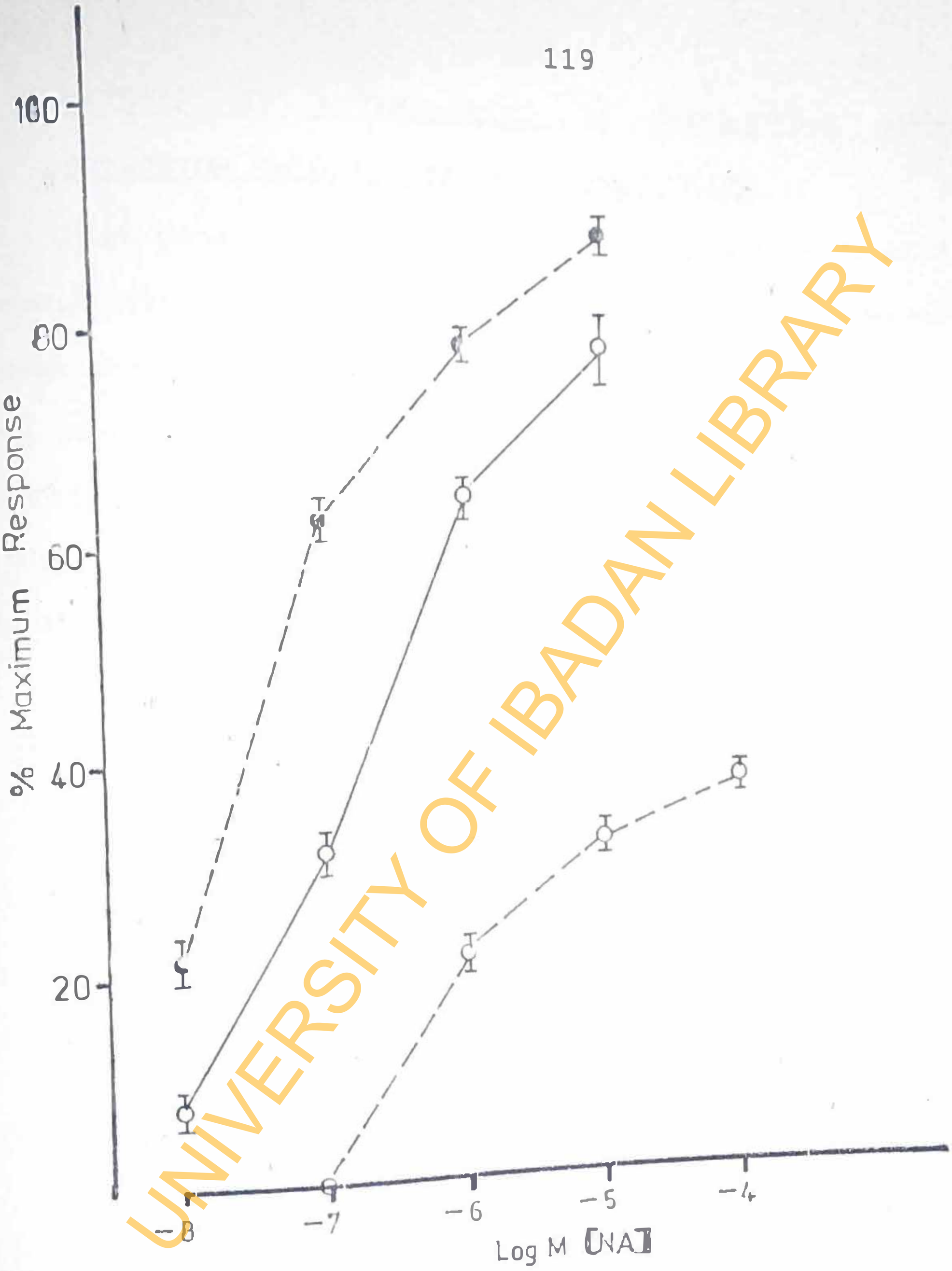
Effect of chloroquine on Noradrenaline evoked contractions of rat aortic strips.

O—O control contractions

● - - ● contractions in the presence of chloroquine 10^{-7}M

O - - O chloroquine 10^{-4}M

Vertical bars indicate S.E.M. (n=3).



3.18 EFFECTS OF ANTIMALARIAL ON CONTRACTION EVOKED BY POTASSIUM CHLORIDE IN RAT AORTIC STRIP

High potassium chloride contracted the aortic strip dose dependently. A control dose response curve was established thereafter the tissue was equilibrated in low dose CQ which was previously shown to potentiate NA induced contraction. The high dose ($10^{-4}M$) which was shown previously to inhibit NA induced contraction markedly, did not produce a marked inhibition of K^+ induced contractions (Fig22 pg 122).

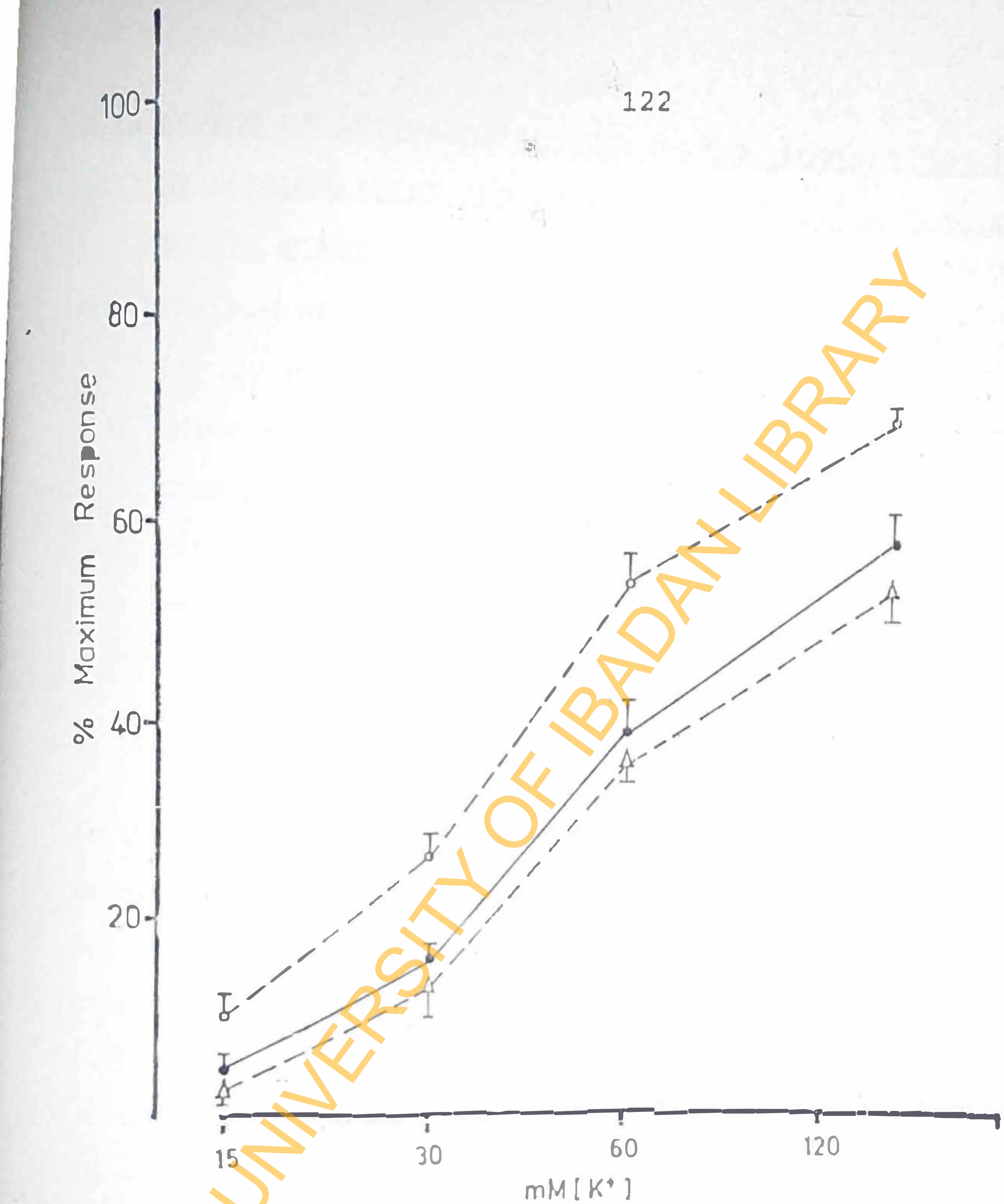
Figure 22 RAT AORTIC STRIP

Effect of CQ on the contractile responses of rat aortic strip to K^+

O- - -O response $10^{-7}M$

control

Δ- - -Δ $10^{-4}M$



3.19 EFFECT OF ANTIMALARIAL (CQ) ON THE CONTRACTIONS INDUCED BY NORADRENALINE AND POTASSIUM CHLORIDE ON ISOLATED RAT AORTIC STRIP

The aim of these experiments were:

(1) To see the actions of antimalarial on two agonist stimulating different calcium channels. Noradrenaline is known to contract muscle via the receptor linked Ca^{2+} channel, whereas K^+ stimulates muscle through potential or voltage operated calcium channel:

(2) And also to see the effect of actions of chloroquine in initiation and sustained contractions in rat aortic strip.

In these experiments Noradrenaline $10^{-6}M$ as a dose producing about 80% of maximum response, while K^+ (50mM) were selected on similar basis and used. Having obtained contractions in normal Krebs solution for both agents, the muscle was incubated in high dose ($10^{-4}M$) antimalarial as was previously described, while in the other experiments high dose CQ was directly added to the sustained contractions using both agonists in different experiment. It can be seen that the action of high dose antimalarial $10^{-4}M$ were markedly inhibited on NA-receptor linked response, below baseline in most experiments, then in K^+ - induced contractions.

3.19 EFFECT OF ANTIMALARIAL (CQ) ON THE CONTRACTIONS INDUCED BY NORADRENALINE AND POTASSIUM CHLORIDE ON ISOLATED RAT AORTIC STRIP

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(1) To see the actions of antimalarial on two agonist stimulating different calcium channels. Noradrenaline is known to contract muscle via the receptor linked Ca^{2+} channel, whereas K^+ stimulates muscle through potential or voltage operated calcium channel;

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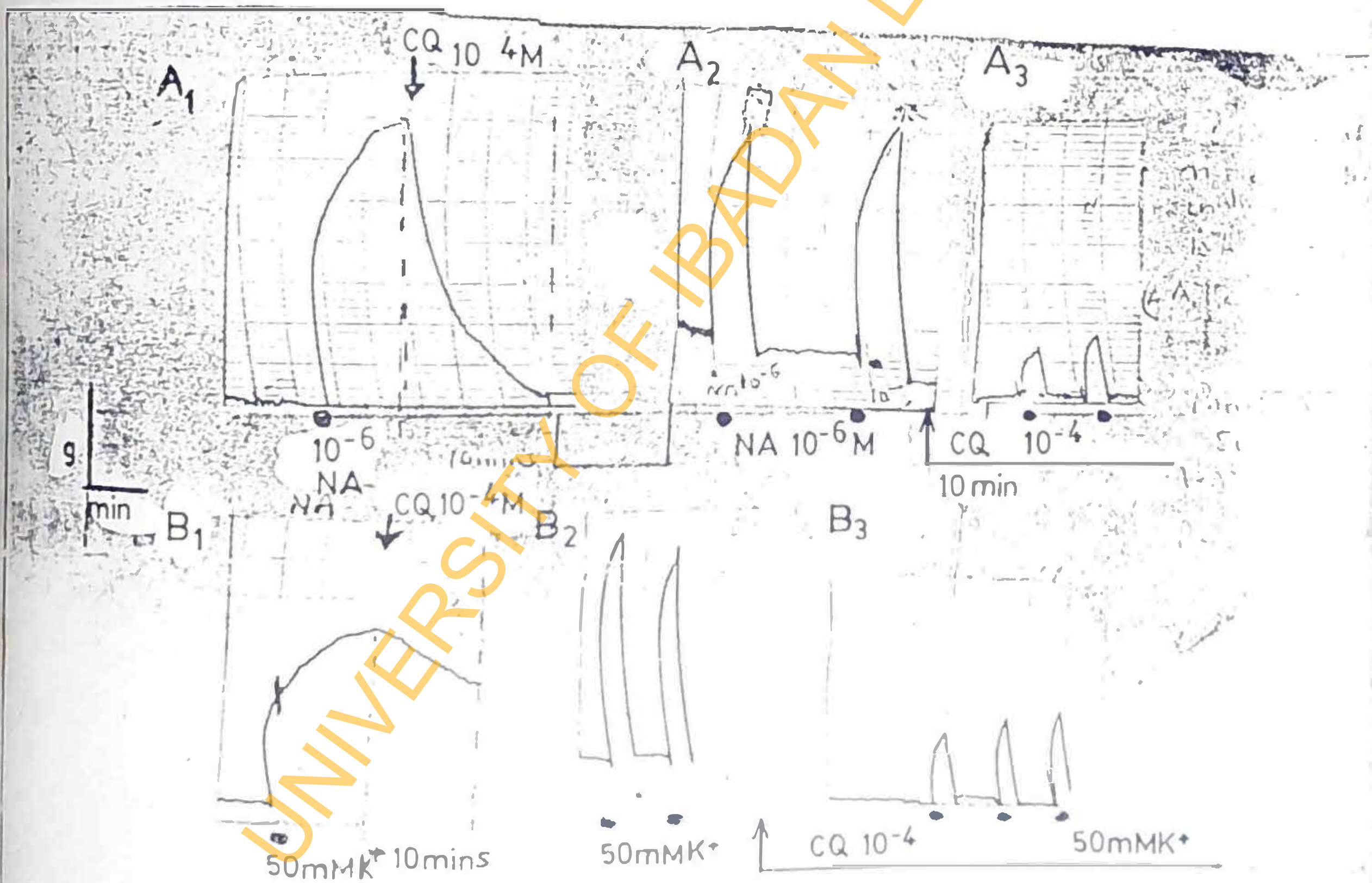
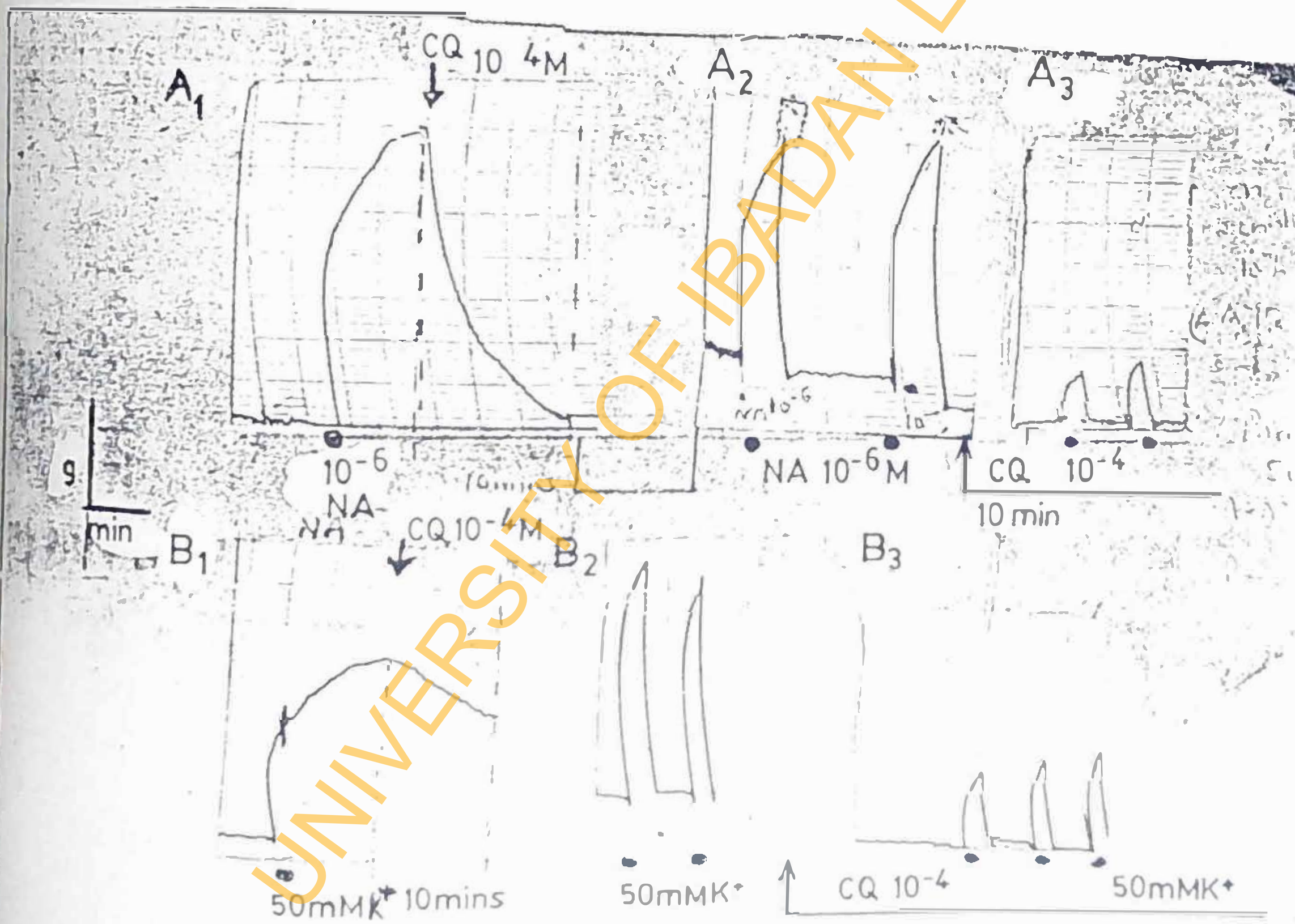
It can also be seen (figure 23 p126) that these effects were more marked on sustained contractions than in the initiation of contraction, particularly on receptor linked responses, as shown in figure .

Figure 23: RAT AORTIC STRIP PREPARATION.

Comparable relaxant effect of 10^{-4}M chloroquine on NA (10^{-6}M) and K^+ (50mM) contraction on both sustained A_1 and B_1 and single doses (NA) A_2 - A_3 and K^+ (B_2 - B_3). The relaxation effect was marked on receptor midiated responses than in K^+ - induced responses as shown in the tracing.





3.20 THE EFFECT OF VARYING THE CONCENTRATION OF Ca^{2+} IN THE KREBS SOLUTION ON THE ACTION OF CHLOROQUINE

The experiment was similar to those already described for the rat stomach strip and the guinea pig ileum. In this experiment, the effects of contractions evoked by NA were studied in Krebs solution containing 1.6mM and 0.4mM calcium, the results are shown in (Figure 24 p 129) It can be seen that CQ enhanced and inhibited NA contraction at 10^{-7}M and 10^{-4}M respectively in 1.6mM Krebs. In Krebs containing 0.4mM Ca^{2+} on the other hand, 10^{-7}M CQ rather than potentiate, inhibited NA contractions, and 10^{-4}M CQ had enhanced inhibition.

Figure 24 RAT AORTIC STRIP

Effect of chloroquine on Noradrenaline evoked contractions of isolated rat aortic strips. Contractions in Krebs solution containing A, 1.6mM Ca^{2+} and B, 0.4mM Ca^{2+} .

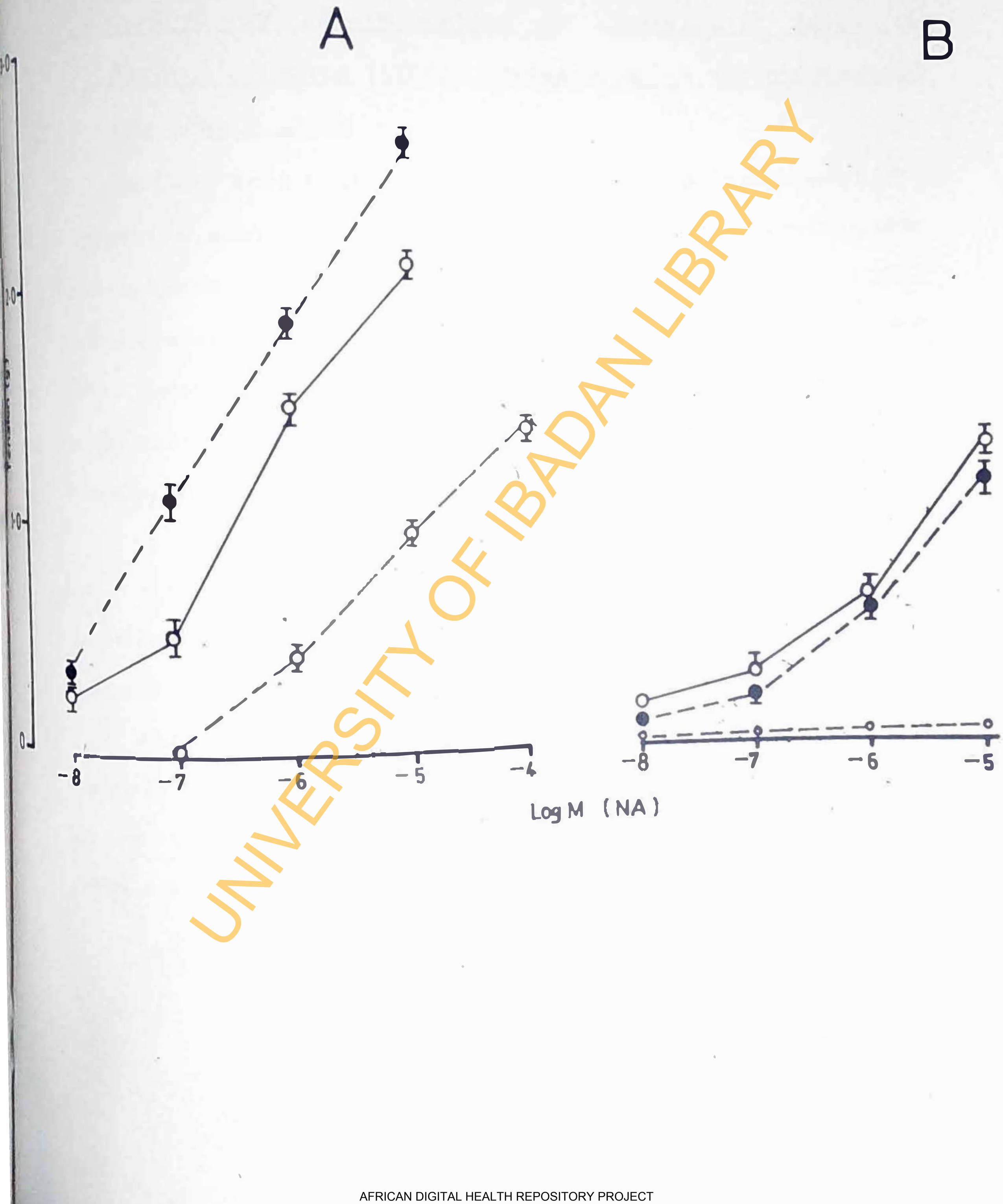
(O——O): Contractions in krebs alone.

Broken lines: Contractions in the presence of chloroquine.

●- - -● 10^{-7}M

O- - -O 10^{-4}M (n=8).

A and B are from separate experiments. Each point is a mean of 3 and 4 measurements in A and B respectively. Vertical bars represent S.E.M. from data generated in each case from one artery preparation. Two other preparations in each case gave similar results.



3.21 EFFECTS OF PRE-INCUBATION OF ANTIMALARIAL DRUGS ON CALCIUM CHLORIDE INDUCED CONTRACTIONS OF THE DEPOLARISED RAT AORTIC STRIP

In Ca^{2+} free high K^+ (55mM) depolarising Krebs solution, cumulative addition of $CaCl_2$ (0.1 - 5mM) caused reproducible contractions dose dependently. The EC_{50} for $CaCl_2$ concentration producing 50% maximum contraction was $3.5\pm0.3\times10^{-3}M$ (n=4). The antimalarials in the concentration range (10^{-6} - $10^{-5}M$) inhibited $CaCl_2$ induced contractions (see Fig 25 pg 132.

It can be seen that $CaCl_2$ concentration response curves were displaced to the right in a non-parallel fashion with depressed maxima in all instances, indicative of non-competitive antagonism.

Mefloquine was the least potent of the antimalarials tested, being almost ineffective at $10^{-6}M$, whereas the other drugs caused marked inhibition of CaCl induced contraction at this concentration.

Figure 25: RAT AORTIC STRIP

Mean log concentration response curves showing the effects of Amodiaquine (A), Mepacrine (B), Mefloquine (C), Quinine (D), on $CaCl_2$ - induced contractile responses in high K^+ depolarising Tyrode solution.

Control ($CaCl_2$ alone)

10^{-6}M

10^{-5}M

Each drug was applied to a different tissue preparation and each symbol is the mean of ± standard error of 4-6 tissues. Vertical bars denote standard error of the mean.

(a)

(b)

(c)

(d)

% Maximum Response

100
80
60
40
20

−4 −3 −2

Log [M] Ca^{2+}

3.22 EFFECT OF ANTIMALARIAL DRUG ON AGONIST CONTRACTIONS IN Ca^{2+} FREE KREBS SOLUTION

The procedure used in this experiment was similar to the one described for rat stomach strip. After establishing a dose response relationship, a submaximal dose of agonist was obtained. Low dose antimalarial did not potentiate agonist effect in Ca^{2+} free Krebs but high dose antimalarial inhibited agonist effect.

Figure 26 ISOLATED RAT AORTIC STRIP

Effects of chloroquine on Ca^{2+} cumulative induced contractions in potassium (55mM) depolarising krebs solution.

(A) Contractions in krebs alone

(B) in 10^{-7}M CQ

(C) in 10^{-4}M CQ

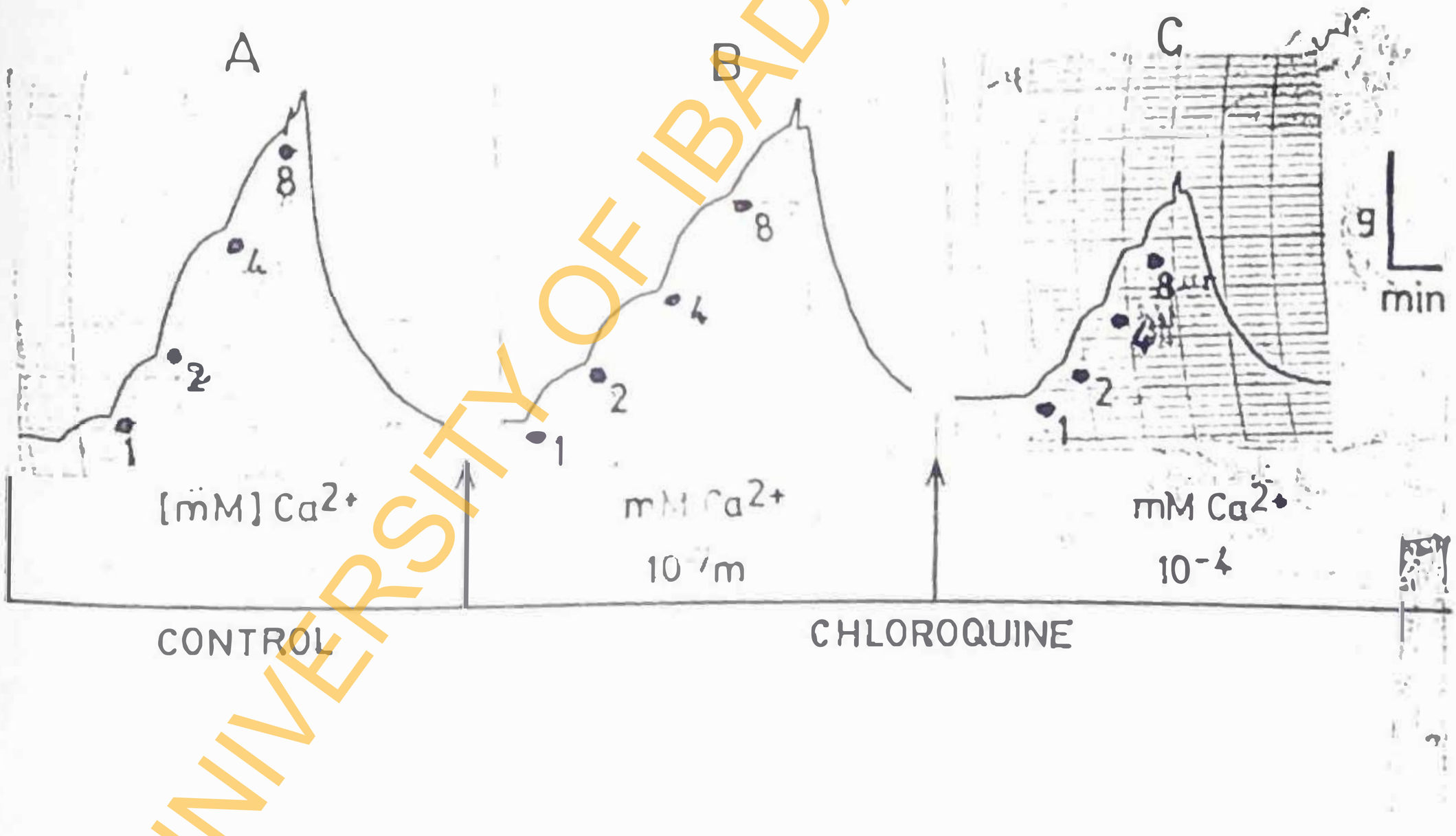
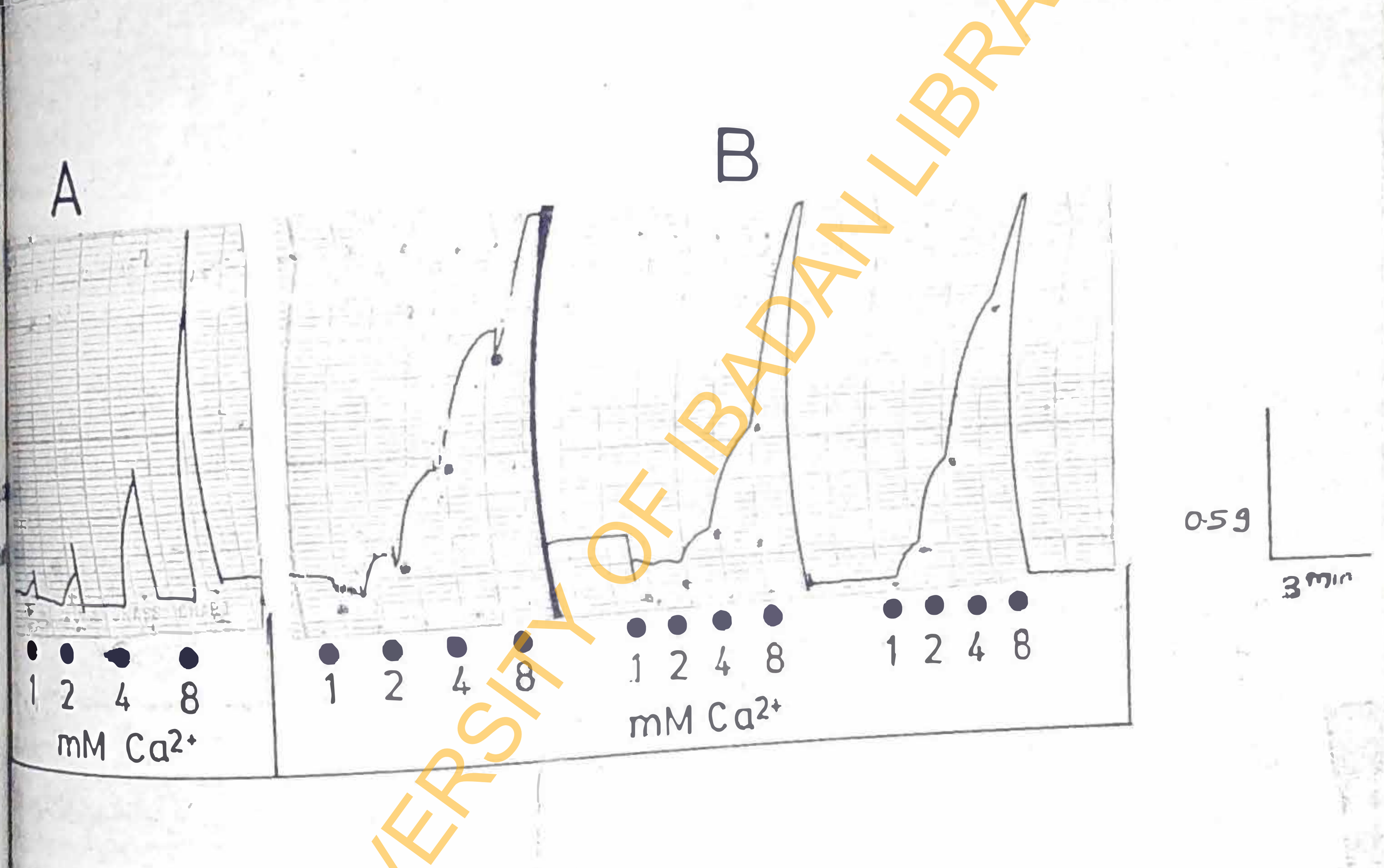


Figure 26a: RAT AORTIC STRIP PREPARATION.

Representative tracings of dose dependent contraction evoked by calcium chloride in potassium (55mM) depolarising krebs medium.

A = single dose responses; B = cumulative DRC.



3.23 EFFECT OF ANTIMALARIAL DRUGS, DILTIAZEM ON PRE-CONTRACTED AORTIC STRIP

Pre-contracted arterial preparations are commonly used to study effect of vasodilators (Kodja et. al., 1992). From earlier experiments, it was found that 10-6M NA gave a good size submaximal contraction (70-80% of the maximum response). This dose was therefore used to precontract the aorta in all experiments in this series. The contraction induced with 60mM KCl were near maximum contraction. In other instances 15mM KCl was used to precontract the vessel (see Yu et. al., 1992).

The aortic strip was allowed to equilibrate for one hour and was then pre-contracted with either agent. Five minutes after the tissue had attained peak contraction, either antimalarial or Diltiazem was added cummulatively. The dilatory effect of the drug was plotted as reversal of the peak contraction where:

$$\% \text{ reversal} = \frac{\text{dilator response}}{\text{peak response}} \times 100$$

The result fo CQ, Quinine and Diltiazem are presented in (figures 27 pg 140). It can be seen that in this model, CQ and Quinine were far more potent than diltiazem. It is noteworthy that CQ consistently relaxed the pre-contracted vessel below its initial baseline.

Figure 27a: RAT AORTIC STRIP PREPARATION.

Dose response curve of relaxation to

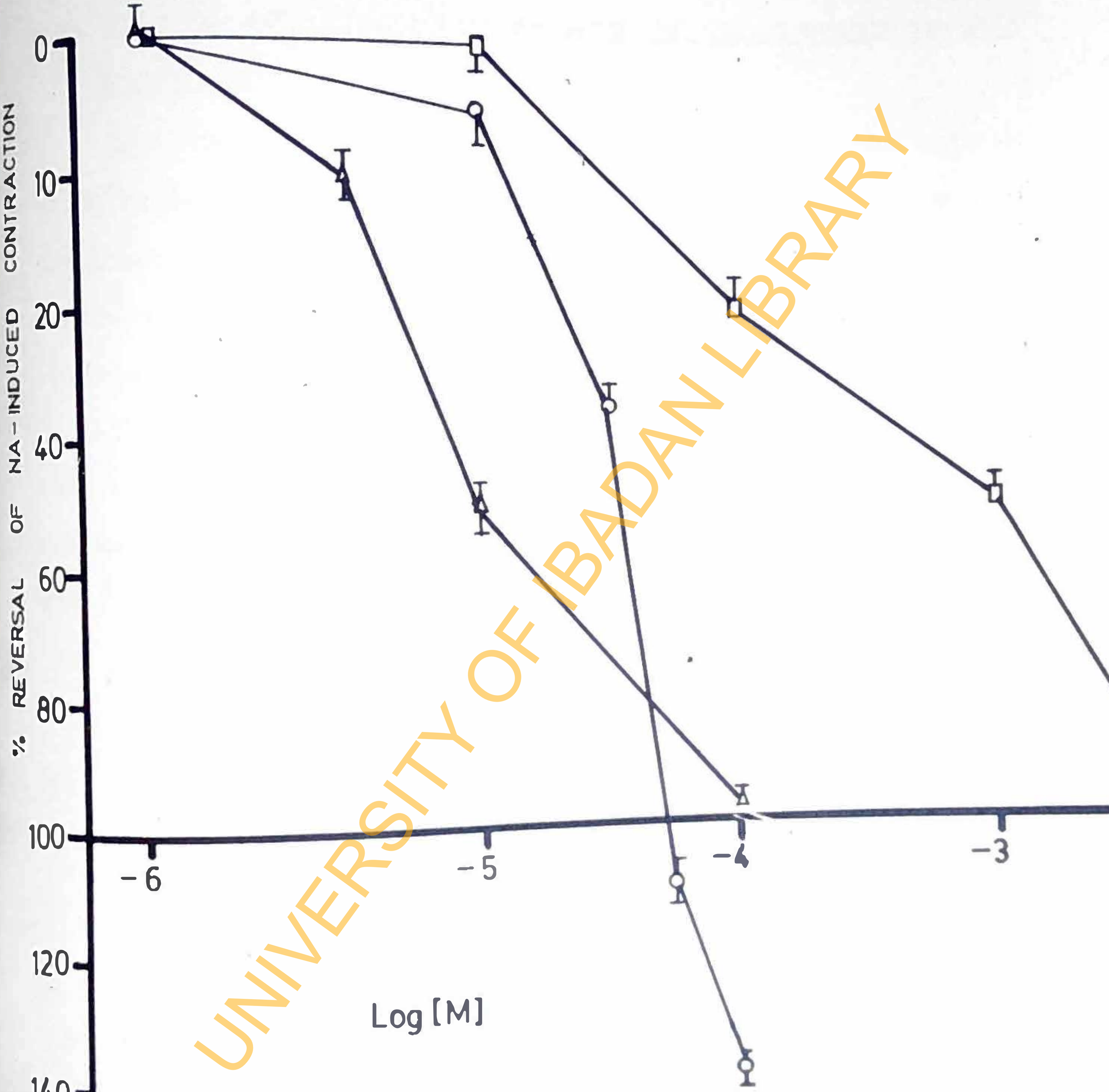
Chloroquine ()——(),

Diltiazem ▯——▯,

Quinine ▲——▲ in NA precontracted rat aortic strip.

Bars represent S.E.M. of 6 experiments.

It can be seen from this graph that CQ, Quinine, were far more potent than Diltiazem. CQ relaxed the precontracted vessel below its initial baseline.



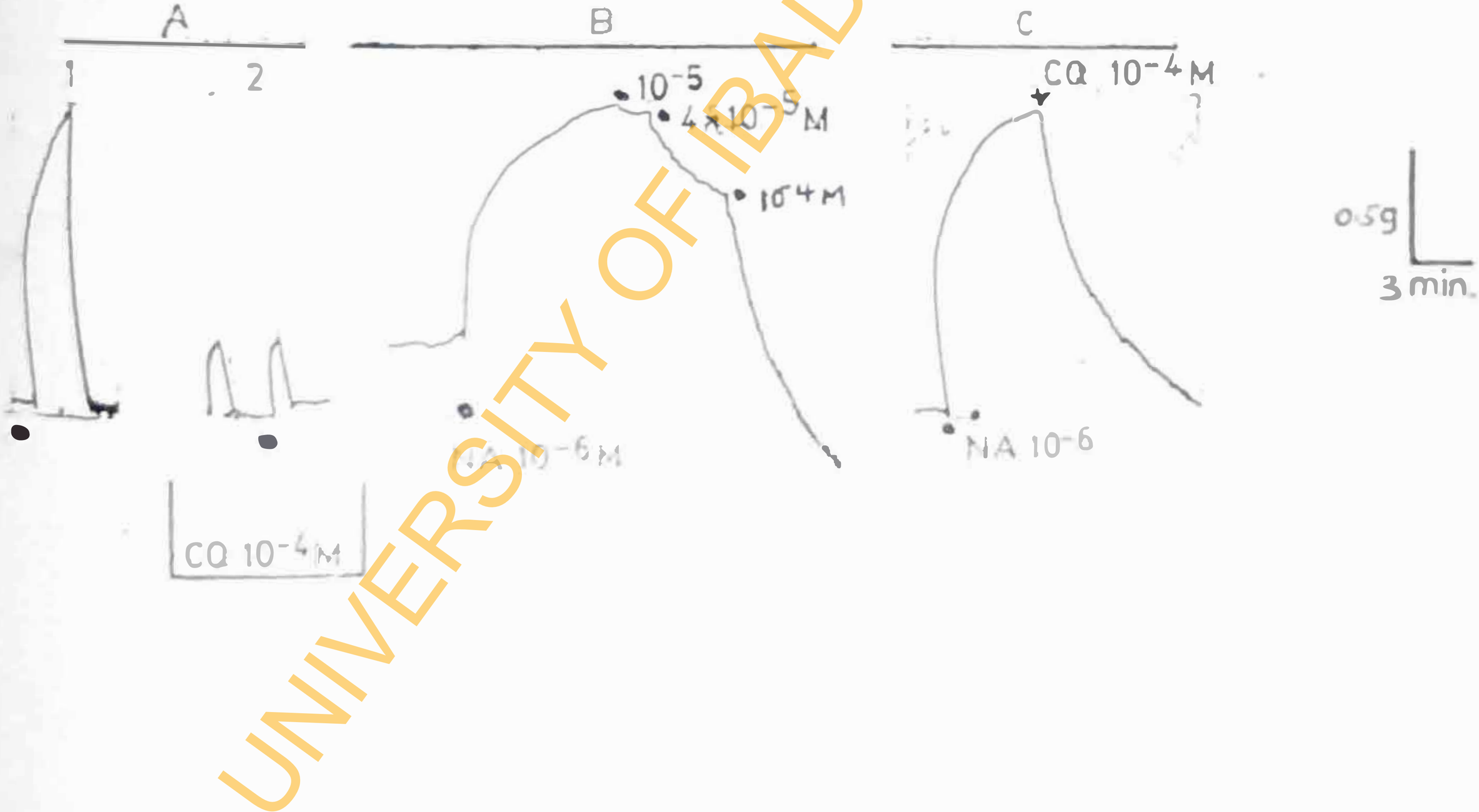
3.24 DILATOR AND INHIBITORY EFFECTS OF CHLOROQUINE IN RAT AORTIC STRIP

In this experiment, dilator effect refers to the action of CQ in dilating the NA-precontracted arterial muscle, while inhibitory effects refers to the action of CQ in reducing NA-contraction after incubating the muscle in physiological krebs solution containing antimalarial for thirty minutes.

The 'Dilator' effect of 10^{-4}M CQ was distinctly greater than inhibitory effect i.e., 10^{-4}M CQ completely relax precontracted vessel whereas the same concentration of CQ preincubated only reduced contraction to NA by about 70% (see fig 27b pg 143)

Figure 27b AORTIC STRIP PREPARATION

Effect of chloroquine on the contraction iduced by Noradrenaline (10^{-6}M); Panel A; contraction before A_1 and after A_2 incubation in chloroquine for 30 minutes. Panel B and C; direct cummulative and single dose relaxation response. The direct relaxatory effect of 10^{-4}M CQ was distinctly, a greater effect than preincubated action of CQ of the same concentration.



3.25 EFFECT OF METHYLENE BLUE (10^{-3}M) ON CQ RELAXATION OF THE RAT AORTIC STRIP ON NORADRENALINE 10^{-6}M SUSTAINED CONTRACTION.

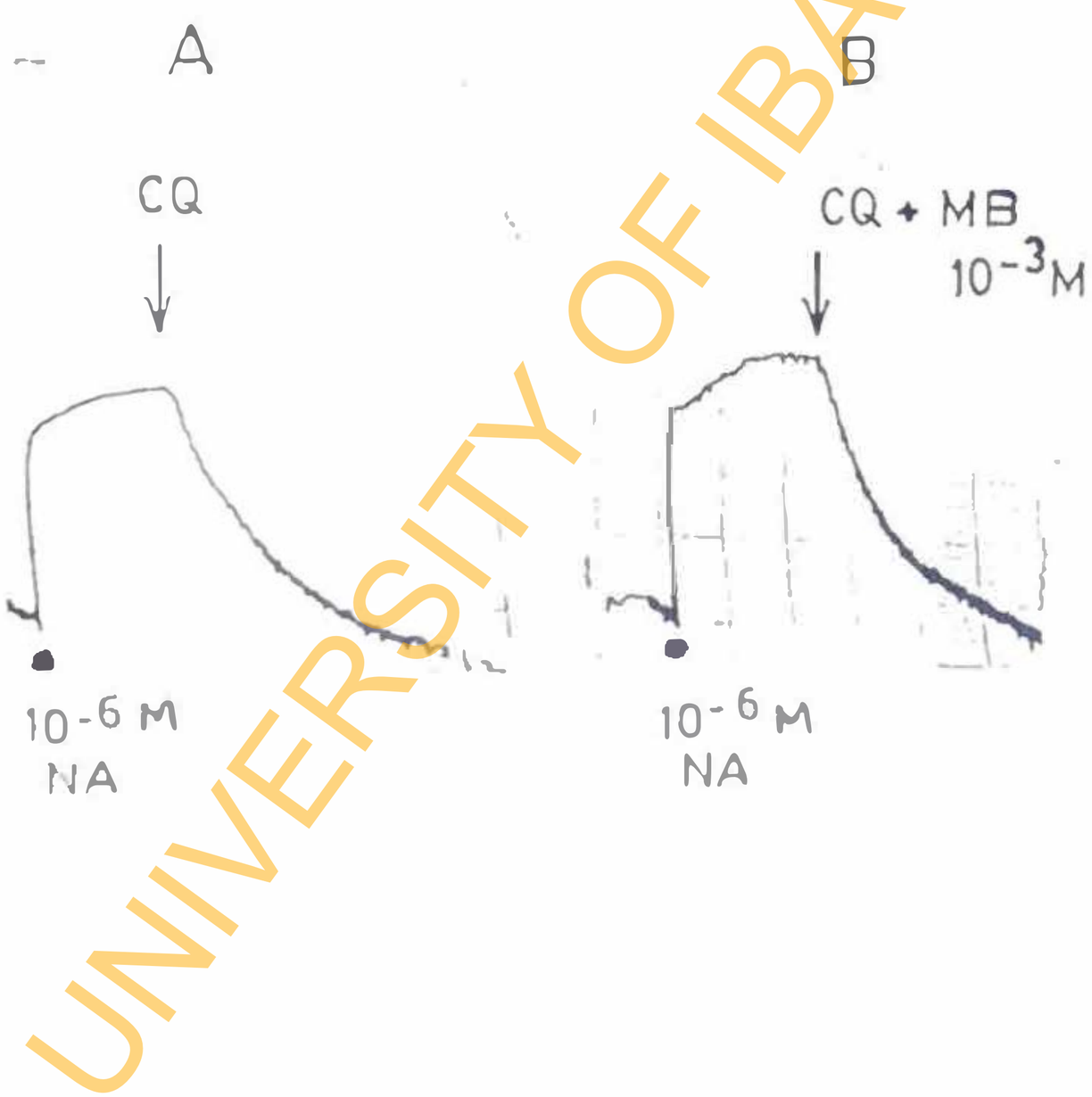
The aim of this experiment was to investigate the mechanism of the relaxant effect of CQ in order to compare it with the classical Vasodilator (Sodium Nitroprusside), whose vasodilatory effect on vascular smooth muscle is attenuated by methylene blue 10^{-5}M (see Karaki et. al., 1988). In this study after obtaining a sustained contraction with NA 10^{-6}M in normal Kreb's solution, the Kreb's solution was replaced with Kreb's solution containing methylene blue 10^{-3}M and left for one hour. Thereafter the tissue was again contracted with NA, 10^{-6}M, the sustained contraction was then challenged with high dose CQ 10^{-4}M as previously done.

The result of this study as presented in figure shows that the sustained aortic contractile response was unaffected by methylene blue 10^{-3}M; suggesting that the mechanism of the relaxant effect or action of CQ in rat aortic strip was unlike those of Sodium Nitroprusside or Ca^{2+} channel blockers.

Figure 27c Rat Aortic Strip

Effect of 10^{-3}M Methylene blue preincubation of rat isolated aortic strip. Methylene blue did not modify the contraction induced by 10^{-7}M Noradrenaline as shown in (figure).

Panel A shows the action of CQ 10^{-4}M; and B action of CQ and Methylene blue on sustained contraction rat aorta.



3.26 DOES THE RELAXATION OF AORTIC STRIP MUSCLE BY CQ INVOLVE OPENING OF K^+ CHANNEL?

In vascular tissues cromakalim a potassium channel opener (Cook et. al., 1988), inhibits contraction elicited by low concentration of K^+ (15mM) but is ineffective against high concentration of potassium K^+ (60mM) (Yu et. al., 1992). In the following experiments, different aortic strips were contracted with 15mM and 60mM concentrations of K^+ and the relaxant effects of CQ determined. The Ca^{2+} channel blocker diltiazem was also investigated. It can be seen that CQ relaxed the aortic strip contracted by either 15mM or 60mM K^+ but the relaxant effect was greater in tissues contracted with 15mM K^+ which again suggested that K^+ channel opening is not an adequate explanation for this action in Rat aortic strip (see figure 28 pg 149-151)

Figure 28a Rat Aortic Strip

Effect of chloroquine reversal of K^+ induced contraction of rat aortic strip.

In this figure, two different K^+ 15mM and 60mM were used to induce contraction. Contractions induced by K^+ 60mM was relaxed up to 78%; while contraction induced by K^+ 15mM was relaxed below baseline more than 160%. Bars represent S.E.M. of 4 experiments.

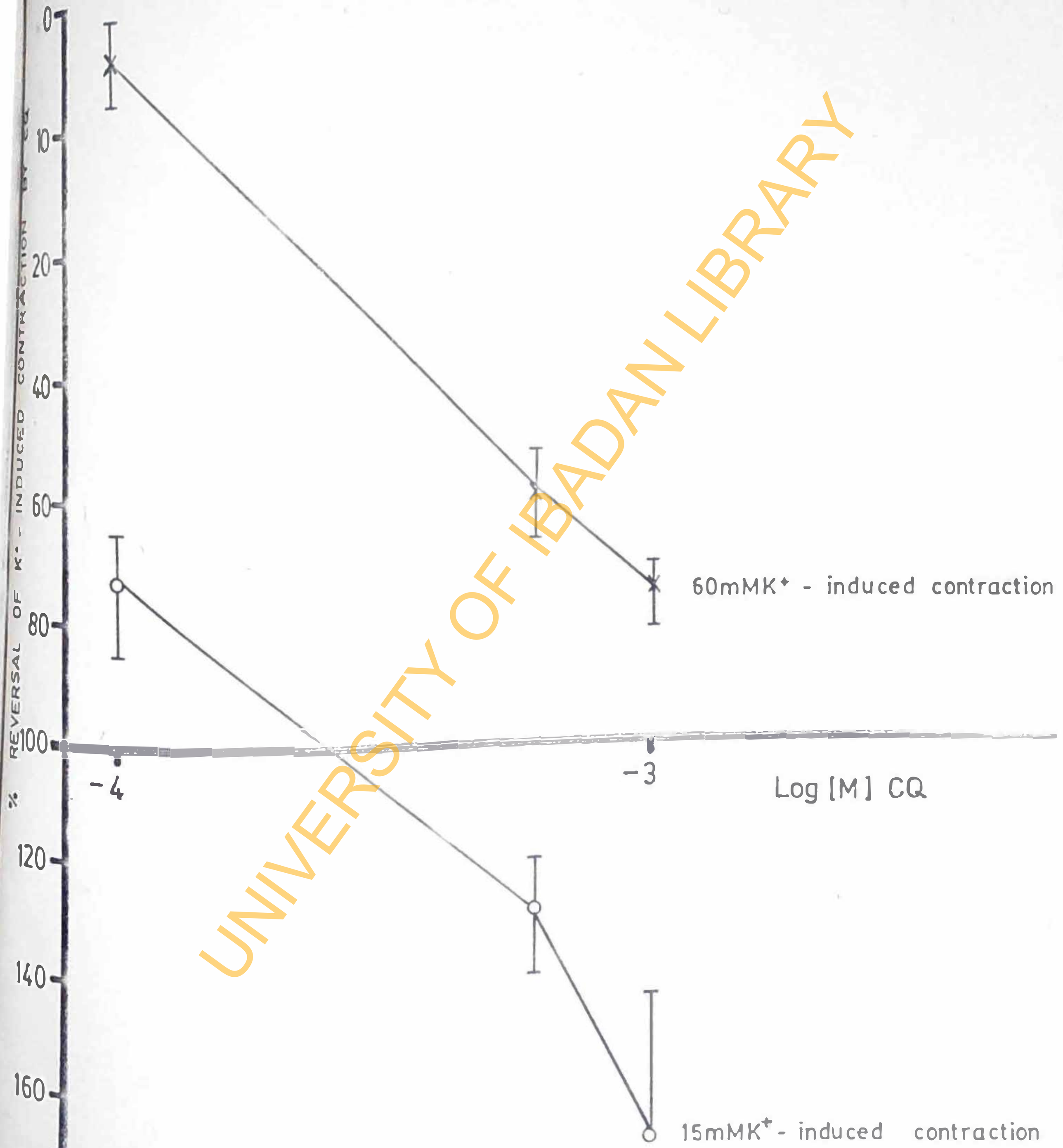
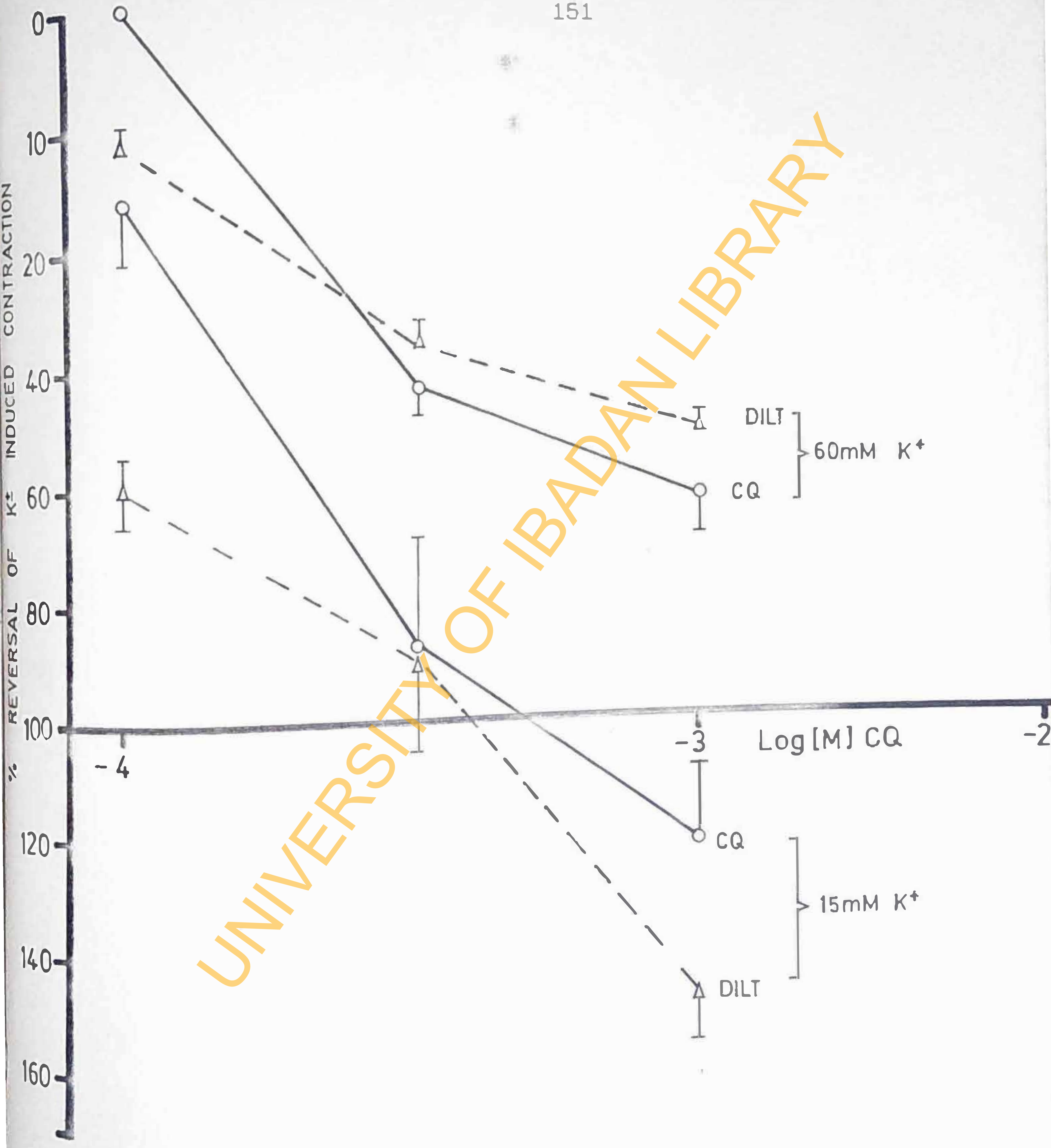


Figure 28b Rat Aortic Strip

Effect of chloroquine, Diltiazem reversal of K^+ induced contraction of rat aortic strip. In this figure, 60mM K^+ relaxant effect on Diltiazem Δ- - - -Δ was > 40%; while CQ O——O relaxant effect was > 50%;

In the experiment with 15mM K^+, the relaxant effect of CQ O——-O was about 120%, while the relaxant effect of Diltiazem Δ------Δ was > 140%. The relaxant effect was more marked on contractions induced by 15mM K^+ than 60mMK^+ in both drugs.



CHAPTER FOUR

DISCUSSION

There have been several reports in the literature of the actions of chloroquine and structurally related compounds on smooth muscles contractions. While some results suggest that the effect of chloroquine on contractions induced by agonists such as Histamine, 5-Hydroxytryptamine (5-HT), Acetylcholine is non-specific but a direct spamolytic action of chloroquine, (Olatunde, 1970), others reported a specific action on Histamine, H_1-recptors (Akubue, 1975). These conflicting reports were observed in gut smooth muscle. There have also been reports of the actions of chloroquine on vascular muscles where it was observed that both a potentiation and inhibition of NA induced contractions at low and high concentrations of chloroquine repectively (Ebeigble, 1982) could be observed. It would therefore appear that the effect of chloroquine on agonists contraction may depend not only on the dose of antimalarial but also on the type of smooth muscle as well as the agonist used.

In this investigation three types of smooth muscles and agonists acting on different types of recptors have been used. The choice of smooth muscle was guided by the results of preliminary experiment where it was found, the effect of removal of Ca^{2+} from the physiological solution bathing the

muscle on the latter's contractility varied between muscle types. In the guinea pig ileum responses to Histamine and Acetylcholine rapidly disappeared (Fig 14a) whereas substantial contractions could be obtained to Acetylcholine in the rat stomach strip (Fig. 14) and to Noradrenaline in the rat aortic strip. It is generally accepted that the contractions of smooth muscle involves Ca^{2+} (Somlyo and Somlyo, 1968; Castell, 1976; Bolton, 1979; Ye et. al., 1992). The differences in response after Ca^{2+} withdrawal would imply that sources of Ca^{2+} for activating the contractile mechanism are similar in rat stomach strip and rat aortic strip but different from those in guinea pig ileum (see also Offiah, 1981). These three muscles were therefore chosen as it seemed from the outset that Ca^{2+} might be involved in the mechanism of action of chloroquine. The agonists used in these studies included Histamine, Acetylcholine, Noradrenaline and Potassium in order to ascertain the specificity or not of the action of chloroquine. Other antimalarial drugs structurally related to chloroquine were included. The aim was to determine whether a pattern of structure-action relationship could be assigned in the action of these drugs. Irrespective of the agonist used to induce contraction. chloroquine had similar excitatory or inhibitory action. It was concluded that the action of

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chloroquine is not receptor specific. The role of calcium was investigated since this cation is a common factor in contractions mediated by different agonists. It is now generally accepted that contractions induced by various agonists involves Ca^{2+} either entering the cell from extracellular stores (Hudgins & Weiss, 1968) or mobilised from intracellular stores (Hinkle, 1965). Intracellular free Ca^{2+} and its regulation are crucial determinants of smooth muscle contractility. The sarcoplasmic reticulum has been identified as the primary intracellular organelle which modulate or buffer Ca^{2+} release (Van Breemen et. al., 1988; Van Breeman & Saida, 1989; Wagner-Mann et. al., 1991).

EFFECT OF DOSES OF CQ AND OTHER ANTIMALARIALS ON CONTRACTILE RESPONSE

Various concentrations of chloroquine and other antimalarials were tested on different agonists induced contraction separately on the three muscles. Irrespective of agonists used to induce contraction, low concentration (10^{-12} - 10^{-7}M) and high concentration (>10^{-6}M) of antimalarials caused potentiation and inhibition respectively, except Halofantrine and Mefloquine where neither effect was distinct (see Fig5.8). Antimalarial potentiation does not appear to be

due to interaction with recptors or with enzymes responsible for the inactivation of the agonists. The evidence for this conclusion is that Histamine, Acetylcholine and Noradrenaline which activate distinctly different receptors and whose inactivating enzyme i.e., acetylcholinesterase and diamine oxidases respectively are quite different, were potentiated to the same extent, and KCl which induces contraction by depolarisation and is not inactivated by any known mechanism was also potentiated.

On the other hand, high concentration of antimalarial inhibited responses to Acetylcholine, Histamine and Noradrenaline; the mechanism of this inhibition is also not receptor specific but likely to be inhibition of utilization of intracellular Ca^{2+}. This inhibitory effect of antimalarial was more marked on receptor mediated responses than K^{+} induced contractions (see Fig 6, p58). it was concluded that CQ exerted a greater effect on receptor-operated than on potential operated calcium channel. The present observation on the dual actions of antimalarials support previous findings of potentiating action of antimalarials in gut as well as vascular smooth muscles, for example, chloroquine in rat Portal vein (Ebeigbe and Aloamaka, 1982); Amodiaquine,

Chloroquine and Mepacrine in guinea pig ileum (Okpako, 1978); Qunine in rat rectal muscle (Savage and Lawal, 1986).

Various studies have shown only inhibitory effects of aminoquinolines antimalarials (Olatunde, 1970; Akubue, 1975; Famaey, 1979; Ebeigbe, 1986). One possible explanation of the greater effect of chloroquine on receptor mediated contraction is that chloroquine at high concentrations blocked Histamine, Acetylcholine and Noradrenaline receptors. However, the characteristics of the inhibition did not suggest receptor blockade. Furthermore, chloroquine at high concentrations has been shown to block a wide range of agonists contraction, for example, PGEs (Okpako, 1978); NA (Ebeigbe, 1982); 5-HT (Olatunde, 1970); electrically induced contractions (Famaey et. al., 1979). It, therefore, seems unlikely that specific receptor blockade can explain the greater inhibitory effect of chloroquine on Histamine, Acetylcholine, Noradrenaline. It seems likely that the inhibitory effect of chloroquine is exerted at a post-receptor site. Previous studies have suggested that the inhibitory effect of aminoquinoline antimalarial on muscle function may be due to interaction with cellular Ca^{2+}. Huddat and Saad (1977) attributed the inhibitory effect of quinine in rat ileum to inhibition of Ca^{2+} influx and Ebeigble et. al. (1982) in vascular tissue.

EFFECT OF VARYING THE CONCENTRATION OF Ca^{2+} IN PHYSIOLOGICAL SALT-SOLUTION ON THE ACTION OF ANTIMALARIAL

In view of the importance of the role of calcium in smooth muscle contraction (Bohr, 1967) coupled with the findings in the present study; it was reasoned that if these effects involved extracellular Ca^{2+} then changing the concentration of Ca^{2+} in the external medium bathing the isolated muscle should affect the degree of potentiation and inhibitions caused by antimalarials under this experimental condition. As the external Ca^{2+} concentration in the medium was lowered from 1.8mM Ca^{2+} to 0.9mM and 0.45mM Ca^{2+}, it was indeed found that the potentiating effect of low concentration of antimalarial decreased in proportion to the decrease in the concentration of Ca^{2+} in the bathing fluid (Fig10-13). In low concentration of chloroquine (10^{-7}M) the effect of Acetylcholine in rat stomach strip was potentiated almost two-fold (PF 0.64±0.3, n=12), when the Ca^{2+} concentration in the external medium was 1.8mM Ca^{2+}. When the Ca^{2+} concentration in external medium was lowered to 0.45mM Ca^{2+}, this dose of chloroquine (10^{-7}M) no longer potentiated acetylcholine contraction but rather a slight inhibition was observed (PF 1.25±0.5, n=12). On the other hand, the inhibitory effect of

high dose chloroquine 10^{-4}M had greater inhibitory effect at 0.45mM Ca^{2+} than 1.8mM Ca^{2+} with acetylcholine as agonist.

It is accepted that contractile agonists utilize Ca^{2+} from two main sources in excitation contraction coupling. These are Ca^{2+} in the external medium i.e., Ca^{2+} in close association with the cell membrane (for review see Bolton, 1979), Brading and Sneddon (1980) and Ca^{2+} from intracellular store (Hinkle, 1965). Against this background one possible explanation of the observed effect of low dose chloroquine is facilitation of Ca^{2+} entry following excitation whereas the inhibition of contractions at higher doses of chloroquine is due to interference with translocation of Ca^{2+} across the membrane. That is, the two effects excitatory and inhibitory are exerted at the same site. There is also the possibility of CQ interfering with mobilisation of intracellular store (Hinkle 1965, Hurwitz & Suria 1971, Bolton 1979, Hurwitz 1975), or other intracellular contractile mechanisms.

The experiments in calcium free PSS containing 0.5mM EGTA were designed to test for the probable sites of action of chloroquine. The results showed that in nominally Ca^{2+} free PSS containing 0.5mM EGTA, acetylcholine could induce successively diminishing contractions of the rat stomach strip which finally stabilised at about 40% of the contraction in

normal Physiological salt solution. This remaining response was referred to as the residual response; NA had similar effects on the rat aortic strip. Under similar conditions the response of the guinea pig ileum to acetylcholine rapidly extinguished and withing 10 minutes of exposure in this medium, no residual response could be recorded. Our result in this respect are similar to those of Offiah (1981); Brading and Sneddon (1980); Okpako and Oladitan (1979). The rapid loss of contractile response in guinea pig ileum in Ca^{2+} free medium may indicate that intracellular Ca^{2+} store in this muscle is limited possibly as a consequence of poor development of Ca^{2+} retaining organelles sarcoplasmic reticulum and mitochondria or may mean the absence of a large store of sequestered Ca^{2+} which can be mobilised by Histamine whereas the response in the rat stomach strip and rat aortic strip may be attributed to a properly developed organelles suggestive of greater intracellular Ca^{2+} holding capacity in these muscles or the sources of activator calcium are different in the different muscles.

Low dose chloroquine which consistently potentiated acetylcholine in normal physiological salt solution had no effect on the residual response in rat stomach strip or rat aortic strip. The residual response is presumed to be due, at

least partly to Ca^{2+} mobilised from intracellular sources (Heaslip and Rahwan, 1982). The failure of low dose chloroquine to potentiate the residual response suggests that the potentiation seen in normal physiological salt solution must be due to facilitation of calcium influx. On the other hand, the residual response was completely abolished by high dose chloroquine. This effect reflects the action of chloroquine in low calcium media (0.45mM) where CQ ($10^{-4}M$) also completely abolishes agonist contraction (see Fig17p108). This result indicates that the inhibitory effects of high dose chloroquine is exerted intracellularly. It could be due to inhibition of any of the events following receptor occupation for example activation of protein kinase C, inositol hydrolysis (Rasmussen et. al., 1987) mobilisation of calcium from intracellular store (Hinkle, 1965). It is noteworthy that in normal Ca^{2+} containing physiological salt solution, chloroquine ($10^{-4}M$) does not produce a complete inhibition of Ach and NA contractions of rat stomach strip and rat aortic strip as can be seen in Fig4:21), whereas in Ca^{2+} free EGTA solution this concentration of chloroquine abolishes the residual response; this observation may be taken to mean that the predominant effect of chloroquine on Ca^{2+} translocation is facilitation, whereas the effect of high dose chloroquine

exerted at an intracellular site is inhibitory. The lesser inhibitory effect of high dose chloroquine in Ca^{2+} containing solution than in Ca^{2+} free EGTA solution is then explained by a counteracting influence of external Ca^{2+}. From the above results it may be concluded that chloroquine in gut and vascular smooth muscles interacts with contractile agents at two sites, one site being the cell membrane where low dose chloroquine causes potentiation probably by facilitating agonist-induced Ca^{2+} translocation. The other site is intracellular where high dose chloroquine inhibits agonist contraction by interference with intracellular mechanisms. This implies that the action of chloroquine on muscle contraction would depend on the concentration of calcium in the bathing physiological solution.

EXPERIMENT IN HIGH DEPOLARISING K+ MEDIUM

Contractile responses of smooth muscle to calcium pre-incubated in a Ca^{2+} free bathing medium and exposed to a depolarising concentration of KCl are often used to identify compounds possessing calcium channel antagonistic properties (Spedding and Cavero, 1984; Godfraind et al., 1986).

In the present experiments high concentration of $CaCl_2$ produced a concentration-related response of the rat aortic

strip preparation. Low dose antimalarial did not show a marked potentiation as can be seen in Fig 26 p135 neither did high dose cause marked inhibition. This was similar to the result obtained when K^+ was used as agonist.

Contractile responses evoked by $CaCl_2$ in depolarising PSS have been proposed to occur as a result of Ca^{2+} entry from the PSS through a Ca^{2+} pathway (Ca^{2+} channels) in smooth muscle membrane, the so-called voltage dependent Ca^{2+} channels (Bolton, 1979; Van Breemen et al., 1979; Meisheri, 1981; Spedding, 1982; Cauvin et al., 1984). The fact that contractions evoked in high Ca^{2+} was not markedly blocked by high dose chloroquine suggest that the antimalarial might not be involved in Ca^{2+} entry through the VOC or POC, while the classical Ca^{2+} antagonist are known to block Ca^{2+} entry via the POC; this finding corroborate previous result with K^+ in this study. Similarly high doses of chloroquine > 10^{-6}M did not markedly block K^+ and Ca^{2+}; this again exclude the possibility of CQ interaction with POC. However, Savage (1987); in comparing the Ca^{2+} blocking activities of four antimalarials (CQ, AMDQ, Quinine, MFQ) with Nifedipine a classical Ca^{2+} blocker (Fleckenstein, 1977; Spedding, 1982) in rat ileum found that AMDQ the most potent and mefloquine the

least potent of the antimalarials showed similar calcium blocking properties at high dose > 10^{-5}M; while Nifedipine in lower doses (10^{-11} - 10^{-9}M) effectively blocked Ca^{2+}. He found that Nifedipine a known Ca^{2+} entry blocker was at least 100,000 times more potent than Amodiaquine on molar basis. The antimalarial concentration used in these studies were similar.

On the basis of molarity, the four antimalarials used did not possess a specific Ca^{2+} channel blockade. The Ca^{2+} and antimalarial interaction was non-competitive, ruling out the possibility of a receptor effect. The concentration response curves where shifted to the right with depressed maxima (see Fig25p139. The result in the present study does not show the action of antimalarial (on maolar basis) when compared to the classical Ca^{2+} blocker (Nifedipine) as a potent or specific Ca^{2+} channel blocker.

STUDIES IN VASCULAR SMOOTH MUSCLE

Over the years, it has been realised that vascular endothelium has an important influence on blood vessel tone via release of endothelium derived relaxing factor (EDRF). Thus in many blood vessels the response of a variety of agonists is dependent on the integrity of the endothelium

(Furchgott, 1983). In the result discussed here, the muscles used were endothelium free. In these studies, an attempt was made to compare the results from the gut smooth muscle with those of the vascular muscle with a view to elucidate the mechanism of action of chloroquine and possibly the cardiotoxic effect associated with chloroquine in malaria therapy.

Contractions of vascular muscle may be initiated by elevating the concentration of free intracellular stores of Ca^{2+} or by influx of Ca^{2+} from the extracellular environment through voltage operated channels activated by agonist receptor combination (Weiss, 1985). Electrophysiological studies have shown that the voltage operated calcium channel can be sub-divided into three types (T, N and L) on the basis of their gating properties and pharmacological sensitivities (Nowycky et al., 1985; McCleskey et al., 1987). The three types of channels differ in their tissue distribution and pharmacological properties (Tsien et al., 1988).

The T-type channel is characterised by a transient time course due to rapid inactivation and a sensitivity to blockade by nickel ions. The L-type or slow calcium channel is slowly inactivated and is the only type sensitive to the dihydropyridines. Another rapidly inactivated channel is the

N-type which in contrast to the T-channel is sensitive to contoxin from Conus geographus. Ca^{2+} influx through N-type channels is more sensitive to Ca^{2+} than to any other divalent cation (Nachshen, 1984).

The evidence for multiple channels has been elucidated in detail by Tsien et. al. (1987). In vascular smooth muscle cells however only two types of Ca^{2+} channels have been identified; an L-type channel and a rapidly inactivated dihydropyridine-resistant channel possibly T-type (Loirand et. al., 1976; Aaronson et. al., 1986; Bean et. al., 1986). The contractile response of rat aorta is not due to an influx of Ca^{2+} ions through L-type VOC. as dihydropyridine failed to modify these response (Cavero and Lawson, 1987).

In the rat isolated aorta, contractions induced by high concentration of Noradrenaline (NA) and high K^{+} are biphasic; the early fast component of contractions depends upon release of intracellular Ca^{2+} while the slowly developing sustained phase of contraction depends upon influx of extracellular Ca^{2+} (Yamashsita et. al., 1977). In Ca^{2+} free conditions a transient contraction may be induced in the rat aorta by NA which is thought to be mediated by intracellular Ca^{2+} (Heaslip and Rahwan, 1982). KCl has been shown to produce a biphasic contracion in the rat aorta (Nghiem et. al., 1982) with a less

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definite separation between the fast and slow components of the KCl induced contraction both depending exclusively upon extracellular Ca^{2+} (Yamashita et. al., 1977; Heaslip and Rahwan, 1982).

Results of the effect of CQ on NA and K^+ induced contraction of the rat aortic strip in the present series of experiments were similar to those of the gut smooth muscle as previously discussed. That is, the predominant effect of doses of CQ of the order of $10^{-7}M$ was a potentiation, but doses $>10^{-5}M$ produced inhibition. This observation is similar to those previously reported in guinea pig atria (Marshal and Ajewole, 1978; Makinde, 1977) where it was suggested that the quinoline drugs (chloroquine, quinine and primaquine) produced positive chronotropic effect. The mechanism of chloroquine-induced potentiation of spontaneous contraction of rat portal vein (Ebeigbe and Aloamaka, 1982) was explained as being due to the release of NA since phentolamine pretreatment prevented this response. The phenomenon of potentiation in contractile responses of vascular smooth muscle to biogenic amines has been attributed to

(i) inhibition of enzymatic degradation of amine (Kalsner, 1969a; Levin and Furchgott, 1970);

(ii) inhibition of amine uptake (Kalsner and Nickerson, 1969a; Kalsner and Frew, 1972) or interference with the rebinding of calcium released into the environment of the contractile element during contraction (Kalsner, 1970c).

The finding in both gut and vascular smooth muscles that low CQ potentiated agonist action involving histamine, acetylcholine and noradrenaline (NA) does not suggest inhibition of enzymatic degradation of amine uptake as a mechanism of action of CQ in this instance. On the other hand, the observation that a variation in the concentration of Ca^{2+} in the bathing fluid had a profound effect on the extent of potentiation is strongly suggestive of a role for Ca^{2+} in this phenomenon. Furthermore, the observation that NA-mediated contractions were more potentiated than K^{+} mediated contractions suggests that the effect of CQ is greater on ROC than on VOC; in doses > 10^{-6}M, inhibitory effect was again more marked in receptor mediated responses than K^{+} mediated responses; this suggests that the inhibitory effect of chloroquine might not involve the opening of calcium operated channel through VOC. The mechanisms by which a drug may inhibit vascular muscle contraction may include the following; membrane hyperpolarisation (Hausler and Thorens, 1975); inhibition of Ca^{2+} entry (Kreye et al., 1975; Zsoter et al.,

1977; Thorens and Haeusler, 1979; Karaki et. al., 1984), increase in Ca^{2+} extrussion or sequestration (Zsoter et. al., 1977; Popescu et. al., 1985); or inhibition of receptor linked phosphoinositide breakdwon (Rapoport, 1986). It may also be due to inhibition of utilization of intracellular Ca^{2+}. The observed inhibition of contraction by high dose CQ in the RAS may be due to one or more of the above mechanisms. However, the failure of low dose CQ to influence the residual response and the total abolition of this response by high dose CQ are indicative of an intracellular site for the inhibitory action of CQ and other antimalarials (see above).

DILATOR AND INHIBITORY EFFECT OF ANTIMALARIAL

The 'Dilator' effect of 10^{-4}M CQ was distinctly greater, i.e., 10^{-4}M CQ completely relaxed the precontracted vessel whereas the same concentration of CQ pre-incubated only reduced contraction to NA by about 70% (see Fig 23 & 27). It is noteworthy that CQ and quinine are more potent than Diltiazem in relaxing precontracted artery. Although Diltiazem is not particularly a potent Ca^{2+} channel blocker (as Nifedipine), the result of the present experiment lends additional support to the view that the 'Dilator' effects of CQ and Quinine are due to mechanisms other than Ca^{2+} channel blockade. The

result further demonstrated an interesting aspect of the Dilator effect of CQ in (Figure 27B pg 143.)

It can be seen that CQ has a greater effect in relaxing the pre-contracted artery than in preventing the initiation of contraction. I have no adequate explanation for this observation but it is possible that CQ has a greater effect in inhibiting the mechanism responsible for sustaining the contraction than in initiating contraction.

RELAXANT ACTION OF CQ IN RAT AORTA

The relaxant action of chloroquine was studied in the rat aortic strip pre-contracted with NA or K^+. The relaxant effect of chloroquine was again more marked on NA than K^+ induced contractions (see Fig 23 p 126). In this regard and in contrast to Ca^{2+} channel blockers, the action of chloroquine was similar to the relaxant action of sodium nitroprusside (Karaki et. al., 1987).

However, methylene blue ($10^{-3}M$) which is known to block the relaxant effect of sodium nitroprusside (Karaki et. al., 1984) did not modify the relaxant effect of CQ (see fig 27C p 144) suggesting that the mechanism of the relaxant effect of chloroquine in vascular smooth muscle might be unique. The Possibility that the relaxant effect of chloroquine might be

due to K^+ channel opening was also investigated. It is known that cromakalim, a K^+ channel opener relaxes arterial muscle precontracted with 15mM K^+ but not when precontracted with 60mM or higher concentration of K^+ (Yu et. al., 1992). The apparent mechanism of cromakalim is to stimulate K^+ efflux which in turn causes hyperpolarisation and subsequent relaxation of arterial muscle.

(Hamilton et. al., 1986; Weir and Weston, 1986; Quast, 1987; Cook et. al., 1986; Quast and Baumlim, 1988) under the condition where arterial strips are contracted with low concentration of K^+. Cromakalim does not produce relaxation in high K^+ (65.9mM). Presumably because high K^+ reduced equilibrium potential of K^+ to a value of membrane potential less negative than that required to close VOC channel (Masuzuwa et. al., 1990). The result in the present study where 15mM K^+ and 60mM K^+ were used to precontract the muscle are in contrast with those of classical K^+ opener which relaxes arterial smooth muscles precontracted with only low dose of K^+ 15mM and not 60mM. CQ in this study was effective in relaxing arteries precontracted by both 15mM K^+ and 60mM K^+ (see fig 28 p 149) suggesting that the mechanism of action of chloroquine may not be due to K^+ channel opening.

CONCLUSION

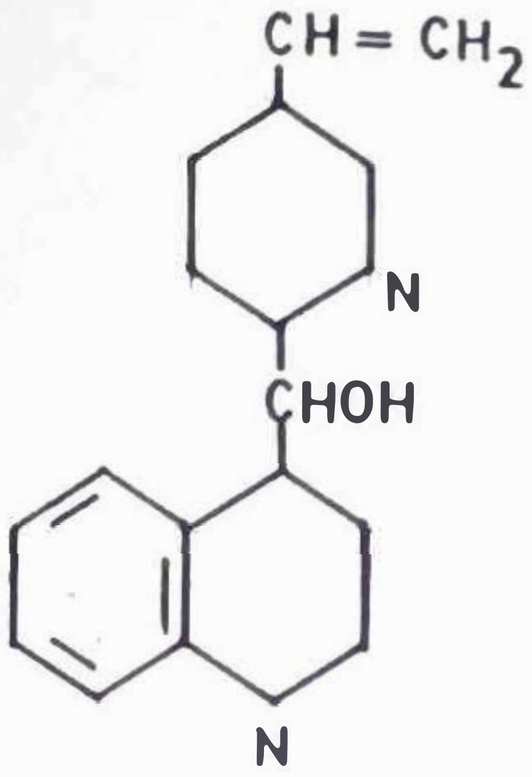
It is concluded from the results of this study that

(a) Chloroquine has two sites of action in smooth muscle. One site is the muscle cell membrane where it appearts to facilitate the influx of calcium; this effect is more marked on receptor linked responses than K^+ induced responses. The other site is intracellular where the action is observed at higher concentration of chloroquine and is manifested as inhibition of agonist contractions. In regard to the actions of the other antimalarial drug studied at these two sites, Amodiaquine, Mepacrine and Quinine showed similar activities to Chloroquine, i.e., they consistently potentiated agonists contractions at low doses and inhibited at high concentrations.

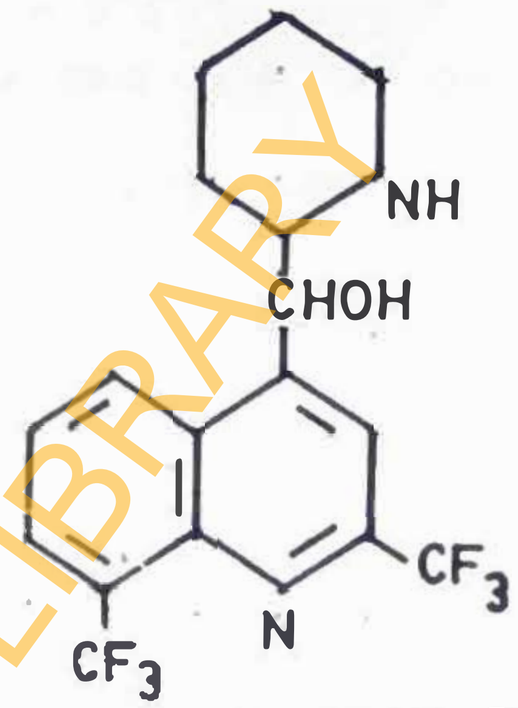
Halofantrine and Melfoquine on the other hand showed neither marked potentiation at low doses nor inhibition at high doses. The common feature in the structure of the antimalarial with similar actions to CQ are Quinoline nucleus, amino substitution in either the 4th or 8th position (CQ, AMDQ, MPC) or substitution in the 6th position chloride (CQ, AMDQ) or CH_3O methoxy (Quinine).

STRUCTURES OF SOME ANTIMALARIAL DRUGS

4 – QUINOLINE METHANOLS

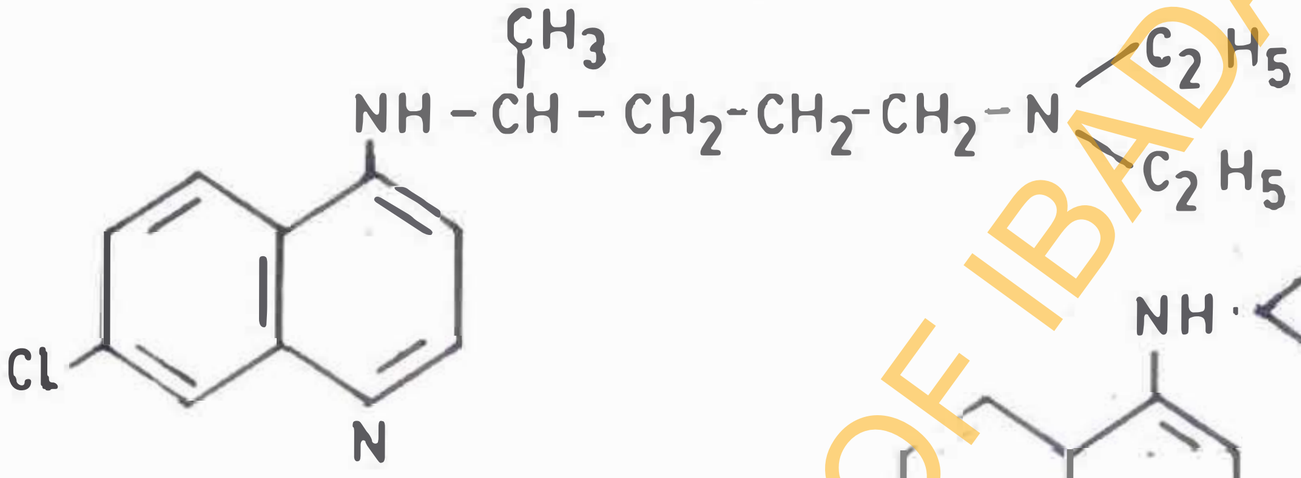


QUININE

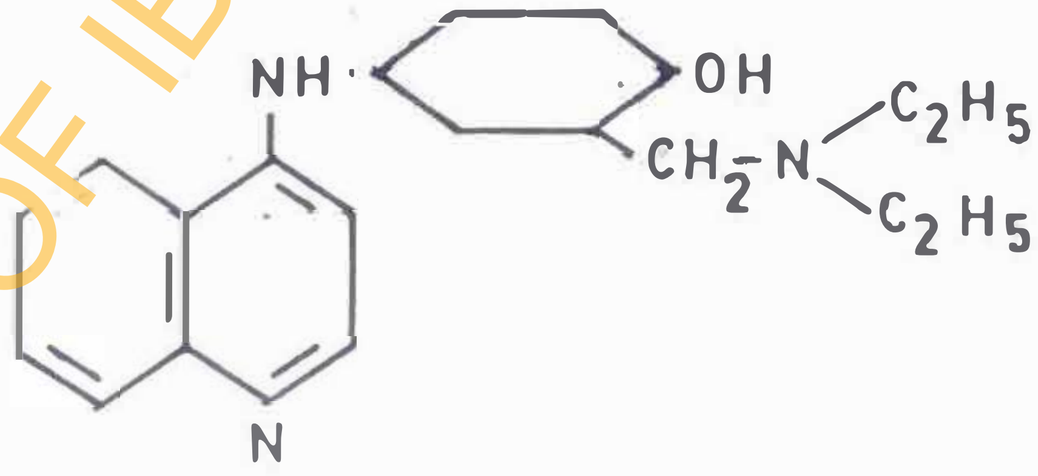


MEFLO QUINE

4 – AMINO QUINOLINES

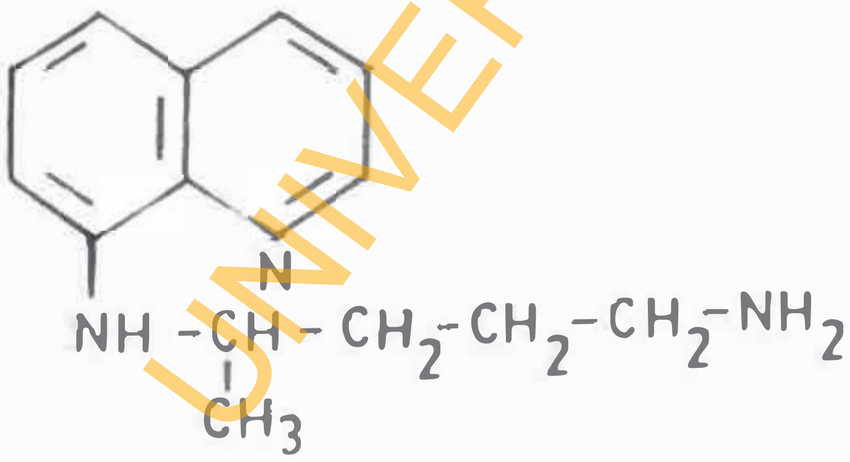


CHLOROQUINE



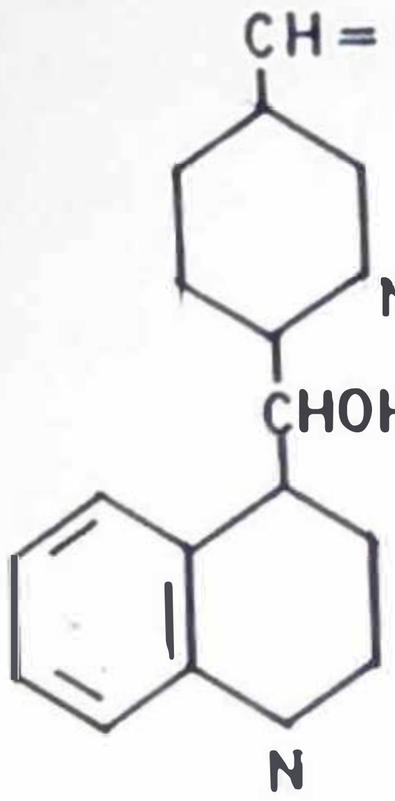
AMODIAQUINE

8 – AMINO QUINOLINE

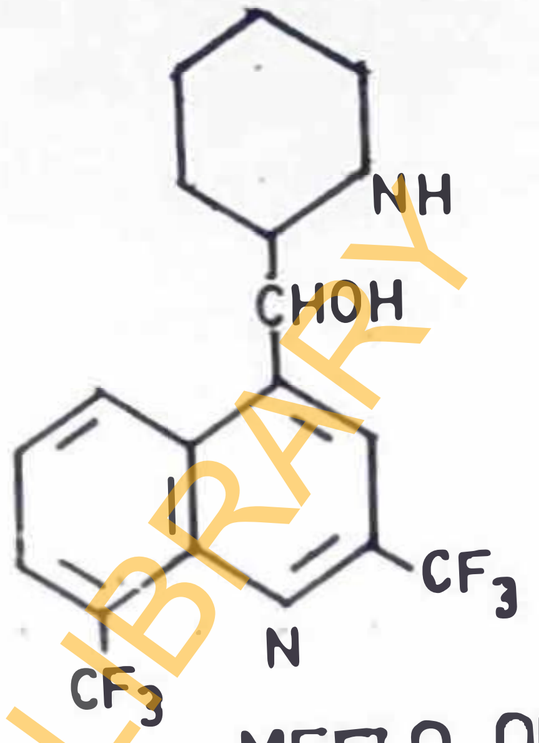


STRUCTURES OF SOME ANTIMALARIAL DRUGS

4 – QUINOLINE METHANOLS

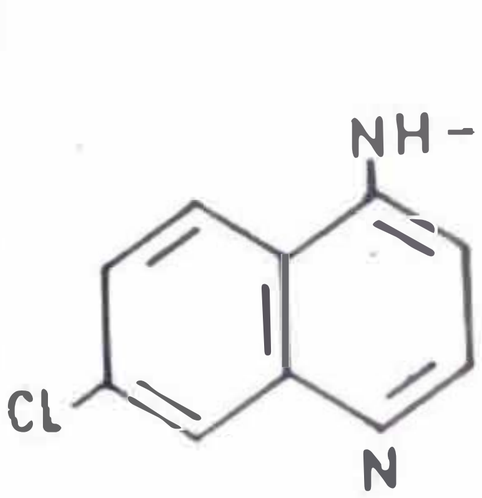


QUININE

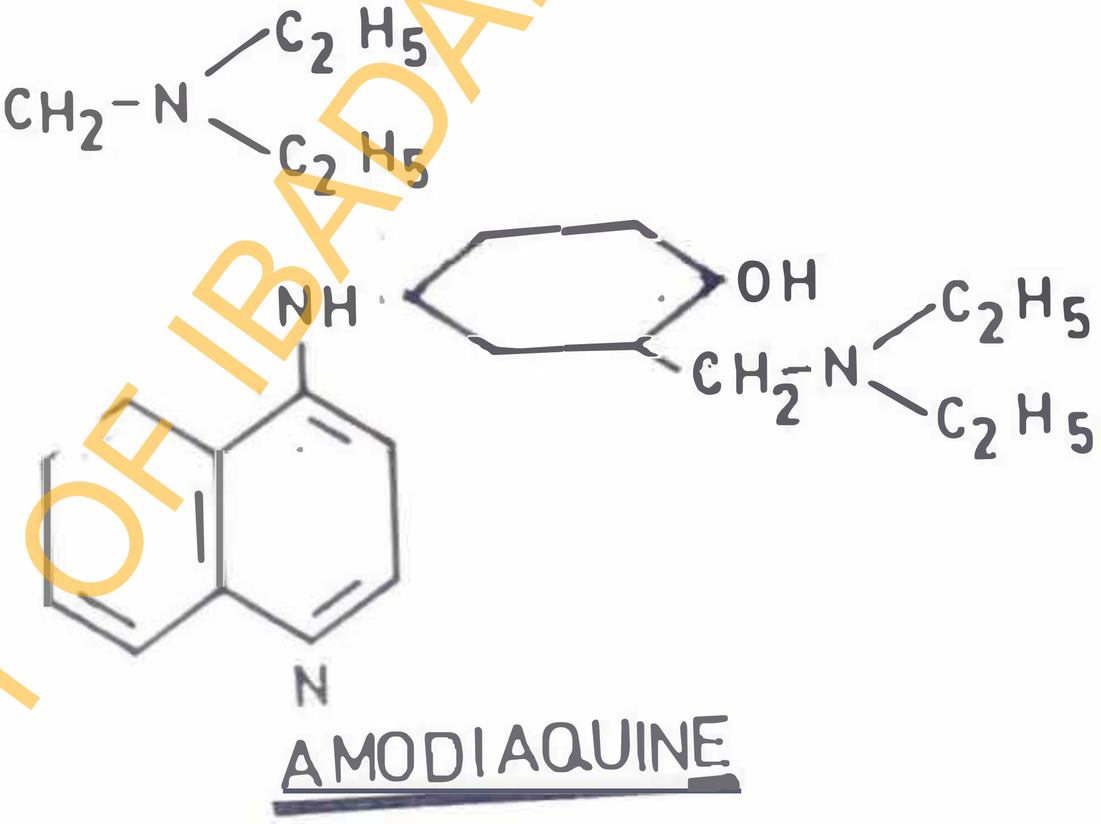


MEFLO QUINE

4 – AMINO QUINOLINES

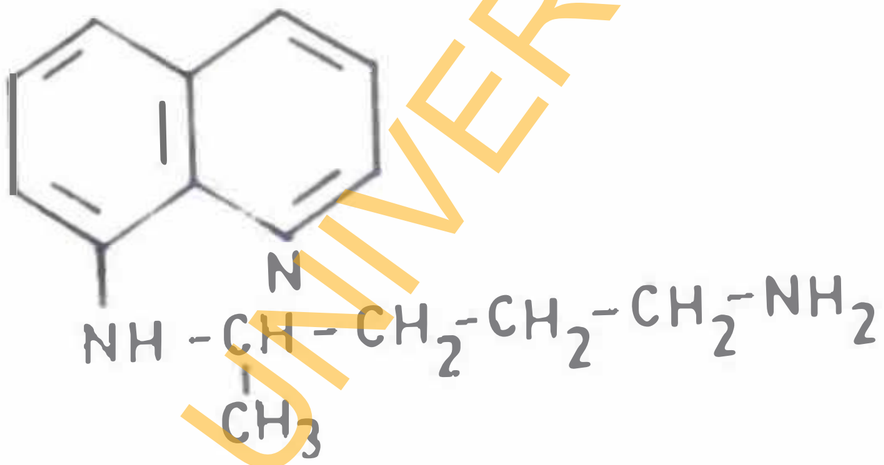


CHLOROQUINE

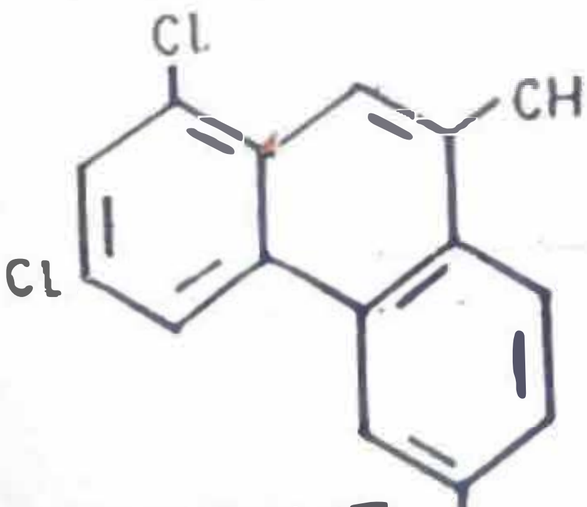


AMODIAQUINE

8 – AMINO QUINOLINE



PHENANTHRENE METHANOL



HALOFANTRINE

(b) The relaxant effect of CQ and antimalarial acting similarly in arteries was most likely not due to calcium channel blockade nor opening of K^+ channels; CQ probably interacted with intracellular contractile mechanisms.

(c) The effect of chloroquine on muscle contraction depends on muscle type, agonist used, and the concentration of Ca^{2+} in the bathing fluid.

These findings suggest that vascular relaxation by chloroquine may contribute to the cardiovascular collapse sometimes encountered in chloroquine therapy.

REFERENCES

AARONSON, P.J., BENHAM, C.D., BOLTON, T.B., HESS, P., LANG, R.J. and TSIEN, R.W. (1986): Two types of single channel and whole cell calcium or barium currents in single smooth muscle cells of rabbit ear arter and the effects of noradrenalin. J. Physiol. 377, 360p.

ADEAGBO, A.S.O. and OKPAKO, D.T. (1980): Mechanism of Noradrenaline potentiation by PGE_2 in rat mesenteric artery. Brit. J. Pharmac. 71, 75-81.

AKODU, O.C. (1985): Effect of quinine on neuro effector transmission and contractility of the isolated rat annococcygeus muscle. B. Pharm. Dissertation, University of Ibadan.

AKUBUE (1975): The action of chloroquine on histamine receptors of isolated guinea pig ileum. West Afric. J. Pharmacol. and Drug Res. 2. 139-143.

ANDERSON, R.G.G. and WILSON, K.B. (1977): Rate of cyclic nucleotide metabolism and mechanical activity in smooth muscle. In the Biochemistry smooth muscle. Ed. Stephens, N.L. pp. 263-292. Baltimore University Park.

ANDERSON, K.E. (1978): Effects of calcium and calcium antagonists on the excitation-contraction coupling in striated and smooth muscle. Acta Pharmacol. Toxicol. 43 (Suppl. I, 1-14).

BATRA, S.C. (1973): The role of mitochondria calcium uptake in contraction and relaxation of the human myometrium. Biochem. Biophys. Acta 305 428-432.

BEVAN, J.A., CASTRA, W., SU, C., and SU, M. (1973): The bimodal basis of the contractile response of the rabbit ear artery to noraphrine and other agonists. Eur. J. Pharmacol. 22, 47-53.

BEVAN, J.A. (1982): The selectvie action of diltiazem on ceresbral vascular smooth muscle of rabbit; antagonism of extrinsic but not intrinsic maintained tone. Am. J. Cardiac. 49, 519-524.

BEVAN, J.A., BEVAN, R.O., HWA, J.J., OWEN, M.P., TAYO, F.M. and WINQUIST, R.J. (1982): Calcium, extrinsic and intrinsic myogenic vascular tone. In: Calcium modulation. Eds. T. Godfraind, A. Albertinin and R. Paoletti. Elsevier Biomed. Amsterdam. pp 125-132.

BLAUSTEIN, M.P. (1974): The inter-relationship between sodium and calcium fluxes across cell membrane. Revs. Physiol. Biochem. Pharmacol. 70, 33-82.

BLAUSTEIN, M.P. (1977): The role of Na-Ca exchange in the regulation of tone in vascular smooth muscle. In: Excitation contraction coupling in smooth muscle. Eds. Casted, R. et. al. Elservier. North Holland.

BATRA, S.C. (1973): The role of mitochondria calcium uptake in contraction and relaxation of the human myometrium. Biochem. Biophys. Acta 305 428-432.

BEVAN, J.A., CASTRA, W., SU, C., and SU, M. (1973): The bimodal basis of the contractile response of the rabbit ear artery to noraphrine and other agonists. Eur. J. Pharmacol. 22. 47-53.

BEVAN, J.A. (1982): The selectvie action of diltiazem on ceresbral vascular smooth muscle of rabbit; antagonism of extrinsic but not intrinsic maintained tone. Am. J. Cardiac. 49. 519-524.

BEVAN, J.A., BEVAN, R.O., HWA, J.J., OWEN, M.P., TAYO, F.M. and WINQUIST, R.J. (1982): Calcium, extrinsic and intrinsic myogenic vascular tone. In: Calcium modulation. Eds. T. Godfraind. A. Albertinin and R. Paoletti. Elsevier Biomed. Amsterdam. pp 125-132.

BLAUSTEIN, M.P. (1974): The inter-relationship between sodium and calcium fluxes across cell membrane. Revs. Physiol. Biochem. Pharmacol. 70. 33-82.

BLAUSTEIN, M.P. (1977): The role of Na-Ca exchange in the regulation of tone in vascular smooth muscle. In: Excitation contraction coupling in smooth muscle. Eds. Casted, R. et. al. Elservier. North Holland.

BATRA, S.C. (1973): The role of mitochondria calcium uptake in contraction and relaxation of the human myometrium. Biochem. Biophys. Acta 305 428-432.

BEVAN, J.A., CASTRA, W., SU, C., and SU, M. (1973): The bimodal basis of the contractile response of the rabbit ear artery to noraphrine and other agonists. Eur. J. Pharmacol. 22, 47-53.

BEVAN, J.A. (1982): The selectvie action of diltiazem on ceresbral vascular smooth muscle of rabbit; antagonism of extrinsic but not intrinsic maintained tone. Am. J. Cardiac. 49, 519-524.

BEVAN, J.A., BEVAN, R.O., HWA, J.J., OWEN, M.P., TAYO, F.M. and WINQUIST, R.J. (1982): Calcium, extrinsic and intrinsic myogenic vascular tone. In: Calcium modulation. Eds. T. Godfraind, A. Albertinin and R. Paoletti. Elsevier Biomed. Amsterdam. pp 125-132.

BLAUSTEIN, M.P. (1974): The inter-relationship between sodium and calcium fluxes across cell membrane. Revs. Physiol. Biochem. Pharmacol. 70, 33-82.

BLAUSTEIN, M.P. (1977): The role of Na-Ca exchange in the regulation of tone in vascular smooth muscle. In: Excitation contraction coupling in smooth muscle. Eds. Casted, R. et. al. Elservier. North Holland.

BOHR, D.F. (1963): Vascular smooth muscle dual effect of calcium. Science 139, 597-599.

BOHR, D.F. (1973): Vascular smooth muscle updated Cir. Res. 32, 665-672.

BOLTON, T.B. (1979): Mechanism of action of transmitter and other substances on smooth muscle. Physiol. Revs. 59, 606-718.

BOLTON, T.B., CLARK, J.P., KITAMURA, K. and LANG, R.J. (1981): Evidence that histamine and carbachol may open the sam ion channels in longitudinal smooth muscle of guinea pig ileum. J. Physiol. (Lond.), 320: 363-399.

BOZLER, E. (1954): Membrane potentials of smooth fibres of the taenia coil of the guinea pig. J. Physiol., 125: 302-315.

BOZZLER, E. (1963): Distribution and exchange of calcium in connective tissue and smooth muscle. Am. J. Physiol. 205, 686-692.

BOZZLER, E. (1969): Role of calcium in initiation of activity of smooth muscle. Am. J. Physiol. 216: No. 3, 671-674.

BRADING, A.F. (1979): Maintenance of ionic composition. Brit. Med. Bull. 35: 227-234.

BRADING, A.F. and SNEDDON, P. (1980): Evidence for multiple sources of calcium for activation of the contractile

mechanism of guine pig taenia coli on stimulation with carbachol. Brit. J. Pharmac. 70: 229-240.

CASTEEL, R. and DROOGMAN, G. (1976): Membrane potential and excitation-contraction coupling in the smooth muscle cells of the rabbit ear artery. J. Physiol. 263: 163-164.

CASTEEL, R. and DROOGMAN, G. (1985): Dependence on calcium of potassium and agonist-induced changes in potassium permeability of rabbit ear artery. J. Physiol. 364: 151-167.

CAUVIN, C.R., LOUT ZENHISER and Van BREEMEN, C. (1983): Mechanism of calcium antagonists-induced vasodilation. Ann. Rev. Pharmacol. Toxical 23: 373-396.

CAUVIN, C., LUKEMAN, S., CAMERON, J., HWANG, O., MEISHERI, K., YAMAMOTO, H. and Van BREEMEN, C. (1984): Theoretical basis for vascular selectivity of Ca^{2+} antagonists. J. Cardo. Pharmacol. 6: 8630-9638.

CAUVIN, C., SAIDA, K. and Van BREEMEN (1984): Extracellular Ca^{2+} dependence and Diltiazem inhibition of contraction in rabbit condint arteries and messenteric resistance vessels. Blood vessels 21: 23-31.

COLUCCI, V.S., GIMBRONE, M.A., (Jnr) and ALEXAMDER, R.W. (1986): Phorbol diester modulates adrenelgic receptor

mechanism of guine pig taenia coli on stimulation with carbachol. Brit. J. Pharmac. 70: 229-240.

CASTEEL, R. and DROOGMAN, G. (1976): Membrane potential and excitation-contraction coupling in the smooth muscle cells of the rabbit ear artery. J. Physiol. 263: 163-164.

CASTEEL, R. and DROOGMAN, G. (1985): Dependence on calcium of potassium and agonist-induced changes in potassium permeability of rabbit ear artery. J. Physiol. 364: 151-167.

CAUVIN, C.R., LOUT ZENHISER and Van BREEMEN, C. (1983): Mechanism of calcium antagonists-induced vasodilation. Ann. Rev. Pharmacol. Toxical 23: 373-396.

CAUVIN, C., LUKEMAN, S., CAMERON, J., HWANG, O., MEISHERI, K., YAMAMOTO, H. and Van BREEMEN, C. (1984): Theoretical basis for vascular selectivity of Ca^{2+} antagonists. J. Cardo. Pharmacol. 6: 8630-9638.

CAUVIN, C., SAIDA, K. and Van BREEMEN (1984): Extracellular Ca^{2+} dependence and Diltiazem inhibition of contraction in rabbit condint arteries and messenteric resistance vessels. Blood vessels 21: 23-31.

COLUCCI, V.S., GIMBRONE, M.A., (Jnr) and ALEXAMDER, R.W. (1986): Phorbol diester modulates adrenelgic receptor

coupled calcium afflux and adrenergic receptor number in cultured vascular smooth muscle cell. Circ. Res. 58: 393-398.

COOK, N.S. (1988): The pharmacology of potassium channels and their therapeutics potential trends. Pharmacol. Sci. 9: 21-28.

COOK, N.S. QUAST, U., HOF, R.P., BAUMLIN, Y. and PALLY, C. (1988a): Similarities in the mechanism of action of two new vasodilator drugs: pinacidil and BRL 34915. J. Cardivasc. Pharmacol., 11: 90-91.

COOK, N.S, SHEILA, W., WEIR and MAX, C. DANSEISEN (1988): Antivasoconstrictor effects of K^+ channel opener, cromokalim on the rabbit aorta - comparison with the calcium antagonist Isradipine. Br. J. Pharmacol. 95: 741-752.

DANIEL, E.E., SEHDEV, H. and ROBINSON, K. (1962): mechanisms of activation of smooth muscle. Physiol. Rev. 42: (Supp. 5): 228-260.

DEVINE, C.E., SOMLYO, A.V. and SOMLYO, A.P. (1972): Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. J. Cell Biol. 52: 690-718.

coupled calcium afflux and adrenergic receptor number in cultured vascular smooth muscle cell. Circ. Res. 58: 393-398.

COOK, N.S. (1988): The pharmacology of potassium channels and their therapeutics potential trends. Pharmacol. Sci. 9: 21-28.

COOK, N.S. QUAST, U., HOF, R.P., BAUMLIN, Y. and PALLY, C. (1988a): Similarities in the mechanism of action of two new vasodilator drugs: pinacidil and BRL 34915. J. Cardivasc. Pharmacol., 11: 90-91.

COOK, N.S, SHEILA, W., WEIR and MAX, C. DANSEISEN (1988): Antivasoconstrictor effects of K^+ channel opener, cromokalim on the rabbit aorta - comparison with the calcium antagonist Isradipine. Br. J. Pharmacol. 95: 741-752.

DANIEL, E.E., SEHDEV, H. and ROBINSON, K. (1962): mechanisms of activation of smooth muscle. Physiol. Rev. 42: (Supp. 5): 228-260.

DEVINE, C.E., SOMLYO, A.V. and SOMLYO, A.P. (1972): Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. J. Cell Biol. 52: 690-718.

DILLON, P.F., AKSOY, M.O., DRISKA, S.P., MURPHY, R.A. (1981): Myosin phosphorylation and the cross bridge circle in arterial smooth muscle. Science 211: 495-497.

DUBE, G., BALK, Y.K. and Van BREEMEN, C. (1987): Effect of isosorbide dinitrate and diltiazem on Ca^{2+} flux and contraction in artery. Eur. J. Pharmacol. 13: 39-47.

EBEIGBE, A.B. and ALOAMAKA, C.P. (1982): Effects of chloroquine on mechanism activity of rat portal vein. Microcirculation 2: 151-159.

EBEIGBE, A.B., ALOAMAKA, C.P., NWABUKO, U.U. (1984): Raised extracellular magnesium enhances norepinephrine induced mobilisation of stored calcium in the rat tail arter. IRCS Med. Sci. 12: 196-197.

EBEIGBE, A.B., ALOAMAKA, C.P., and ALOHAN, F. (1986): Mechanism of chloroquine induced inhibition of smooth muscle contraction. Arch. Int. Pharmacodyn. Ther. 280: 254-263.

EGGERMONT, J.A., RAEYMAKERS and CASTEELS, r. (1989): Calcium transport by smooth muscle membrand and its regulation. Biomed. Biochem. Acta 48: 5/6: 370-381.

ENDO (1977): Calcium release from the sarcoplasmic reticulum. Physiol. Revs. 57: 71-108.

FAMAEY, J.P., FONTAINE, J. and REUSE, J. (1975): Inhibiting effects of morphine, chloroquine, non-steroidal and steroidal anti-inflammatory drugs on electrically induced contractions of guinea pig ileum and reversing effect of prostaglandins. Agents and Actions Vol. 5/4, 354-358.

FAMAEY, J.P., FONTAINE, J. and REUSE, J. (1977a): The effect of various non-steroidal anti-inflammatory drugs on cholinergic and histamine induced contractions of the guinea pig isolated ileum. Brit. J. Pharmacol. 60: 165-171.

FAMAEY, J.P., FONTAINE, J. and REUSE, J. (1977b): An analysis of the inhibitory effects and of possible prostaglandin antagonism of chloroquine in the guinea pig isolated ileum. J. Pharm-pharmacol. 29: 761.

FAMAEY, J.P., FONTAINE, J. and REUSE, J. (1980b): The effect of anti-inflammatory steroids on the response of the guinea pig isolated ileum to Acetylcholine, Histamine, Nicotine, 5-Hydroxytryptamine and electrical stimulation. A study of the effects of Amodiaquine on the guinea pig isolated ileum. Nauym. Schmiedeberg. Arch. Pharmac. 313: 165-170.

FLECKENSTEIN, A. (1977): Specific pharmacology of calcium in myocardium cardiac pacemakers and vascular smooth muscle. Ann. Rev. Pharmacol. Tox 17: 149-166.

FURCHGOTT, R.F. and BHADRAKOM (1953): Reactions of strips of rabbit aorta to epinephrine, isoproternol, sodium nitrite and other drugs. J. Pharmac. Exp. Ther. 142. 39-58.

FURCHGOTT, R.F. (1983): Role of endothelium in the responses of vascular smooth muscle. Circ. Res. 53: 557-573.

FURCHGOTT, R.F. and ZAWADZKI, J.V. (1980): The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373-376.

GODFRAIND, T. (1976): Calcium exchange in vascular smooth muscle: action of noradrenaline and lanthanum. J. Physiol. (Lond.) 260: 21-35.

GODFRAIND, T. and KABA, A. (1972): The role of calcium in the action of drugs on vascular smooth muscle. Arch. Int. Pharmacodyn Ther. 196: Suppl. 35-49.

GODFRAIND, T. and DIEU, D. (1981): The inhibition of flunarizine of the norepinephrine evoked contraction and calcium influx in rat aorta and messenteric arteries. J. Pharmacol. Exp. Ther. 217: 510-515.

GOODFORD, P.J. (1970): In: Smooth muscle. Bulbring. E., Brading, A.F., Jones, A.W. and Tomita, T. (eds.) pp 100-121 Arnold, London.

GOODFORD, P.J. (1975): In: Smooth muscle. Bulbring, E., Brading, A.F., Jones, A.W. and Tomita, T. (eds.), pp 100-121. Arnold, London.

GRIENDLING, K.K., RITTENHOUSE, T.A., BROCK, L., SEKSTEIN, M.A., GIMRONE (Jnr.) and ALEXANDER, R.W. (1986): Sustained diacylglycerol formation from inositol phospholipids in angiotestion II stimulated vascular smooth cells. J. Biol. Chem. 261: 5901-5906.

HAEUSLER, G. and THORENS, S. (1975): The pharmacology of vasoactive antihypertensive. In: Vascular newroeffector mechanisms. Eds. J.A. Bevan, G. Bunstock, B. Johnson, R.A. Maxwell and O.E. Needergard (S. Karger Basal p. 232).

HAMILTON, T.C., WEIR, S.W. and WESTON, T.H. (1986): Comparison of the effect of BRL 34915 and Verapamil on electrical and mechanical activity in rat portal vein. Br. J. Pharmacol. 88: 103-111.

HAMILTON, T.C., WEIR, S.W. and WESTON, T.H. (1989): Cromakalise nicoranil and pinacidil: Navel drugs which

open potassium channels in smooth muscle. Gen. Pharmacol. 20: 1-9.

HEASLIP, R.J. & RAHWAN, R.G. (1982): Evidence for the existence of two distinct pools of intracellular calcium in rat aorta accessible to mobilisation by norepinephrine. J. Pharmacol. Exp. Ther, 221, 7-13.

HERMSMEYER, K. (1988): Pinacidil actions on ion channels in vascular arteries. J. Cardiaras. Pharmacol. 12: (Suppl. 2), 517-522.

HIATT, E.P (1948): Effects of repeated oral dose of quinine and qunidine on the blood pressure and renal circulation of dogs with experimental neurogenic hypertension. Am. J. Physiol. 115: 114-117.

HIATT, E.P. (1950): Sympatholytic effects of quinidine and quinine. Am. J. Physiol. 160: 212-216.

HIATT, E.P. and SHURIE, V. (1948): Effects of repeated oral dose of quinine and quinidine on the blood pressure and renal circulation of dogs with experimental neurogenic hypertension. Am. J. Physiol. 115: 114-117.

HIATT, E.P. (1950): Sympathetic effects of quinidine and quinine. Am. J. Physiol. 160: 212-216.

HINKLE, J.A.M. (1965): Calcium requirements for noradrenaline and high potassium ion concentration in arterial smooth

muscle. In muscle (ed.) Paul, W.M., Daniel, E.E., Kay, C.M. and Monckton, G. p. 269.

HOFF, R.P., MULLER-SCHINEINITZER, E. and NSUMANN, P. (1980): In-vitro and in vivo effects of a new poereful calcium antagonist. Br. J. Pharmacol. 73: 196p.

HOFF, R.P., VUORELA, H.J. (1983): Assessing calcium antagonism in vascular smooth muscle: A comparison of three methods. J. Pharmacol. Meth. 19: 41-52.

HUDDAT, H. and SAAD, H.H.M. (1977): Quinine and lanthanum effects on contractility and calcium movements of rat ileal smooth muscle. Gen. Pharmacol. 8: 341-347.

HUDDAT, H. and SAAD, K.H.M. (1978): The effects of sodium and magnesium and their interaction with quinine and lanthanum or spontaneous activity and related calcium movements of rat ileal smooth muscle. J. Comp. Physiol. 126b: 233-240.

HUDGINS, P.M. and WEISS, G.B. (1968): Differential effects of Ca^{2+} removal upon vascular smooth muscle contraction induced byt norepinephrine, histamine and K+. J. Pharmac. Exp. Ther. 159: 91-97.

HURWITZ, L., FITZPATRICK, D.F., DEBBAS, G. and LANDON, E.J. (1973): Localisation of calcium pump activity in smooth muscle. Science. 177: 384-385.

HURWITZ, L. and JOINER, P.D. (1969): Excitation-contraction coupling in smooth muscle. Fed. Proc. 28: 1629-31.

HURWITZ, L. and JOINER, P.D. (1970): Mobilisation of cellular calcium for contraction in intestinal smooth muscle. Amer. J. Physiol. 218: 12-19.

HURWITZ, L. and SURIA, A. (1971): The link between agonist action and response in smooth muscle. A. Rev. Pharmac. II: 303-326.

HURWITZ, C. (1975):Characterisation of calcium pools utilised for contraction in smooth muscle. Inserm. 50: 369-380.

HURWITZ, L., JOINER, P.D., VON HAGEN, S. and DAVENPORT, G.R. (1969): Calcium accumulation and mechanical response of ileal muscle from rat and guinea pig. Amer. J. Physiol. 216: 215-219.

IKHINMWIN, M.K., SOFOLA, O.A. and ELEBUTE, O. (1981): The effects of calcium ions on the depression of cardiac contractility by chloroquine and quinine. Eur. J. Pharmacol. 69: 507-510.

IMAI, S., KITAGAW, A.T. (1981): A comparison of the effects of nitroglyceric, nifedipine and papaverine on contractures induced in vascular and intestinal smooth muscle by potassium and lanthanum. Japan. J. Pharmacol. 31: 193-199.

HURWITZ, L. and JOINER, P.D. (1969): Excitation-contraction coupling in smooth muscle. Fed. Proc. 28: 1629-31.

HURWITZ, L. and JOINER, P.D. (1970): Mobilisation of cellular calcium for contraction in intestinal smooth muscle. Amer. J. Physiol. 218: 12-19.

HURWITZ, L. and SURIA, A. (1971): The link between agonist action and response in smooth muscle. A. Rev. Pharmac. II: 303-326.

HURWITZ, C. (1975):Characterisation of calcium pools utilised for contraction in smooth muscle. Inserm. 50: 369-380.

HURWITZ, L., JOINER, P.D., VON HAGEN, S. and DAVENPORT, G.R. (1969): Calcium accumulation and mechanical response of ileal muscle from rat and guinea pig. Amer. J. Physiol. 216: 215-219.

IKHINMWIN, M.K., SOFOLA, O.A. and ELEBUTE, O. (1981): The effects of calcium ions on the depression of cardiac contractility by chloroquine and quinine. Eur. J. Pharmacol. 69: 507-510.

IMAI, S., KITAGAW, A.T. (1981): A comparison of the effects of nitroglyceric, nifedipine and papaverine on contractures induced in vascular and intestinal smooth muscle by potassium and lanthanum. Japan. J. Pharmacol. 31: 193-199.

ITOH, K., KURIYAMA, H., UENO, H. (1983): Mechanism of the nitroglycerine induced vasodilation in vascular smooth muscles of the rabbit and pig. J. Physiol. (Lond.) 343: 233-252.

JAMES, D.M. and GILES, H.M. (1985): Trypanosomiasis. In: Human antiparasitic drugus: Pharmacology and usage, pp. 72-91. John Wiley and Sons Limited.

JOB, D.D. (1969): Ionic basis of intestinal electrical activity. Am. J. Physiol. 217: 1534-1541.

KALSNER, S. (1969a): Mechanism of hydrocortisone potentiation of responses to Epinerphrine and Norepinephrine in rabbit aorta. Circ. Research 24: 383-395.

KALSNER, S. (1970): Potentiation of responses to histamine in aortic strips by steroids. Can. J. Physiol. Pharmac. 48: 443-449.

KALSNER, S. (1970): The potentiating effects of ethanol on responses to an inhibition of extraneuronal metabolism of catecholamines by veratrine. J. Pharmac. 24: 493-496.

KALSNER, S. and FREW, R.D. (1972): Potentiation responses to and inhibition of extraneuronal metabolism of catecholamines by veratrine. J. Pharmac. 24: 493-496.

KAORU Masuzawa, TOMOHIRO Amatsuda and MASAHISA Asand (1990): Evidence that pinacidil may promote the opening of ATP-

sensitive K^+ channels yet inhibit the opening of Ca^{2+} activated K^+ channel in K^+ contracted canine messenteric artery. Brit. J. Pharmacol. 100: 143-149.

KARAKI, H., MURAKAMI, K., NAKAGAWA, H., URAKAWA, N. (1984): Nitroglycerine induced biphasic relaxation in vascular smooth muscle of rat aorta. Brit. J. Pharmacol. 81: 387-392.

KARAKI, H., NAKAGAWA, H. and URAKAWA, N. (1984): Comparative effects of verapamil and sodium nitroprusside on contraction and $45Ca^{2+}$ uptake int he smooth muscle of rabbit aorta, rat aorta, and guinea pig taenia coil. Br. J. Pharm. 81: 393-400.

KARAKI, H. and WEISS, E.B. (1984): Calcium release in smooth gastroentology. 87: 960.

KARAKI, H. (1987): Use of tension measurements to delineate the mode of action of vasodilator. J. Pharmacol. Method 18: 1.

KARAKI, H.K. and OZAKI (1988): Different effects of Norepinephrine and KCl on cytosolic Ca^{2+} tension relationship in vascular smooth muscle of rat aorta. Eur. Pharmacol. 151: 325.

KARAKI, H. and WEISS, G.B. (1988): Calcium relezse in smooth muscle. Life Scie. 42: 111.

KITAMURA, K., KURIYAMA, H. and SUZUKI, H. (1976): Effect of noradrenaline on the membrane properties and on the contraction of the rabbit main pulmonary artery. J. Physiol. 263: 164-165.

KOJDA, G., KLAUS, W., WERNER, G., and FRICKLE, U. (1992): Intervascular and stimuls selectivity of Nitrendipine and related derivatives in KCl and PGF2d precontracted porcine arteries. Brit. J. Pharmacol. 106: 85-90.

KOSTERLITZ, H.W., LYDON, R.J. and WATT, A.J. (1970): The effects of adrenaline, noradrenaline and isprenaline on inhibitory and adrenoceptors in the longitunal muscle of the guinea pig ileum. Br. J. Pharmac. 39: 398.

KREYE, V.A.M., BARON, G.D., JUTH, J.B., and SCHMIDT, H. (1975): Mode of action of sodium nitroprusside on vascular smooth muscle. Naunyn-Schmiedeb. Arch. PHarmacol. 288: 381.

KUKOVETZ, W.R., POCH, G. and HOLZMANN, S. (1981): Cyclic nucleotide and relaxation of vascular smooth muscle. In: Vanhoute, P.M. Leusen I, (eds.) Vasodilation. New York, Raven 339-353.

LEVIN, J.A. and FURCHGOTT, R.F. (1970): Interaction between potentiating agents of adrenergic amines in rabbit aortic strip. J. Pharmacol. Pharmoc. 172: 320-331.

LOIRAND, G., PACAUD, P., MIRRONEAU, C. and MIRRONEAU (1986): Evidence for two distinct Ca^{2+} channels in rat vascular smooth muscle cells in short term primary culture. P. Flugers Arch. 407: 566-568.

MAKINDE (1977): In vitro studies on the mechanisms of cardiotoxic effects of chloroquine. University of Ibadan Ph. D. Thesis.

McCHESNEY, E.W., ROTHFIELD, N.E. (1964): Comparative metabolic studies of chloroquine and hydroxychloroquine. Arthritis Rheuma 7, 328-332.

McCLESKEY, E.W., FOX, A.P., FELDMAN, D.H., CRUZ, L.J., OLIVERA, B.M. and TSIEN, R.W. (1987): W-conotoxin: direct and persistent blockade of specific types of calcium channels in neurons but rat muscle. Proc. Natl Acad. Sci. U.S.A. 84: 4327-4331.

MARSHALL, R.J. and OJEWOLE, J.A.O. (1978): Comparative effects of some antimalarial drugs on isolated cardiac muscle of the guinea pig. 46: 759-768.

MEISHERI, K.O., HWANG, O. and Van BREEMEN, C. (1981): Evidence for two separate Ca^{2+} pathways in smooth muscle plasmalemma. J. Memb. Biol. 59: 19-25.

MEISSNER, G. (1986): Ryanodine activation and inhibition of the Ca^{2+} released channel of sarcoplasmic reticulum. J. Biol. Chem., 261: 6300-6306.

NACHSHEN, O.A. (1984): Selectivity of the Ca^{2+} binding site synaptosome Ca^{2+} channels inhibition of influx by multivalent metal cation. J. Gen. PHysiol. 83: 941-967.

NAGAO, T., NARITA, H., SATO, M., MAKAJIMA, H. and KIYOMOTO, H. (1992): Development of diltiazem, a calcium antagonist: Coronary vasodilating and antihypertensive action. Clin. Exp. Hypertens. A4: 285-296.

NELSON, E.E. (1927a): Studies on quinine and quinidine III. The antagonism of quinine and quinidine for the circulatory effects on epinephrien. Arch. Int. Pharmacodyn Ther. 33: 197-203.

NELSON, E.E. (1928): Studies on quinine and quinidine V. Do they have specific action on autonomic nerve ending? Proc. Soc. Exp. Biol. Med. 25: 499-501.

NGHIEM, C., SWAMY, V.C. & TRIGGLE, D.J. (1982): Inhibition by D600 of horepinephrine and clonidine-induced responses of the aortae from normotensive (WKY) and spontaneoulsy hypertensive rats (SHR). Life Sci, 30: 45-49.

NOGUERA, M.A. and D'OCON, M.P. (1992): Different and common intracellular calcium stores mobilised by noradrenaline

and caffeine in vascular smooth muscle. Naumyn Schiederbers Arch. Pharmacol. 343: 331-343.

NOWYCKY, M.C., FOX, A.P. and TSIEN, R.N. (1985): Three types of neuronal calcium channel with different calcium against sensitivity. Nature. 316: 440-443.

OFFIAH (1981): Studies on the source of activator calcium in isolated preparation of guinea pig ileum and taenia caecum. University of Ibadan M.Sc. Dissertation.

OLATUNDE, I.A. (1970): Quantitation of the degree of antagonism of chloroquine to histamine, acetylcholine and serotonin (PA_2 values). Arch. Int. Pharmacodyn 185: 66-70.

OLATUNDE, I.A. (1970): Parenteral chloroquine in children. W. Afric. Med. J. 19: 93-96.

OKPAKO, D.T. (1978): Interactions of aminoquinolines and mepacrine with prostaglandin E_2 in tissues of rats and guinea pig. Gen. Pharmacol. Vol. 9, No. 1, pp. 25-28.

OKPAKO, D.T. and OLADITAN, F.O. (1979): The effect of varying the concentration of calcium ions on the actions of indomethacin and PCE_1 in guinea pig ileum. Gen. Pharmac. 10: 327-329.

OKWUSABA, F.K., OTUBU, J.A.M. and UDOH, F.V. (1990): Mode of inhibitory actions of actue and chronic chloroquine

administration on rat diaphragm. Br. J. Pharmacol. 101: 133-139.

PALMER, R.M.J., FERRIDGE, A.G & MONCADA, S. (1987): Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor, Nature, 88: 411-415.

PATON, W.D.M. and ABOO, Zar (1968): The origin of Ach released from guinea pig intestine and longitudinal muscle strip. 194: 13-33.

PERRY, S.V. and GRAND, R.J.A. (1979): Mechanisms of contraction and the specialised protein components of smooth muscle. Phil. Trans. R. Brit. Med. Bull. 35: 219-226.

PFIZER, G.L., MERKEL, J.C., RUEGG and HOFMCIN, F. (1986): Cyclic GMP dependent protein kinase relaxes skinned fibre from guinea pig taenia coli but not from chicken gizzard. Pflugers. Arch. 407: 87.

POPESCU, L.M.C., PANORU, M., HINESCU and NATU, O. (1985): The mechanism of CGMP induced relaxation in vascular smooth muscle. Eur. J. Pharmac. 107: 393.

PROSSER, C.L. (1974): Smooth muscle, Ann. Rev. Physiol. 36: 503-506.

QUAST, U. (1987): Effectof the K^+ efflux stimulation vasodilator BRL 34915 or ^{86}Rb efflux and spontaneous

activity in guinea pig portal vein. Br. J. Pharmacol. 91: 5.

QUAST, U. and BAUMLIN, Y. (1988): Comparison of the effluxes of ^{42}K and ^{86}Rb elicited by cromokalim (BRL 34915) in tonic and phasic vascular tissue. Maunyn Schmiederberg. Arch. Pharmacol. 338: 319-326.

RAPOPORT, R.M. (1986): Cyclic guanosine monophosphate inhibition of contraction may be mediated through inhibition of phosphophatidylinositol hydrolysis in rat aorta. Circ. Res. 58: 407.

RASMUSSEN, H., TAKUWA, Y., and PARK, S. (1987): Protein kinase C in the regulation of smooth muscle contraction FASEB J. I, 177-185.

REUTER, H., BLAUSTEIN, M.P. and HAEUSLER, G. (1973): Na-Ca exchange and tension development in arterial smooth muscle. Phil. Trans. R. Soc. Lond. (Biol.) 265: 87-96.

ROBERTSON, P.A. (1960): Calcium and contractility in the depolarised smooth muscle. Nature. 186: 316-317.

SALAKO, L.A., ADEROUNMU, Fadeke, LAOYE, J., MAKINDE Modupe and ADIO, R. Aina (1988): Sensitivity of plasmodium falciparum to mefloquine-sulphadoxine-pyrimethonine (Femsimef) *in vivo* and to mefloquine *in vitro* in Nigeria.

Annals of Trop. Meda Parasitology. Vol. 82: No. 4, 325-330.

SAVAGE, A.O. and AKINLALU, C.A. (1985): Actions of quinine on the rat isolated rectum. Arch. Int. Pharmacodyn and Therap. 276: 162-176.

SAVAGE, A.O. and LAWAL, O.B. (1986): Influence of calcium lack and nifedipine on potassium, barbachol and quinine induced contractions of lizard rectum. Comp. Biochem. Physiol. (Lond.) 235: 341-343.

SCHATZMANN, H.J. (1973): Dependence on calcium concentration and stoichiometry of calcium pump in human red cells. J. Physiol. (Lond.) 235: 551-569.

SCHUMANN, H.J., GORLITZ, B.D. and WAGNER, J. (1985): Influence of papaserine, D600 and nifedipine on the effects of noradrenaline and calcium on isolated aorta and mesenteric artery of the rabbit. Naunyn Schiedeberg. Arch. Pharmacol. 289: 409-418.

SHINE, K.I. (1973): Contractile calcium in rabbit myocardium observations with nicotine and quinine. Am. J. Physiol. 224: 1024.

SOFOLA, O.A. (1980): The cardiovascular effects of chloroquine in anaesthesised dogs. Card. J. Physiol. Pharmacol. 58: 836-841.

SOMLYO, A.V., DEVINE and SOMLYO, A.P. (1971): Thick filaments in unstretched mammalian smooth muscle. Nature (Lond.) 233: 218-129.

SOMLYO, A.V. and SOMLYO, A.P. (1968a): Electromechanical and pharmacomechanical coupling in vascular smooth muscle. J. Pharmac. Exp. Therap. 159: 129-145.

SOMLYO, A.V. and SOMLYO, A.P. (1971): Strontium accumulation by sarcoplasmic reticulum and mitochondria in vascular smooth muscle. Science 174: 955-957.

SPARROW, M.P. and SIMMONDS, W.J. (1965): The relationship of the calcium content of smooth muscle to its contractility in response to different modes of stimulation. Biochem. Biophys. Acta 109: 503-511.

SPEDDING, M. and CAVERO, I. (1984): "Calcium antagonists". A class of drugs with a bright future part II. Determination of basic pharmacological properties. Life Sci. 35: 575-587.

SPERELAKIS, N. (1962): ^{45}Ca and ^{89}Sr movement with contraction of depolarised smooth muscle. Am. J. Physiol. 203: 860-866.

TAKUWA, Y., TAKUWA, N. and RASMUSSEN (1986): Carbachol induces a rapid and sustained hydrolysis of polyphospho-

inositades in bovine tracheal muscle. J. Biol. Chem. 261: 14670-14675.

TAYO, F.M. and BEVAN, J.A. (1987): Extracellular calcium dependence of contraction and endothelium dependent relaxation varies along length of the aorta and its branches. J. Pharmacol. Exp. Therap. 240; 594-601.

THORENS and HAEUSLER, G. (1979): Effects of some vasodilators on calcium translocation in intact and fractionated vascular smooth muscle. Eur. J. Pharmacol. 54: 79.

TRIGGLE, O.J. (1981): Calcium antagonists: Basic chemical and pharmacological aspects. In: New perspectives a calcium antagonists. Ed. G.B. Weiss, pp 1-18. Amer. Physiol. Soc.

TSIEN, R.W., HEISS, P., McCLESKEY, E.W. and ROSENBERG, R.L. (1987): Calcium channels mechanisms of selectivity, permeation and block. Ann. Rev. Biophys. Chem. 16: 265-290.

TSIEN, R.W., LIPGCOMBE, D., MADISON, D.V., BLEY, K.B. and FOX A.P. (1988): Multiple types of neuronal calcium channels and their selective modulation. Trends. Neuroscience 11, 431-437.

Van BREEMEN, C. (1977): Calcium requirements for activation of intact aortic smooth muscle. J. Physiol. (Lond.) 272: 317-329.

Van BREEMEN C., AARONSON, P. and LOUTZENHISER, R. (1979): Sodium calcium interaction in mammalian smooth muscle. Pharmacol. Rev. 30: 167-208.

Van BREEMEN, C. and DANIEL, E.E. (1966): The influence of high potassium depolarisation and acetylcholine on calcium exchange in the rat uterus. J. Gen. Physiol. 1299-1317.

Van BREEMEN, C., HWANG, O. and MEISHERI, K.O. (1981): The mechanism of inhibitory action of diltiazem on vascular smooth muscle contractility. J. Pharmacol. Exp. Therap. 218: 459-464.

VANE, J. R. (1957): A sensitive method for the assay of 5-Hydoxytrptamine. Brit. J. Pharmacol. chem. 12: 344-349.

WAGNER-MANN, C.C., ROWMAN, L and STUKER, M. (1991): Primary action of endothelin on Ca^{2+} release in bovine coronary artery. Blood vessels. 21: 267-278.

WAGNER-MANN, C.C., and STUKER, M. (1991): Endothelin mediates Ca^{2+} influx and release in porcine coronary smooth muscle cells. Amer. J. Physiol. Cell Physiol. 260: C771-C777.

WAUD, D.R. (1968): Pharmacological receptor. Pharmacol. Rev. 20: 49-88.

WESTON, A.H. (1990): Antihypertensive agents which open smooth muscle K^+ channels. In: Handbook of experimental pharmacology Vol. 93. Pharmacology of antihypertensive therapeutics. Ed. Canten, D. and Mulrow, P.J. pp. 644-676. Berlin. Springer Vertag.

WEBB, R. and BOHR, D. (1978): Mechanism of membrane sterilisation by calcium in vascular smooth muscle. Am. J. Physiol. 235: C227-C232.

WORLD HEALTH ORGANISATION: Technical report series Vol. 529. 1973.

WOLF, M.H., LEVINE, I., MAYS, W.S. (Jnr), CAUTRECASES, P. and SAHYOUN, N. (1985): A model for intracellular translocation of protein kinase C involving synergism between Ca^{2+} and phorbol esters. Nature (Lond.) 317: 546-549.

YAMASHITA, K., TAKAGI, T. & HOTTA, K. (1977): Mobilisation of cellular calcium and contraction-relaxation of vascular smooth muscle. Jap. J. Physiol, 27, 551-564.

YU SHEU-MEEI, SHENG-CHU KUO, LIU-JIAU HUANG, SELMASIU-MAN SUN, TUR-FU HUANG AND CHE-MING TENG (1992): Vasorelaxation of

rat thoracic aorta caused by two Ca^{2+} channel blockers HA-22 and HA-23. J. Pharm. Pharmacol 44: 667-671.

ZSOTER, T.T., HENEIN, N.F. and WOLCHINSKY, C. (1977): The effect of sodium nitroprusside on the uptake and efflux of ^{45}Ca from rabbit and rat vessels. Eur. J. Pharmac. 45: 7.