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INTERACTIONS OF β -BLOCKERS WITH CELL SUSPENSIONS

A Thesis submitted in partial
fulfilment of the requirements
for the award of the Degree of
DOCTOR OF PHILOSOPHY

by

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Finally, I am very grateful to Mrs. J. F. Owen and J. A. Brennan for their technical assistance, and my typist, Mrs. M. M. Brooks, without whom this work would not have been completed.

(ii)

DEDICATION

To my parents,
brother
and
sisters

ORIGINALITY

All the work presented in this thesis has been carried out by the author except where acknowledged and has not previously been presented for a degree at this University or any other institution.

.....

T. C. Abanzukwe

ABBREVIATIONS

When appropriate the following abbreviations have been used in this thesis.

<u>Ca</u>	about
CFM	Carbohydrate free medium
CMC	Critical micelle concentration
DM	Defined medium
HTAB	Hexadecyl tri methyl ammonium bromide
MIC	Minimum inhibitory concentration
O	Oil
OD	Optical density
RPM	Revolutions per minute
SEM	Scanning electron microscope
TCA	Trichloroacetic acid
TTC	2,3-5 triphenyl tetrazolium chloride
>	Greater than
<	Less than
=	Equal to

Other abbreviations were defined as used in the text.

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CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

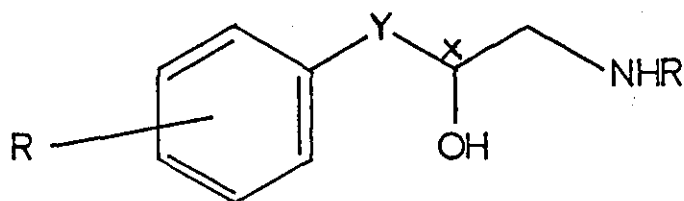
SECTION ONE Beta adrenergic blocking agents (β -blockers)

1.1.1 Chemistry of β -blockers

The development of β -blockers, followed the discovery by Ahlquist (1948) of β -receptors as one of the sites with which catecholamines interact. 3,4-Dichloro analogues of noradrenaline, adrenaline and isoprenaline were first used as β -antagonist (Powell and Slater, 1958), but these agents possess intrinsic agonist activity as well as having carcinogenic effects (Fitzgerald, 1969).

The first useful, clinically important, β -adrenergic blocking agent was designed by Black (1960). Since then hundreds of analogues have been synthesized with only slight structural modifications (Fig. 1.2).

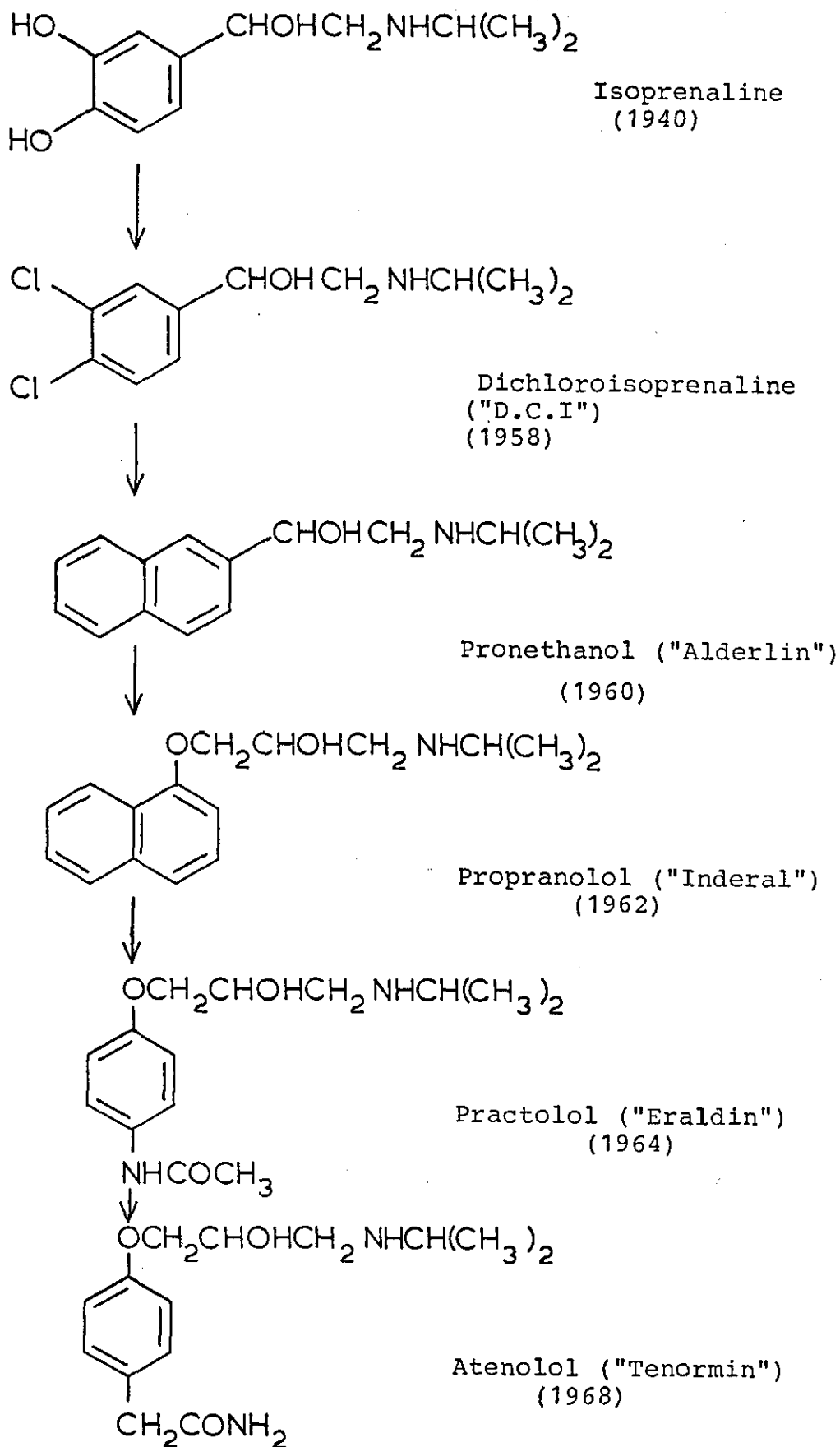
β -blockers consist of two types: the aryl ethanolamine, and the aryloxypropanolamine series. Pronethanol is representative of the former type, while propranolol is typical of the latter. Considering the generalised structure (Fig. 1.1) the main difference between the two types of β -blockers is at position Y. For aryloxyethanolamines, Y is a direct link while for aryloxypropanolamine Y is $-\text{OCH}_2-$. In both types, the two main components of the structure are the β -aminoethanol chain and the aromatic ring (Fig. 1.1). The aromatic ring need not necessarily be



x denotes chiral carbon centre

Fig. 1.1

Fig. 1.2: Modification of Catecholamines



benzenoid but can be heterocyclic (for example, timolol) or benzheterocyclic (for example, pindolol; (Fig. 1.3a,b)).

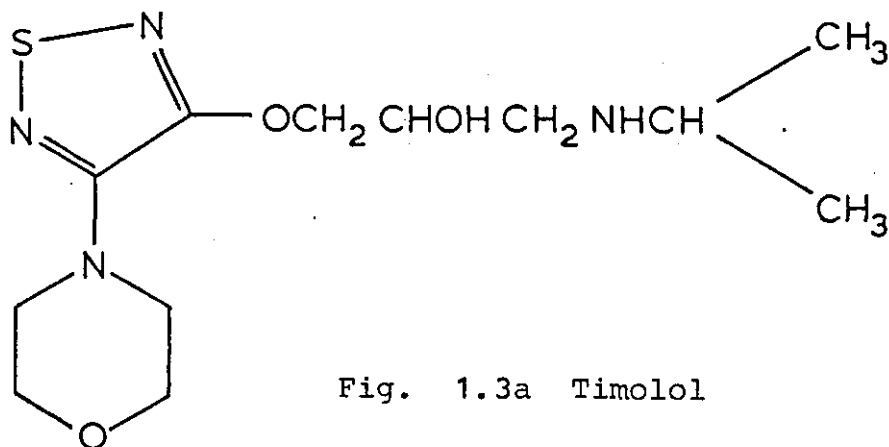


Fig. 1.3a Timolol

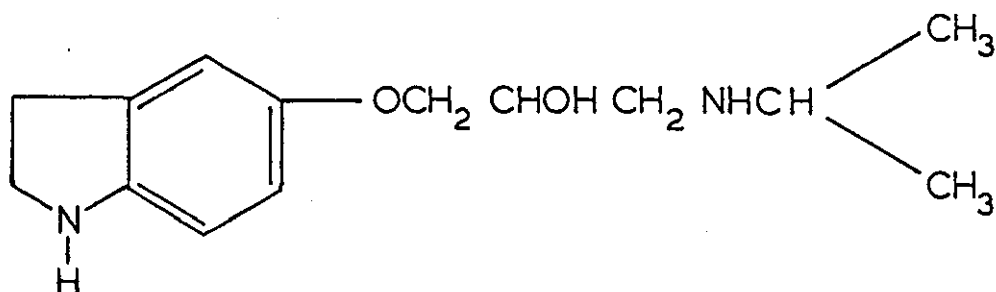


Fig. 1.3b Pindolol

The nature of and the position of the substituents on the aromatic ring give rise to the characteristic property of an individual beta blocker and plays an important role in the pharmacological profile of the molecule.

The chiral carbon atom is directly linked to Y (Fig. 1.1). Its presence means that β -blockers can exist in either (+) or (-) configuration as shown in Fig. 1.4.

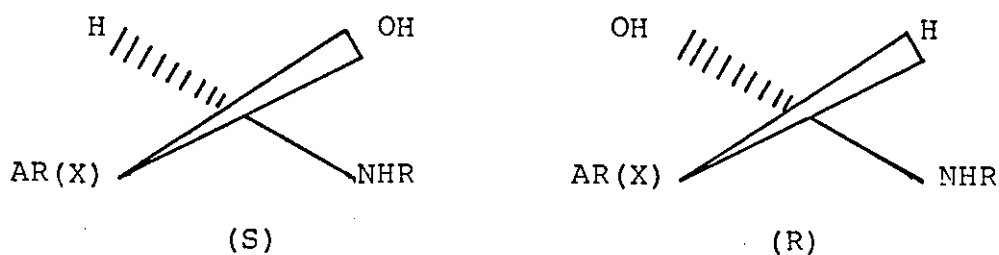


Fig. 1.4 Configuration of β -blockers

The particular configuration each β -blocker adopts is important in the pharmacological properties of the molecule.

1.1.2 Pharmacological actions of beta-blocking drugs

The pharmacological actions of the beta blocking drugs are based on their affinity for beta receptors, normally occupied by catecholamines. Consequently, various kinds of beta responses elicited by catecholamines are inhibited. Because β -receptors fall into two groups (Ahlquist, 1948), known as β_1 and β_2 , beta responses also fall into two groups. One group of responses, including cardiac stimulation and lipolysis, are mediated by β_1 -receptors. The other initiated by β_2 -receptors include relaxation of the smooth muscle of blood vessels and bronchi and glycogenolysis.

Many of the beta blockers inhibit both β_1 and β_2 receptors, (e.g. propranolol) and are therefore non-selective. Many others, such as practolol and acebutolol are selective for the β_1 receptor (Table 1.1).

Several beta blockers have partial agonist activity or "intrinsic sympathomimetic activity" (ISA). Oxprenolol, alprenolol, practolol and pindolol possess this property and are therefore capable of causing β -stimulation of the heart. Oxprenolol and pindolol have ISA at dose levels required for β -blockade. Practolol and alprenolol, however, exhibit ISA at doses lower than those necessary for β -blockade. Usually, stimulation precedes antagonism, and in clinical conditions these compounds may initially aggravate or worsen disease conditions before controlling them (Epstein and Borer, 1976).

β -blockers are used clinically where elevated levels of catecholamines bring about various disease conditions. They are useful in hypertension, angina, trauma, thyrotoxicosis (an enlargement of the thyroid gland), phaeochromocytoma (tumour of the adrenal gland) and anxiety.

Table 1.1 Properties of representative β -receptor blocking drugs

Drug	"Quinidine like activity"	Partial agonist activity	Selectivity	Intrinsic sympathomimetic activity	Relative cardiac specificity
Propranolol	+	-	-	-	-
Alprenolol	+	+	+	+	-
Oxprenolol	+	+	-	+	-
Pindolol	-	++	-	-	-
Practolol	-	+	+	+	+
Timolol	-	-	-	+	-
Sotalol	-	-	-	-	-
Acebutolol	-	-	+	-	-
Atenolol	-	-	-	-	+

After Gibson, D. G. (1974).

β -blockers are also useful in conditions where the mechanism of action is not related to β -blockade. Somani (1965), observed the antagonism by pronethalol and propranolol on cardiac arrhythmias induced in the dog by sympathomimetic amines. Furthermore these drugs, including DCI, also antagonised glycoside induced arrhythmias by a non-specific action (Zwieten, 1969; Hashimoto and Saton, 1979). The mode of action of these drugs resembles that of quinidine. For example, the inhibition of lipid-facilitated transport of Ca^{2+} from Ringer's buffer into chloroform by propranolol is linked with the anti-arrhythmic activity of β -blockers to membrane stabilizing and negative inotropic activities (Luria and Anderson, 1967). This negative inotropic effect of β -blockers was shown to correlate with changes in myofibrillar ATPase activity and changes in the availability of adenosine triphosphate (Nayler and Stone, 1966). This may lead to reduced rate of rise and overshoot of the action potential, increased duration of action potential and prolongation of the refractory period. Related to this is the local anaesthetic activity of beta adrenergic drugs demonstrated by Gill and Williams (1964). Pronethalol was found to be twice as effective as procaine as a local anaesthetic and propranolol slightly more so. Local anaesthetic activity is characterized by inhibition of myocardial Na^{+} channels, slowing of neural conduction in, for example, frog sciatic nerve or diminished corneal responses (Aguilera and Williams, 1965).

These pharmacological properties are related to the "membrane stabilizing activity" (MSA) of β -blockers, a concept first introduced by Guttman (1940). This property is characterized by the physical stabilization of membranes (first described in nerves) and protection against lysis. The concept of membrane stabilization is compatible with the idea of mechanism of local anaesthesia. It was suggested that both membrane stabilization and local anaesthetic action can be correlated closely with physical stabilization and mechanical strengthening of membranes (Seeman, 1966).

Propranolol has been shown by Seppala and Saris (1971) to have activity against phospholipase A. They suggested the possible interaction of propranolol with membrane phospholipids. A number of "non-specific membrane activities" apply to several β -blockers. The fact that none of these effects are associated with the β -blockers atenolol, practolol or pindolol (Smith, 1982) suggests a different mode of pharmacological action other than β -blockade. Furthermore, these ancillary properties are found in both isomers whereas in β -blockade the (L) isomer is about 40 times more active as a β -blocker than the dextro isomer (Howe, 1963).

1.1.3 Structure - activity relationship

Pharmacological parameters such as the inhibition of isoproterenol induced relaxation of smooth muscle, reduction of myocardial contractile force induced by isoproterenol, and the increase in the rate and contractile force of guinea-pig or rabbit atria, have been used to determine the potency and specificity of potential β -blockers. Since β -blockers competitively inhibit β_1 and β_2 -receptors, affinity for these receptors have been used as an indication of blocking potency or the inhibition of the second messenger to which β -receptors are linked, namely adenyl cyclase. Inhibition of lung adenyl cyclase (mainly β_2 tissue) is correlated with bulk along the face of the β -blocker molecule (Unger (1980)) especially by position 5 down the alkyl chain from the -NH (Fig. 1.5).

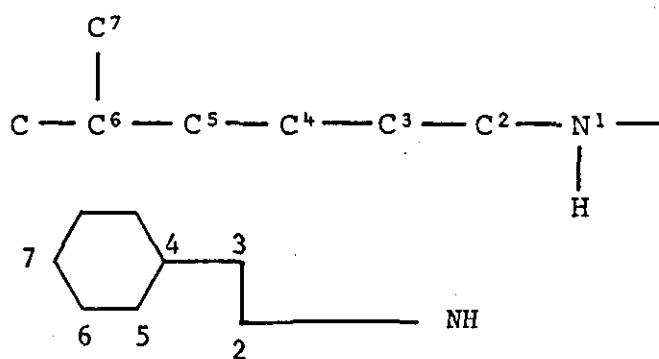


Fig. 1.5

In contrast, heart activity (mainly a β_1 tissue) is increased by bulk of the side chain on the aromatic ring. Cyclic substituents on the aromatic ring are preferred (position 6 and higher), and was explained as the difference in β_1/β_2 selectivity amongst β -blockers. Further investigations by Unger (1980) suggest that β_1 and β_2 receptors exists in slightly different conformations, resulting in varied shapes and sizes of these receptor sites. These findings are in agreement with the findings of (Barrett and Carter, 1970).

Main and Tucker (1985) suggested that β -blockers with cardioselective property are more hydrophilic than propranolol, suggesting a relatively hydrophilic β_1 receptor compared to a more lipophilic β_2 -receptor. However, Gibson (1974) showed that selectivity resided with the para substituent only. Further evidence on the significance of substituent position on the benzene ring resulted from the findings of (Unger, 1980)) when he introduced a methylene ($-\text{CH}_2$) group between the amide group and the aromatic ring resulting in the formation of atenolol which is devoid of ISA, but was as potent as propranolol in terms of cardioselectivity. In addition, the related $p\text{-CH}_2\text{NHCOR}$ and $p\text{-CH}_2\text{NHCONHR}$ analogues were cardioselective but devoid of ISA. It is possible that cardioselectivity results from an interaction of a para substituent, through perhaps hydrogen bond formation with a complementary site on the β_1 -receptor.

The ISA activity of a number of ortho substituted phenoxypropanolamines was correlated with specific conformations by noting the increase in heart rate when 2.5 mg/kg of the compound was given intravenously to rats depleted of catecholamines (Levy, 1968a). Structural variations showed the size of the ortho substituent to be very important in determining the extent of ISA levels. It appears that the smaller the ortho substituent the longer is the ISA observed. The influence of aromatic ring substituents was provided by using hydroxy substituents. The aryloxypropanolamine analogue of isoprenaline (Fig. 1.6a) is a β -agonist, however, the dihydroxy

analogue of the same compound is a partial agonist (Fig. 1.6b). The metahydroxy (Fig. 1.6b1) and the para-hydroxy (Fig. 1.6a1) analogue are also partial agonists (Main and Tucker, 1985).

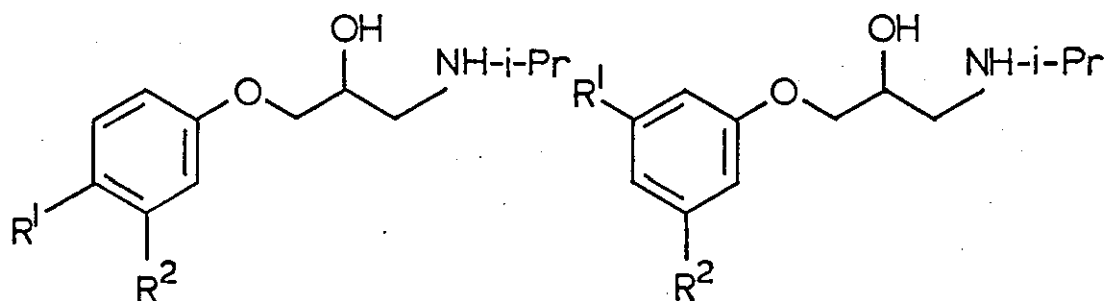


Fig. 1.6a, $R_1 = R_2 = \text{OH}$

Fig. 1.6b, $R_1 = R_2 = \text{OH}$

Fig. 1.6a1, $R_1 = \text{OH}$; $R_2 = \text{H}$

Fig. 1.6b1, $R_1 = \text{OH}$; $R_2 = \text{H}$

In general, amino substituents that give blocking potency include: 5-butyl > isopropyl > secbutyl isobutyl ~ sec amyl. The activity increases with bonding and decreases with chain lengthening or with alkyl groups smaller than C_3 (Barret and Carter, 1968). Further substitution on the α - or β -carbon atoms decreases activity; removal or alkylation of the hydroxy group or acylation of the amine resulted in great loss of biological activity. A model for β -blockers is shown in Fig. 1.7.

Levy (1968a) attempted to relate the cardiodepressant effects of some β -blockers to their non-specific membrane effects and thus to physicochemical properties. He observed that the more lipophilic propranolol and pronethalol were stronger local anaesthetics than the hydrophilic sotalol or practolol. However, he did not observe any quantitative correlation between lipophilicity and specific β -blocking activity or non-specific depression of myocardial contraction on isolated rabbit atria.

He also observed that (+)-propranolol (a very weak β -blocker) was equipotent with its racemate as a local anaesthetic. In addition he observed that β -blocker

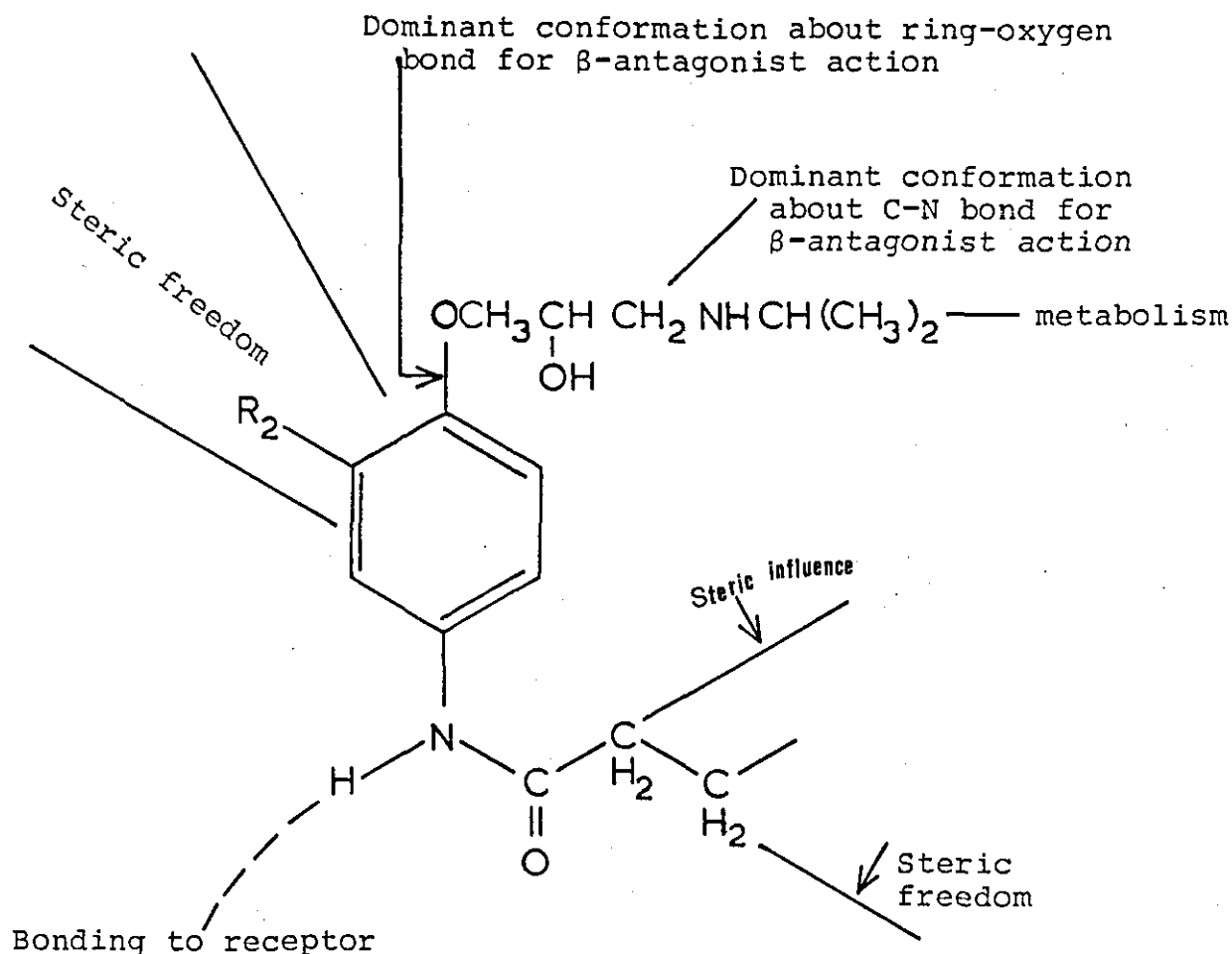


Fig. 1.7 Model for β -blockers (after Davies 1979)

surface activity is related to negative inotropism, in agreement to the findings of Hellenbrecht (1973), and Lemmer, (1974). Furthermore, Wiethold (1972) observed that the uptake of [^3H] serotonin in human platelets was inhibited by several β -blockers; the extent of inhibition followed the order: propranolol, > sotalol > practolol. Because of this correlation, Wiethold (1972) proposed that the non-specific membrane effects of all β -blockers could be predicted simply from their partition coefficients. Similar results with propranolol, pindolol,

and practolol, were obtained by Akiyama and Igisu (1979) in a study of fluorescence polarization of 1,6-diphenyl-hexatriene (DPH) labelled human erythrocyte ghosts. Where as propranolol was effective and induced a significant (11%) fluorescence polarization, practolol and pindolol had no effect. Lee and Carter (1977) showed that the transition temperature of dipalmitoyl phosphatidylcholine (DPPC) and dipalmitoylphosphatidyl ethanolamine (DPPE0) was reduced by propranolol, while practolol was ineffective even at very high concentrations.

The negative inotropic receptor is a protein tropomyosin complex Ca^{2+} tropomyosin, and actomyosin (Honig and Reddy, 1973). Rauls and Baker (1979) showed that there is a close correlation between antiarrhythmic activity of β -blockers membrane stabilization events and the negative inotropic activity and depletion of Ca^{2+} stores in the cardiac sarcoplasmic reticulum. Hellenbrecht and Lemmer (1973) observed that the membrane stabilising activity of a series of β -blockers was related to their partition coefficients, the more lipophilic propranolol being more effective local anaesthetic compared to hydrophilic β -blockers. Lipophilic β -blockers are thought to bind to the lipophilic cell membrane at physiological pH. The protonated aliphatic nitrogen at this pH, interacts with a polar group of cell membrane in such a way that Ca^{2+} is displaced, while the lipophilic aromatic position is still embedded in the lipid bilayer. The resulting effect is that Ca^{2+} transport across the cell membrane is prevented thereby producing the desired antiarrhythmic activity.

Löfgren (1948) arranged local anaesthetic according to the following simple scheme (Fig. 1.8).

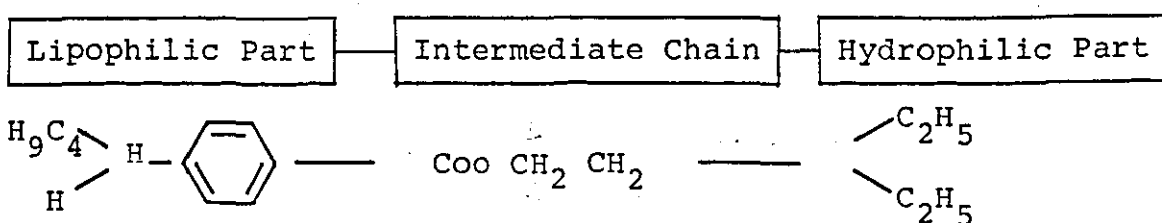


Fig. 1.8 Schematic representation of local anaesthetic structure

The lipophilic and the hydrophilic part consist of aromatic and amine positions respectively as shown for tetracaine (Fig. 1.8).

Structurally, β -blockers are similar to local anaesthetics. Fig. 1.9 illustrates the main chemical features of β -blockers which may be represented as the lipophilic, intermediate and hydrophilic positions.

The intermediate and the hydrophilic positions are the same with respect to the constituent parts. However, changes in the lipophilic positions brought about by substituents on that part of the molecule may have a profound effect on the membrane activity. Substituents which reduces the lipophilicity on the aromatic ring will reduce membrane activity, while substituents which increases the lipophilicity on the benzene ring will increase membrane activity.

Hellenbrecht and Lemmer (1979) arrived at the following conclusion on requirements for lipophilicity:

- (1) Prediction of blocking and membrane activity requires sufficient lipophilicity and bulk or para ring substituents.
- (2) Electronic - withdrawing substituents on the ring and the lipophilicity and size of amine substituent influence β -mimetic activity but diminish membrane activity of these compounds.

Thus changes in the hydrophile/lipophile balance as well as the molecular bulk of the β -blocker will have profound effects on the membrane activity of these compounds.

1.1.4 Toxicity of β -blockers

The relatively minor side effects of β -blockers over other antihypertensive agents often makes them the drug of choice in the control of blood pressure. They do not cause orthostatic hypotension, thereby providing a very even control of pressure (Lucchesi, 1969). The side effects associated with β -blockade are due mainly to

Fig. 1.9: Schematic arrangement of β -Blocker Structure
(Tetracaine and Procaine included for comparison)

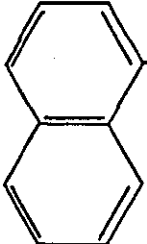
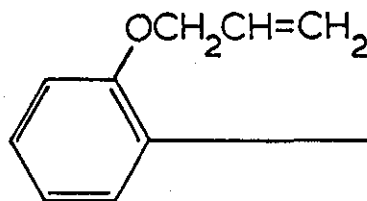
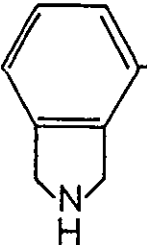
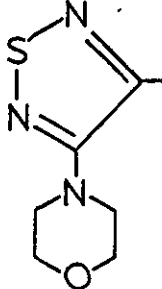
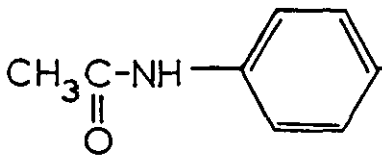
LIPOPHILIC	INTERMEDIATE CHAIN	HYDROPHILIC
PROPRANANOL 	$\text{OCH}_2\text{CHOHCH}_2$	$\text{NHCH} \begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$
OXPRENOLOL 	$\text{OCH}_2\text{CHOHCH}_2$	$\text{NHCH} \begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$
PINDOLOL 	$\text{OCH}_2\text{CHOHCH}_2$	$\text{NHCH} \begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$
TIMOLOL 	$\text{OCH}_2\text{CHOHCH}_2$	$\text{NHCH} \begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$
PRACTOLOL 	$\text{OCH}_2\text{CHOHCH}_2$	$\text{NHCH} \begin{matrix} \text{CH}_3 \\ \text{CH}_3 \end{matrix}$

Fig. 1.9 Continued

LIPOPHILIC	INTERMEDIATE CHAIN	HYDROPHILIC
ATENOLOL <chem>NC(=O)Cc1ccc(cc1)COCC(O)CN(C)C</chem>		
ACEBUTOLOL <chem>CC(=O)c1ccc(cc1)NC(=O)CC(C)COCC(O)CN(C)C</chem>		
METOPROLOL <chem>COCCc1ccc(cc1)COCC(O)CN(C)C</chem>		
TETRACAINE <chem>CCCCNc1ccc(cc1)C(=O)OCCN(C)C</chem>		
PROCAINE <chem>Nc1ccc(cc1)C(=O)OCCN(CC)CC</chem>		

their non-selective inhibition of β_1 and β_2 receptors. It is possible to precipitate asthma following the inhibition of β_2 receptors of the lung. The vasoconstriction will reduce lung airways and hence precipitate asthma.

Associated with virtually all the β -blockers is the risk of cardiac failure, since the result of β -blockade is a reduction in the heart rate, which may lead to fall in cardiac output.

Various syndromes such as psyche, including dreams, hallucinations, insomnia, and depression are associated with β -blockers. These side effects reflect the ability of the lipophilic β -blockers to cross the blood brain barrier (Cruickshank and Neil-Dwyer, 1985). Practolol has no C.N.S. side effects because of its hydrophilic nature. It is a safe drug for asthmatics, in addition it brings about a smaller reduction in cardiac output compared with propranolol. However, it was withdrawn from clinical use, because of eye problems which may lead to blindness. (Main and Tucker, 1985).

β -blockers are likely to precipitate hypoglycemia, since they interfere with adrenergic stimulation of glycogenolysis in skeletal muscle likely to result in an increase in plasma lactate. However, lactate may be converted to glucose which is added to plasma pool.

Propranolol does not interfere with pregnancy though it is capable of crossing the placenta to the foetal circulation. This could lead to foetal cardiac failure, or could impair foetal cardiac responses to stresses of labour, thus affecting delivery (Reed, 1974).

It is also likely that reduction in cardiac contractility associated with β -blockers may lead to increase in ventricular size (Lucchesi, 1976), as well as filling pressure, wall tension and end diastolic pressure (Parker, 1968). Adverse effects associated with these changes in normal cardiac function may lead to the following: failure in compromised heart, excessive withdrawal of β tone in pulmonary and peripheral regions causing possible bronchoconstriction and vascular claudication respectively; precipitation of acute myocardial infarction after sudden

withdrawal (Miller, 1975). Additionally a variety of less severe side effects have been noticed such as nausea, lethargy, oxygen haemoglobin dissociation, inhibition of free fatty acid release from adipose tissue and loss of mechanism *in vivo* to regulate pulmonary collagen production which may result in fibrosis (Lindenschmidt, 1984).

SECTION TWO

Comparative Cytology

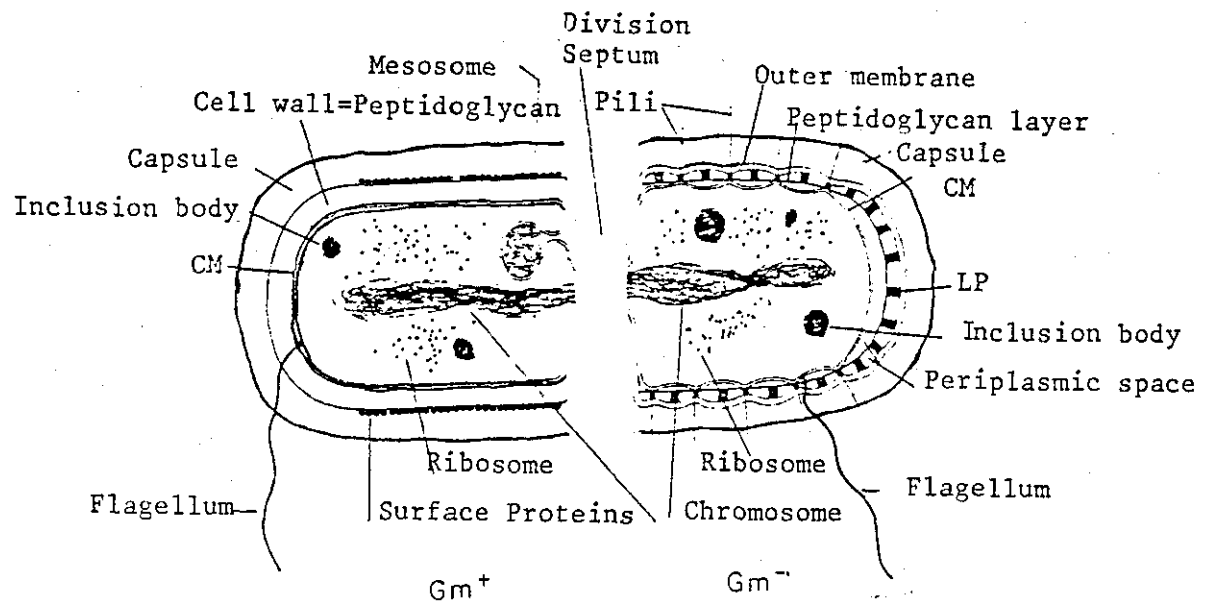
Soon after the discovery of β -adrenergic blockers by Black (1960), it was found that several β -blockers had membrane activity as discussed in Section One. Thus, it is appropriate to provide a comparative account of the surfaces presented by prokaryotic and eukaryotic cells to their surroundings in order to appreciate the likely site of primary interactions between cells and the β -blocking drugs.

1.2.1 Comparative Account of Cell Surfaces

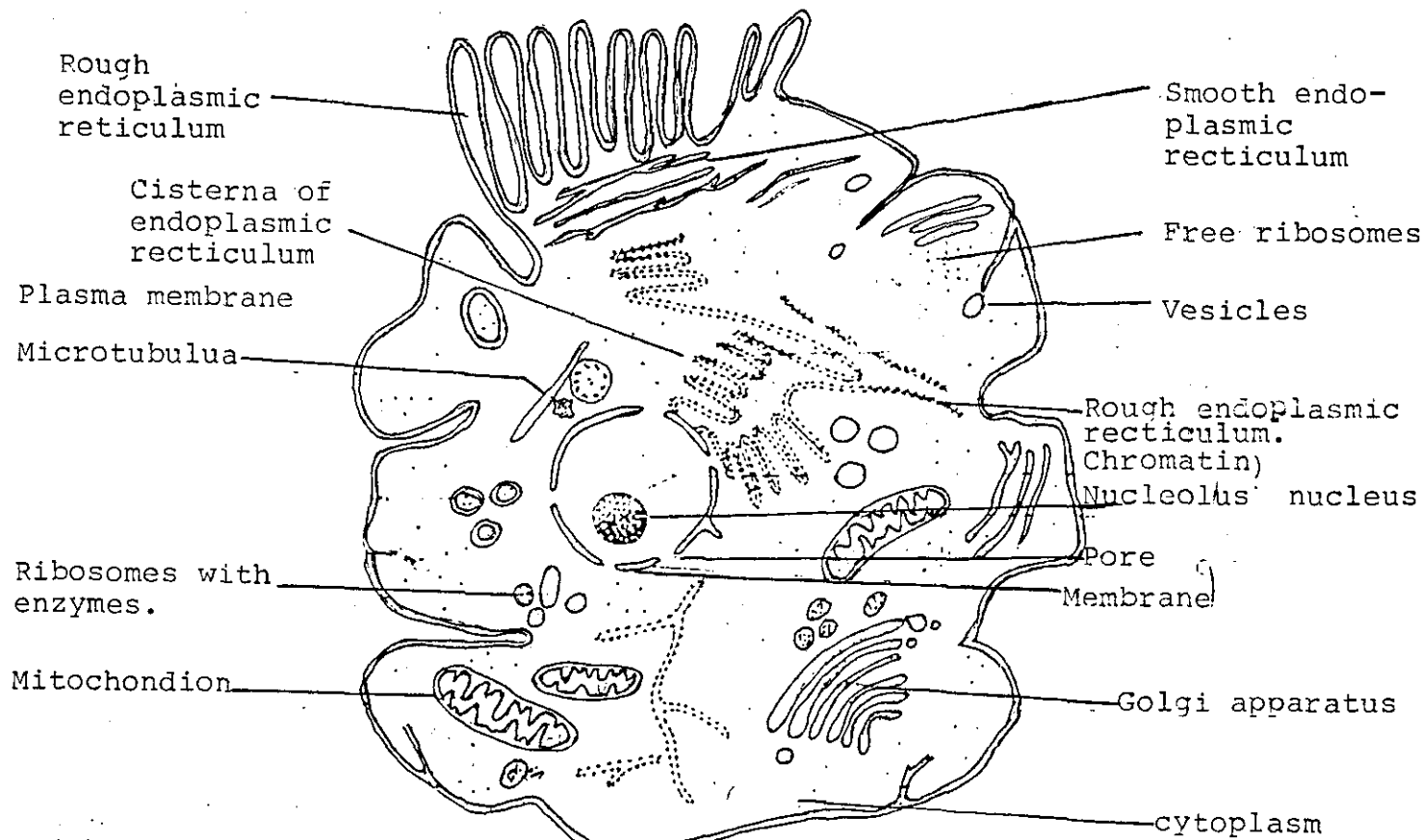
Cells of both eukaryotes, and prokaryotes possess a cell membrane. In animal cells, for example the erythrocyte, the cell is delimited by the plasma membrane. However in some eukaryotes such as the fungi and in the prokaryotes, the situation is rather complex, involving a cell envelope which makes contact with the immediate environment (Fig. 1.10). The nature and chemical composition of the envelope is different in gram-positive and gram-negative bacteria as well as in fungi.

The outer most layer of the cell envelope of Gram-positive bacteria is the cell wall. It is a homogenous layer 10 - 15 nm wide, consisting mainly of peptidoglycan (Murien). Teichoic acids, polysaccharides and proteins are also present (Salton, 1967). Peptidoglycans are heteropolymers, based on repeating subunits of N-acetylglucosamine and N-acetylmuramic acid (Fig. 1.11a) and a small number of amino acids. The two acetyl sugars occur alternatively in glycan strands in β -1,4 linkage. Each strand consists of disaccharide units, the number of which depends on the individual species.

The peptidoglycan of Gram-positive bacteria is additionally linked to teichoic acid (a phosphate polymer of glycerol or ribitol) which appears in different forms depending on the nature of the repeating backbones. It is this, as well as the nature and position of the phospho-



(A) Diagram of "Earliest form of organism" that from which others are developed. Gram-positive (Gm^+) cell on left and Gram-negative cell (Gm^-) on right, showing similarities and cell envelope differences. CM, cytoplasm membrane, LP, lipoprotein.
(After Joklik, 1984).



(B) Generalised Eukaryotic animal cell (After Wilson, 1977).

diester linkage and various substituents such as D-alanine, glucose or succinate which is responsible for conferring individuality in the surface structure of the different Gram-positive strains.

The peptidoglycan content of "walls" of Gram-negative bacteria is comparatively low (up to 5 to 10 per cent of the wall; Salton, 1967). It is located in the inner most portion of the wall (Ghuysen, 1968, Salton, 1967). As in gram-positive bacteria, the two acetyl sugars (N-acetylglucosamine and N-acetylmuramic acid) are joined by β -1, 4-glycosidic bonds. In addition the acetyl sugars repeat alternatively, but the glycan strand of peptidoglycan is covalently linked to lipoprotein and to a layer of lipopolysaccharide (Fig. 1.12). Various substituents such as amino acids are crosslinked to the glycan strands but the nature of these peptide chains is different from those of Gram-positive bacteria (Fig. 1.11a,b).

In both Gram-positive and Gram-negative bacteria, the glycosidic bonds and peptide bonds hold together the peptidoglycan subunits in a molecular mesh or fabric, enabling it to withstand tumor pressure which originates from the protoplast. In Gram-positive bacteria, teichoic acid binds to Mg^{2+} and may have a responsibility for transporting this ion into the cell. In addition it acts as the surface antigen of Gram-positive bacteria (Vanderkooi and Green (1967)).

The outer part of the "wall" of Gram-negative bacteria is termed the outer membrane (Mühlradt and Goleck, 1975) approximately 7-8 nm wide, with fine structure, and overall appearance similar to that of unit membrane. It consists of lipopolysaccharide, phospholipids and proteins (Salton, 1967; Ghuysen, 1968; Mühlradt and Golecki, 1975).

However, lipopolysaccharide is the major component of the outer membrane. It is located at the cell surface, the polysaccharide chain extending externally (Fig. 1.13a). It is an oligomer, consisting of three subunits; Lipid A, the R core region, and the O-side chain (Stanier, 1976), linked together through pyrophosphate bridges in the lipid A region. The lipophilic A region consists of disaccharide and esterified fatty acids. The hydrophilic R core

Fig. 1.11(a)

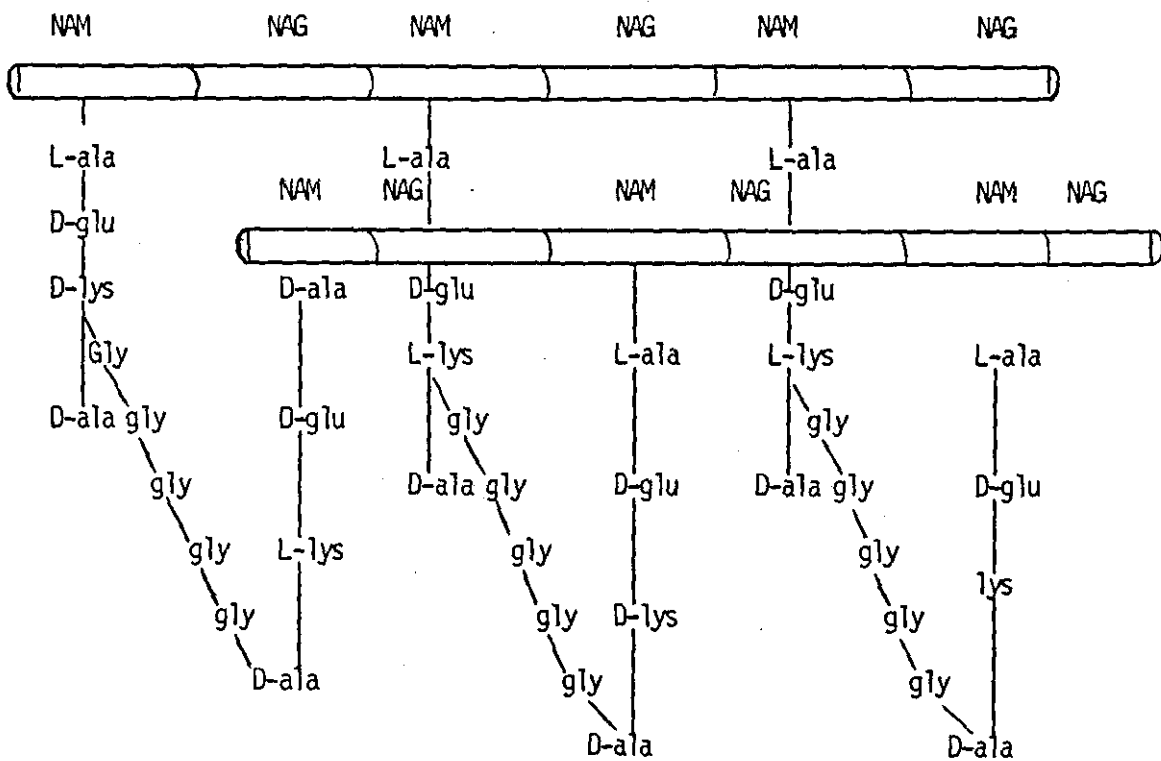


Fig. 1.11(a)

Cross-linked mucopeptide (peptidoglycan) arrangement in *S. aureus*.

Abbreviations: NAM = N-acetylmuramic acid. NAG = N-acetylglucosamine.
L-ala = L-alanine. L-lys = L-lysine, gly = glycine, D-ala = D-alanine, D-glu = D-glutamate.

Fig. 1.11(b)

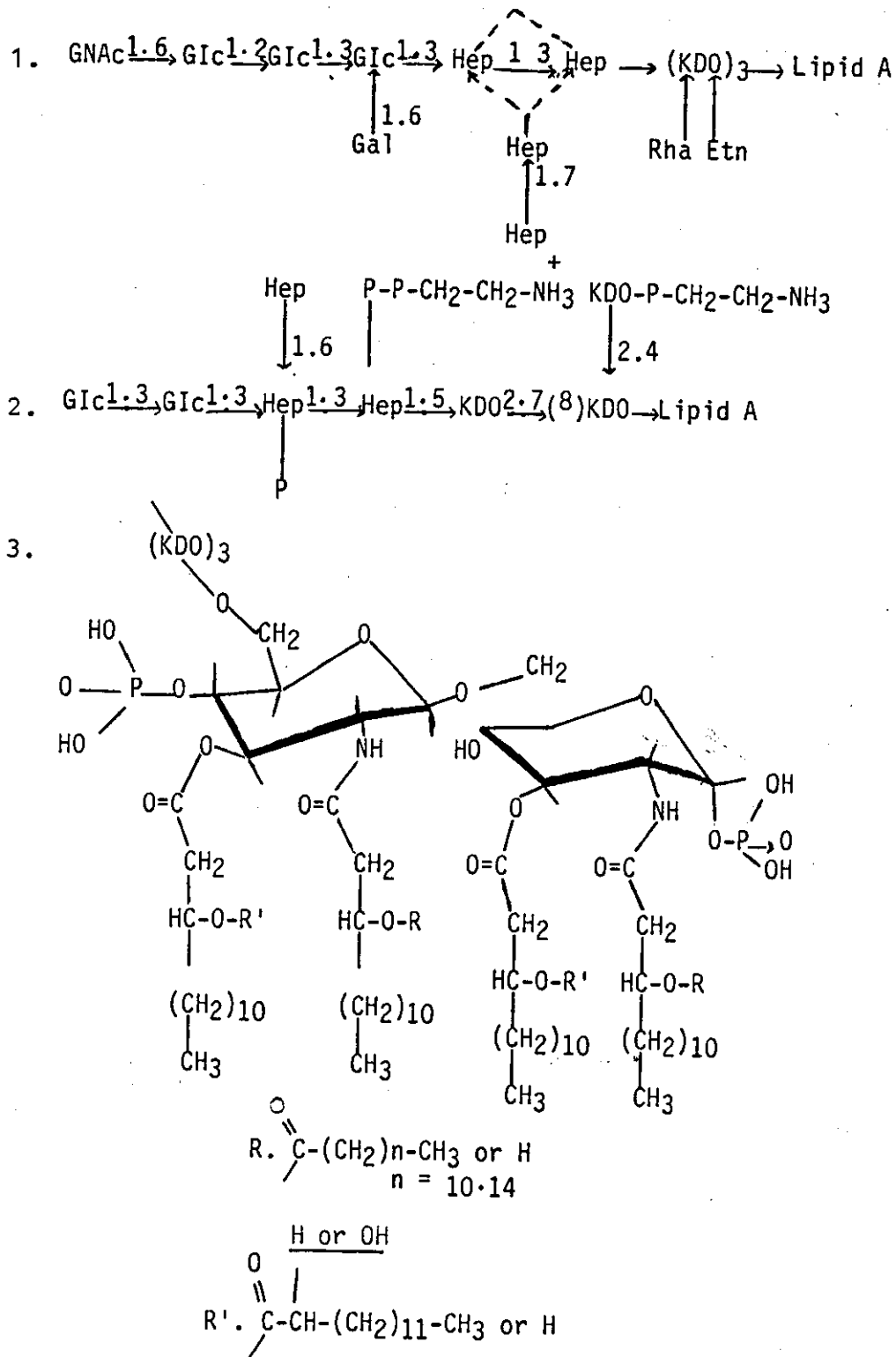


Fig. 1.11(b)

Chemical structure of lipopolysaccharide. (1) E.coli K12; (2) E.coli B; (3) a proposed structure of lipid A region. Glc, glucose; GNAc, N-acetylglucosamine; Gal, galactose; Hep, heptose; KDO, 2-keto-3-deoxyoctonate; Rha, rhamnose; Etn, ethanolamine; P, phosphate.

consists of sugars and repeating tetra-or-pentasaccharide depending on the strain (Schneider and Rosenbush, 1981).

Lipopolysaccharide interacts with major lipoproteins of the outer surface, in a manner that results in a hexagonal lattice structure (Datta and Arden, 1970). Many other functional aspects result from the interaction between lipopolysaccharide and specific proteins of the outer leaflets:

- (1) The functioning of the outer membrane protein F (Ompf) pore function) requires the presence of lipopolysaccharide (Schneider and Rosenbush, 1981; Barbara and Angus, 1983).
- (2) The presence of lipopolysaccharide and OMPC in the K₁₂ strain of E.coli (Mutoh, 1978; Wilson and Luftig, 1970; Yu, 1981; Yu and Mizushima, 1982) is very important in the functioning of bacteriophage T₄ and tub for which it acts as a receptor.
- (3) The interaction between lipopolysaccharide and OMPA protects the former from random hydrolysis by proteases (Yu, 1981). This observation is in agreement with the findings of Datt and Arden, 1974; Ames, 1974; Lugtenberg, 1976 and Beher, 1980).

Lipoprotein may interact with themselves to form oligomeric form (Inouye, 1974 and Sato, 1977) giving rise to a trimer (Yu, 1984) on a hexagonal lattice with 7.7 nm repeat. Recently Rosenbush isolated the three dimensional structure, this envelope protein having a pore function (Gravito, 1983). In antibiotic resistant bacteria, for example, Pseudomonas aeruginosa, the proportion of pore forming proteins in a closed state is much higher than those in an open state, thus reducing the amount of drugs penetrating through the hydrophilic pores (Angus and Carey, 1982).

The lipoprotein so far described are referred to as major proteins, and some are covalently bound to peptidoglycan layer (Braun and Rehn, 1969; Braun and Gnirke, 1973) Fig. 1.12, while others are present in a free form (Inouye and Shaw, 1972). Other major proteins (Mizuno, 1979;



Fig. 1.12:

The chemical structure of the bound form of the major lipoprotein DAP, diaminopimelic acid MNAC, N-acetylmuramic acid; GNAC. N-acetylglucosamine, R₁, R₂, and R₃ represent carbon chains of fatty acids.

(After Rogers and Perkins, 1980a)

Ichihara, 1981) also covalently bound to peptidoglycan (Mizuno, 1979, and Yu, 1981 are involved in surface extrusion of F.plasmid (Narayarunm and Minkley, 1984).

Phospholipid molecules are present in the outer membrane in small numbers in Gram-negative bacteria (Miura and Mizushima, 1968; Osborn, 1972) localised in the inner leaflet of the surface (Donohue and Schaechter, 1930) and consist of phosphatidyl ethanolamine, phosphatidyl glycerol, and cardiolipin.

In Gram-negative bacteria, outside the relatively thin peptidoglycan layer is an aqueous compartment referred to as the periplasmic space, containing various sequestered proteins (Fig. 1.13a). Some are hydrolytic enzymes (proteins, RNA, and DNA nucleases, phosphatases, phosphodiesterase and β -lactamase which assist in nutrient acquisition. Others are specific binding proteins for sulphate, galactose, maltose, glutamine, and many other amino acids (Ito, 1981; Mutoh, 1982).

Fungal envelopes are essentially similar to those of Gram-positive bacteria which consist of a rigid wall layer (Fig. 1.13b). The major macromolecule of fungal walls is polysaccharide (Aronson and Preston, 1960; Braun and Calderon 1981), consisting of the following chemical component: chitin, mannan, glucan and sometimes cellulose (Sekeya and Nozawa, 1983). There are proteins and lipids, although the latter represents only a small proportion (Hunsley and Burnett, 1970).

In the dimorphic yeast form of Candida albicans, chitin is the major component of the septum which separates the bud and mother cell in the yeast form, as well as playing an important role in the formation of the septa and of the primary apical wall of the hyphal form (Gilpatrick, 1981 and Rogers and Perkin, 1980). Glucan and mannan are highly branched, showing high degree of polymerization (Hassid and Joslyn, 1941; Chattaway and Homes, 1968; Rogers, 1963). The product of hydrolysis (Citonelli and Smith, 1955) show a 1 $\rightarrow$ 6 D-glucose linkage. 1 $\rightarrow$ 6 mannose linkage, in addition to 1 $\rightarrow$ 3 linkage in

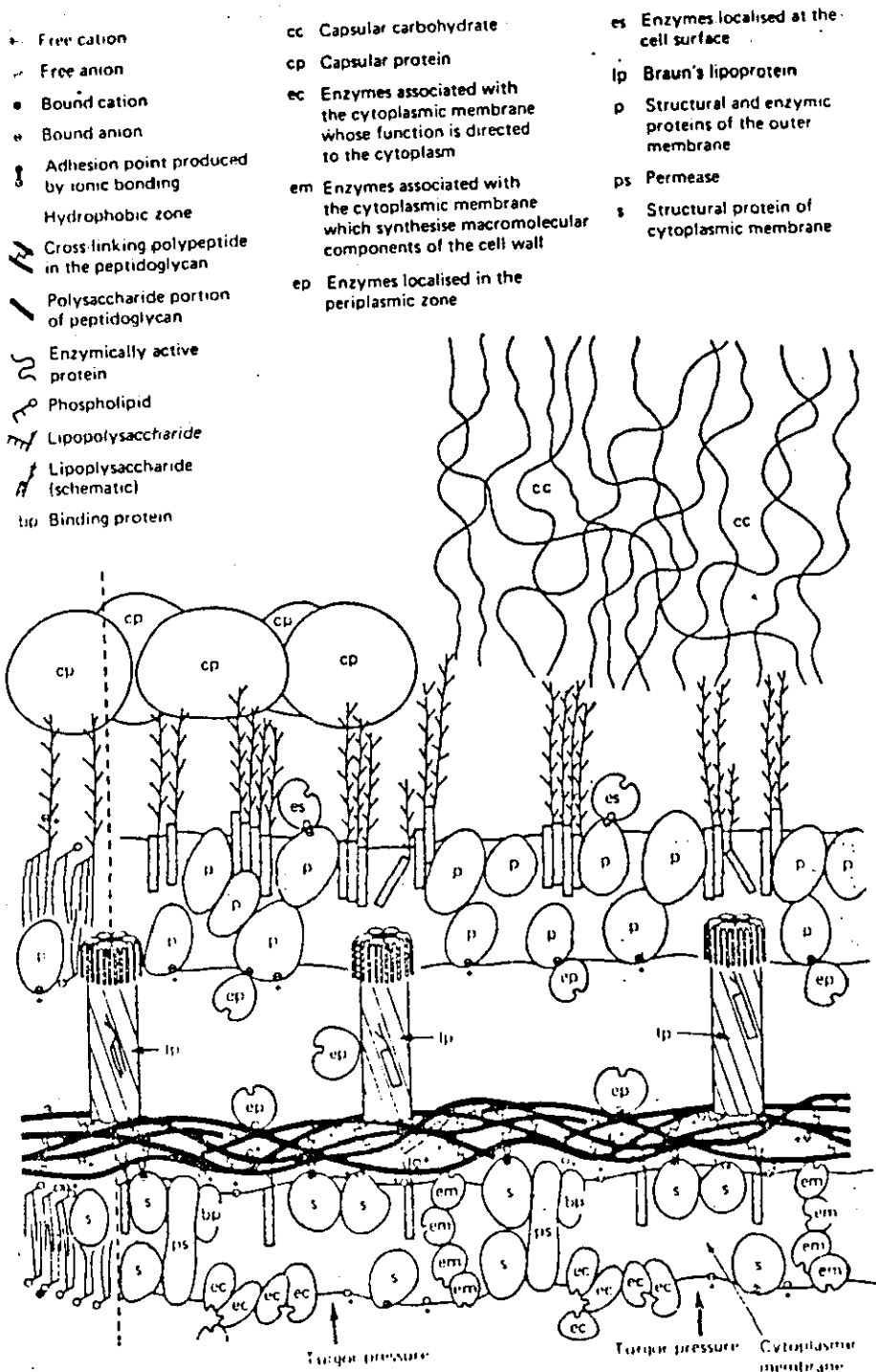


Fig. 1.13a:
 Structure of Gram-negative cell wall.
 (After Costerton and Cheng, 1975).

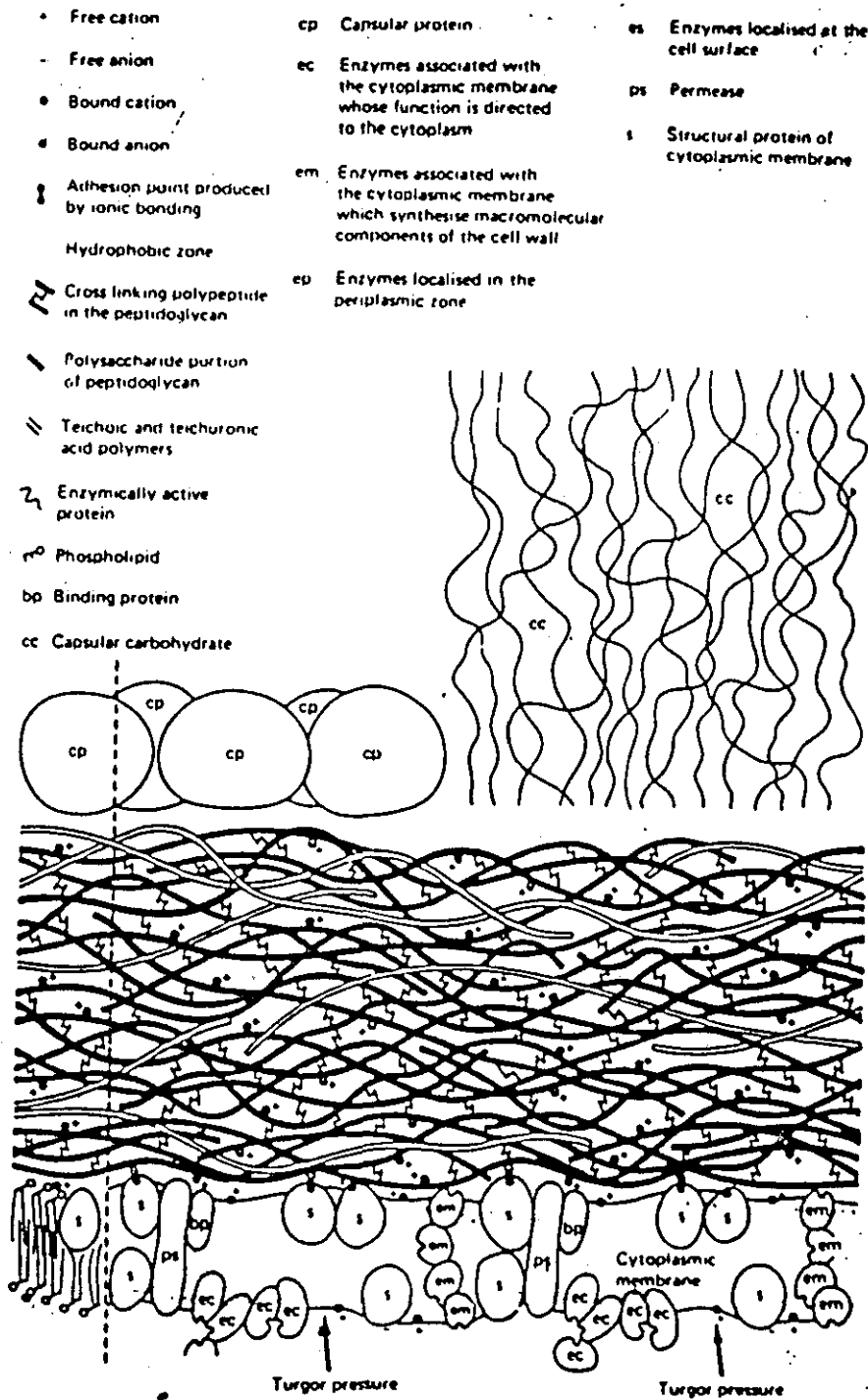


Fig. 1.13b:
Structure of Gram-positive cell wall.
(After Costerton and Cheng, 1975).

glucose and mannose units in non-reducing and reducing terminal units respectively. In addition, relatively short chains of α 1 $\rightarrow$ 2 linked units are joined together by α 1 $\rightarrow$ 6 linkages in mannose unit (Gorin and Perin, 1956; Hunter and Rose, 1971). All the linkages are firmly held by glycosidic bonds.

The presence and nature of these linkages confer individuality to different classes of fungi (for example, the absence of 1 $\rightarrow$ 3 linkages and relatively high degree of branching of mannose units in C.albicans) and constitute differences between the mannan from C.albicans and those examined from other yeasts (Citonelli and Smith, 1955). Furthermore, the glucan from C.albicans differs from those of Saccharomyces cerevisiae in being more highly branched and having 70% of β -1-6 linkages (Rose, 1976; Rogers and Perkins, 1986).

These polysaccharides are protective in function as in the bacterial cell wall and protect the protoplast from osmotic and other changes in the environment. In addition they are responsible for the characteristic shape of the cell and have to be modified during cell changes such as: growth of hyphal tip, initiation of branching hypha, change from mycelial to yeast form or vice versa (cardiospore) (Braun and Caldevon, 1981; Bloch, 1983).

Thus, the differences in Gram-positive and Gram-negative bacteria as well as in fungi in terms of surfaces presented to the outside environment can be seen as one of chemical composition, associated to each group of organisms. The outer surface of Gram-negative organisms is a series of layers, the outermost of which is membrane-like and encloses a periplasmic space. Beneath this lies the peptidoglycan (wall material) which is linked to the cell membrane, thus the outer membrane-like layer of Gram-negative bacteria provides an extra protection against the passage of drugs. On the other hand, the outermost layer of Gram-positive bacteria and fungi is the thick homogenous wall, which in case of Gram-positive is mainly the heteropolymer peptidoglycan and in fungi are chitin, glycan, and mannose. This major difference between these organism have

been implicated as the consequence of greater antibiotic susceptibility of Gram-positive organisms and some fungi compared to Gram-negative organisms (Brown and Melling, 1975).

Flagella, pili and coating material (slime) are appendages of bacterial surfaces. Flagella interact with both lipopolysaccharide and peptidoglycan layer of bacterial cells (Osborn, 1972) and functionally, are responsible for bacterial movement. Pili are very important in both surface adhesion and genetic exchange from cell to cell (Stanier, 1976). Coating material is protective in function. Coating material such as the capsule is a loose layer of polysaccharide extending the cell diameter. In addition to providing a means of cell adhesion, it is also very important in enabling the cell to compete favourably in its environment.

Both eukaryote and prokaryote cells possess a cytoplasmic membrane. Physico chemical and comparative chemical studies of the cytoplasmic membrane of bacterial cells with those of other biological membranes have shown that they are very similar (Salton, 1967). The fluid mosaic model (bilayer "unit membrane") proposed by Singer and Nicholson, (1972) is characteristic of all biological membranes (Fig. 1.14a). The model provides a dynamic asymmetric structure in which, lipids and proteins are distributed in an asymmetric manner, allowing control of movements of molecules into and outside the cell.

The major chemical constituents of the cell membrane are phospholipid and proteins. Phospholipids are arranged as a bilayer with some proteins intercalated within it; other proteins adhere more or less strongly on both sides in an asymmetric manner (Lenaz and Parenti, 1974). Phospholipids are derived from phosphatidic acid. Phosphatidyl ethanolamine (PE) is the major phospholipid; phosphatidyl glycerol (PG); and cardiolipin or diphosphatidyl glycerol (di PG) is present in a smaller amount compared to either (PE or PG) (Lenaz and Parenti, 1974; Freedman, 1981).

In the eukaryotic membrane, the major phospholipids in addition to (PE), (PG), and (di PG) includes lecithin (phosphatidylcholine), phosphatidylserine, sphingomyelin,

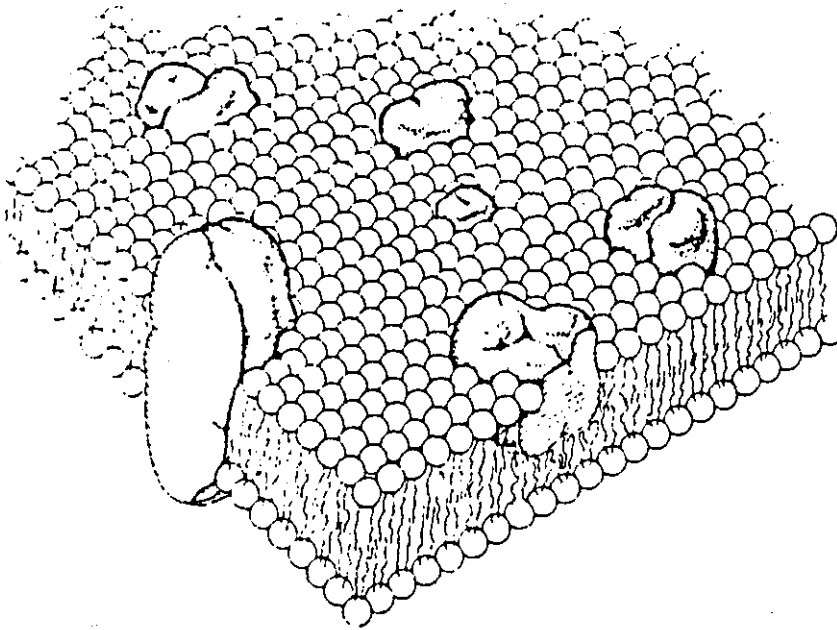


Fig. 1.14a:

The fluid-mosaic model for membrane structure. The matrix of lipid bilayer is shown with the stippled bodies inserted into it as it is imagined integral membrane proteins exist.

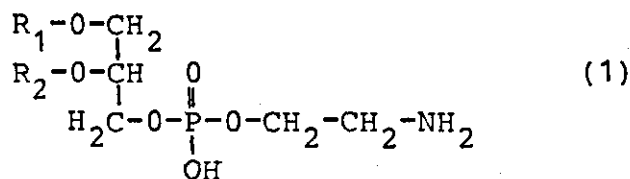
(After Metcalfe and Kornberg, 1978).

phosphoinositide (Fig. 1.14). These compounds are of special interest, as they bear structural and functional significance and may represent the component of membrane responsible for the biconcave shape of erythrocytes (Kates and Allison, 1961). The other class of lipids known as sterols are particularly present in the eukaryotic cell membrane. The interactions between cholesterol and lecithin is such that the former condenses the latter. The resulting arrangements or packaging stabilizes the erythrocyte membrane, and allows the rotational movement of the lecithin, which depends on the right proportions of cholesterol (De Bernard, 1958). The proportion of phospholipids differs from one cell type to the other. It also differs among different strains belonging to the same group of bacteria. In addition, the proportion of phospholipids in the outer membrane of Gram-positive organisms differs from those in the cytoplasmic membrane. (Wovikoff, 1961; Rogers and Perkins, 1980a). (Table 1.2a,b).

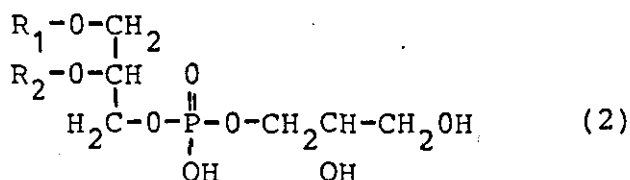
The fatty acid composition of phospholipids include: palmitic, myristic and lauric acids (Bell and Northcote, 1971; White and Lenaz, 1971). In eukaryotic cell membrane there is high proportion of unsaturated fatty acid such as linoleic, arachidonic, pentanoic, and oleic acids (Stanier, 1976). They stabilize the hydrocarbon chains in the membrane, by virtue of the presence of olefinic bonds, which exert stronger intermolecular attraction than paraffinic hydrocarbon bonds (Dawson and Henington, 1960). Branched fatty acid chain are more common with the Gram-positive bacteria, whereas, in Gram-negative bacteria, variation in straight chain fatty acids are predominant (Wovikoff, 1961).

The ability of lipid molecules to migrate from one monolayer to the other is known as "flip-flop", a mechanism responsible for continuous transport and assembly of lipid molecules (Kornberg and McConnell, 1971; Lenard and Rothman, 1976; Renooiji and Van Golde, 1977; Donohue-Rolf and Schaechter, 1980). "Flip-flop" is more rapid in bacterial cells compared to erythrocytes (Donohue-Rolfe and Schaechter, 1980). Micellar or hexagonal structures appear responsible for the rapid "flip-flop" movements of

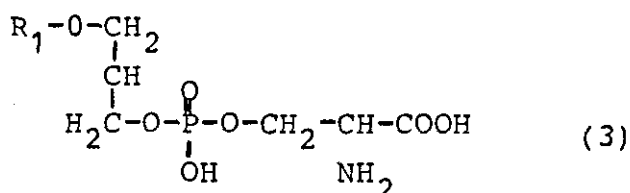
Fig. 1.14:



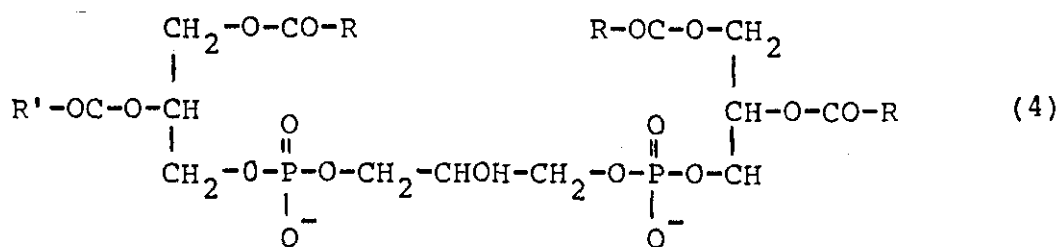
Phosphatidylethanolamine



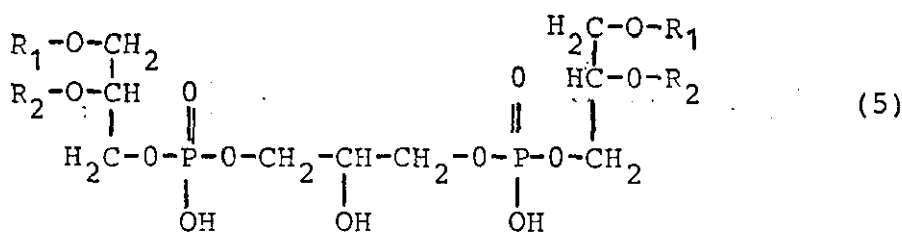
Phosphatidylglycerol



Phosphatidylserine

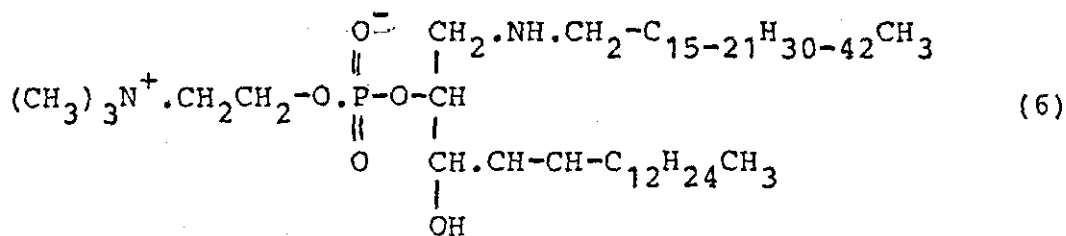


Diphosphatidyl glycerol

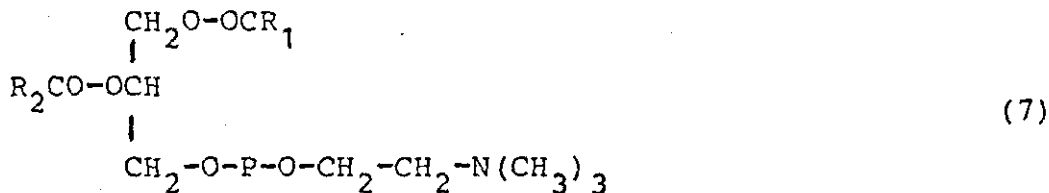


Cardiolipin

Continued/...



Sphingomyeline



Phosphatidylcholine

Fig. 1.14:

Structures of common prokaryotic and eukaryotic phospholipids, 1, 2, 3, 4 and 5 are common in both prokaryotic and eukaryotic cells. 6 and 7 are only common in eukaryotic cells.

lipid molecules in biological membranes.

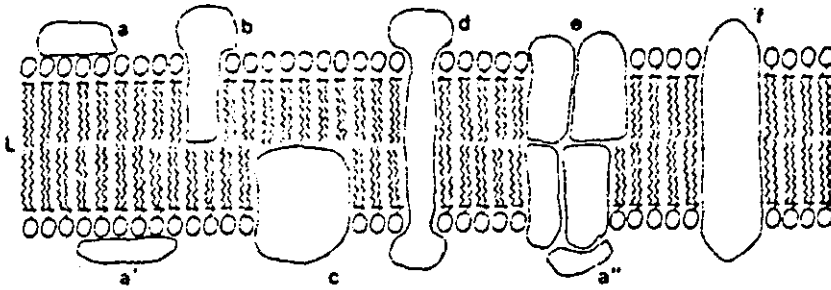
There are large number of biological membrane proteins, which arrange themselves in the lipid bilayer either by penetrating deeper into the bilayer, referred to as intrinsic protein, or some may be found at the surfaces of the bilayer and are referred to as peripheral proteins (Vaniderkooi and Green, 1970; Lenaz, 1971 and Sleek, 1972) (Fig. 1.15a). Where as the peripheral proteins are associated with the acylations of lipids through electrostatic forces, integral proteins interacts more strongly by hydrophobic forces. Accordingly the former are detached from membranes by such means as variation in ionic strength or of pH (Juliano, 1973), and the latter by detergents (Hatefi and Hanstein, 1969; Ichihara and Hussain, 1975).

Membrane proteins are distributed asymmetrically with the N-terminal part facing the outside and the C-terminal part facing the cytoplasm. From a functional point of view (Ohkawan and Webster, 1981), such proteins would have vectorial characteristics which could maintain the active conformation of enzymes in the membranes.

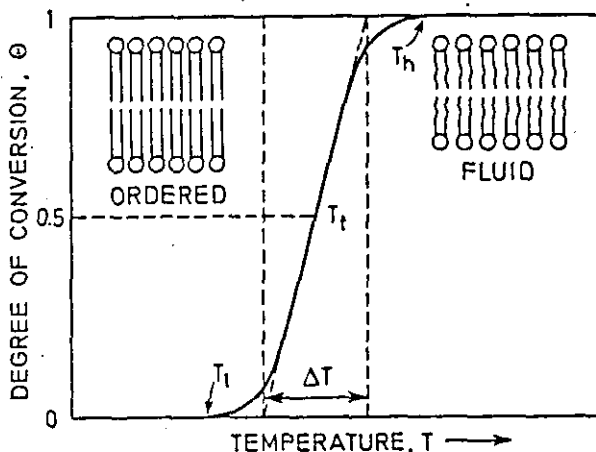
Membrane fluidity is influenced by the dynamic physical state of lipids and is temperature dependent (Fig. 1.15b). At a particular temperature, the hydrocarbon chain of lipids "melt" (fluidity) with the polar ends still functional (Luzzati, 1968). A change from membrane fluidity (liquid state) to crystalline state will result in changes in the functional characteristics of the membrane (e.g. lipid-protein interactions) (Overath and Schairer, 1974). Studies on the influence of changing temperature on lipid bilayer have yielded results in which solid and fluid phases co-exist in the same bilayer and this can be represented by phase diagrams (Shimshick and McConnell, 1973) (Fig. 1.15b).

Lipids containing short-chain or polyunsaturated fatty acids as well as proteins that interacts electrostatically with the polar heads of phospholipids (Lenaz and Parenti, 1974), will reduce mobility of the polar cell surfaces of the membrane as well as polar regions at all depths in the bilayer (Lenaz and Curatola, 1976).

Fig. 1.15



- (A) Arrangement of integral and peripheral proteins in a biological membrane, a, a', a'', peripheral proteins; b-f; integral proteins having different dispositions and extent of penetration.
(After Lenaz, 1979)



- (B) Schematic presentation of the lipid bilayer in the fluid and ordered states. The degree of conversion (Θ) gives the average fraction of lipid molecules in the fluid state as a function of temperature. T_l and T_h indicate the low and high temperature end of the phase transition respectively. T_t is defined as the temperature at which half of the lipids are in the fluid state. ΔT is a measure of the transition width.
(After Metcalf and Kornberg, 1978).

The influence of membrane fluidity on passive diffusion of hydrophilic substance across biological membranes has been observed by Eze and McElhaney (1981). Their observations show that passive diffusion of glycerol across biological membranes supplemented with various unsaturated fatty acids is dependent upon the nature of the unsaturated fatty acids above the transition point.

Many of the membrane proteins are enzymes (Table 1.3). Some are involved in transport of molecules across biological membranes. Membranes contain proteins referred to as permeases, which play a specific role in mediating the passage of certain compounds. Like many enzymes the synthesis of permeases is induced by the presence of the penetrating substance in the mechanism (Lehninger, 1975). For example, E.coli grown on a galactose-containing medium will induce the synthesis of the permease β -galactosidase, which is necessary for the hydrolysis of galactose (Lehninger, 1975). Such catalyzed permeability suggest a specific interaction involving membrane permease and the penetrant.

In another type of transport mechanism (active transport) also involves the interaction between penetrants and specific membrane proteins, but in addition "couples" a source of energy to the process of catalyzed transport, so that penetrants can diffuse inside the membrane across a concentration gradient (Lehninger, 1975; Cassey and Broger, 1981).

The process of respiration involves the mechanism of active transport, in which the synthesis of ATP is normally achieved from the transmembrane electrochemical gradient generated from electron transfer in respiratory system (Mitchell, 1979; Hooper and Dispirito, 1985; Lambert, 1978).

The process of formation of proton gradient appears identical in both eukaryotic and prokaryotic cells (Hooper and Dispirito, 1985), in which the oxidation of substrates results in the formation of protons from the substrate or water or both in the extra-cytoplasmic compartment Fig.1.16. The energy derived from electron transport across biological membrane may be used in

Table 1.2a: Comparison of inner and outer membranes from
Gram-negative species

Organism	Phospholipid	Membrane	
		Cytoplasmic	Outer
<u>Salmonella</u> <u>typhimurium</u>	Total phospholipid (PL) (mg PL/mg protein)	0.53-0.61	0.3
	Phosphatidylglycerol (% total PL as glycerol)	33	17
	Diphosphatidyl glycerol (% total PL as glycerol)	6.8	1.7
	Phosphatidylethanolamine as ratio	1	1.3
<u>E.coli</u>	Total phospholipid (mg/g cells)	9.1 ± 0.4	3.4±0.2
<u>Proteus mirabilis</u>	Total phospholipid (% of membrane dry wt)	38	18
	Phosphatidylglycerol, diphosphatidylglycerol (% total polar lipid)	30	15-20
	Phosphatidylethanolamine (% total polar lipid)	55-60	75-80

After Costerton and Ingram, 1974.

Table 1.2b: Chemical Composition of Membrane from Gram-positive bacteria and micro-fungi

Organism	Protein (%)	Total Lipid (%)	Hexose (%)	Ref
<u>Bacillus megaterium</u>	58-75	20-28	0.2-8	)
<u>Micrococcus lutea</u>	52-68	23-28	16-19	) a
<u>Staphylococcus aureus</u>	69-73	30	1.7	)
<u>Saccharomyces cerevisiae</u>	46-47.5	37.8-45.6	3.2	)
<u>Candida utilis</u>	)			) b
<u>Candida albicans</u> (mycelial foun)	) 45.0	31.0	25	)
<u>Fusarium culmorum</u>	25.0	40.0	30	) c

(a) (After Salton, M. R. J., 1967)

(b) (After Hunter, K. and Rose, A. H., 1971)

(c) (After Roose, A. H., 1976)

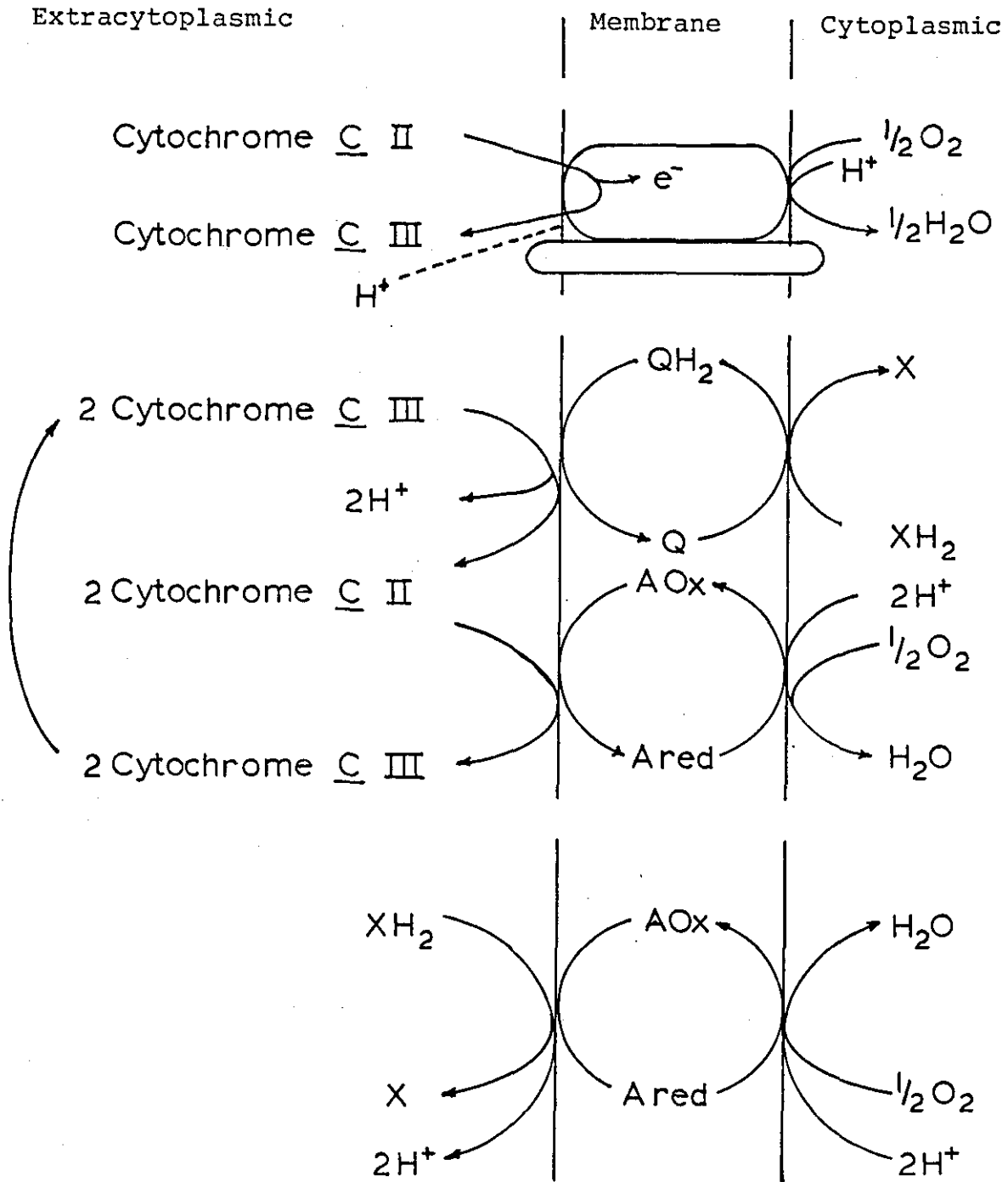


Fig. 1.16: General means for generation of a proton gradient. (1) Direct translocations of protons by cytochrome C oxidase. (2) Redox loops. (3) Extracytoplasmic oxidation. Q, H^+ carrier such as coenzyme A; A; electron carrier. (After Hooper and Dispirito, 1985).

various transport systems (Hamilton, 1975), such as transport of metabolites and ion against gradients (Eilam and Lavi, 1985).

Various active transport systems in bacterial membrane have been correlated with membrane fluidity (Shimshick and McConnel, 1973; Wilson, 1970; Shecher, 1974) in which the Arrhenius representations of various active transport show that change in slope occurs at mid-temperature of the order - disorder transition. Furthermore, the increase in slope at lower temperature, (Overath and Shaiker, 1970) has been attributed to an increase in activation of energy of transport, during which membrane lipid hydrocarbon are in ordered state.

Various metabolic activities are carried out by many different kinds of membrane proteins, each of which are probably involved in specific enzymic action. The synthesis of membrane lipids, proteins and various classes of macromolecules that make up, for example, the bacterial cell wall (peptidoglycans, teichoic acids, lipopolysaccharides, and polysaccharides, such as mannan, chitin and glycan as in fungal wall are mediated by biosynthetic enzymes which integrate themselves with membranes (Duran and Carbib, 1979; Bloch, 1983). In addition various metabolic processes such as proteins are mediated by many kinds of membrane enzymes.

1.2.2 The importance of Cations in Membrane Structure and Function

In the last section (1.2.1) various chemical macromolecule have been described in terms of structural arrangements and functions. These large molecules can interact with themselves or with another macromolecule to give definite types of arrangements. However, such arrangements may not be complete without the involvement of cations, likely to act as a "bridge" in which either a particular molecule interacts with itself or with another species. Cations are thus part of the membrane constitution, within which their presence influences stability.

The conformation of protein may require the

Table 1.3: Some hydrolytic enzymes requiring divalent cations for activity

Enzyme	Reaction	Metal ion
Carboxypeptidase	Hydrolysis of C-terminal peptide residue	Zn^{2+}
Leucine aminopeptidase	Hydrolysis of leucine N-terminal peptide residue	Zn^{2+}
Dipeptidase	Hydrolysis of dipeptides	Zn^{2+}
Neutral protease	Hydrolysis of peptides	Zn^{2+} , Ca^{2+}
Phospholipase C	Hydrolysis of phospholipids	Zn^{2+}
β -lactonase II	Hydrolysis of β -lactan ring	Zn^{2+}
Thermolysin	Hydrolysis of peptides	Zn^{2+} , Ca^{2+}
Alkaline phosphatase	Hydrolysis of phosphate esters	Zn^{2+}
Carbonic anhydrase	Hydration of CO_2	Zn^{2+}
α -Amylase	Hydrolysis of glucosides	Ca^{2+} , Zn^{2+}
Inorganic pyrophosphatase	Hydrolysis of pyrophosphate	Mg^{2+}
Phospholipase A_2	Hydrolysis of phospholipids	Ca^{2+}
ATPase	Hydrolysis of ATP, and Inorganic phosphate	Mg^{2+}
$\text{Na}^+ \text{K}^+ \text{ATPase}$) $\text{Mg}^{2+} \text{Ca}^{2+} \text{ATPase}$)	Hydrolysis of phosphate with transport cations	Mg^{2+} , Ca^{2+}
Various phosphatases e.g. fructose diphosphatase	Hydrolysis of phosphate	Mg^{2+} , (Zn^{2+})

participation of divalent cations. The activity of phosphorylating enzymes produced by P.aeruginosa requires magnesium ions for activity, although Mn^{2+} , Zn^{2+} and Co^{2+} can replace Mg^{2+} in this respect (Hughes, 1981). The ATPase enzymes are activated by divalent cations. They may be either Mg^{2+} - or Ca^{2+} - activated adenosine triphosphatases (Abrams and McNamara, 1960; Ishikawa and Lehninger, 1962). In erythrocytes, membrane bound Ca^{2+} activated ATPase participates in the transport of inorganic cations (Abrams, 1965; Bhattacharyya and Barnes, 1978) in such processes as action potential and transport of sugars and amino acids (Belliveau and Lanyi, 1978). Cation-activated ATPase linked transport-systems are characteristically similar in both eukaryotes and prokaryotes (Juliano, 1973). Some other membrane bound hydrolytic enzymes requiring divalent ions for activity are listed in Table 1.3.

Apart from activation of membrane enzymes, cations may bind directly to either the cell surface or cell membrane, where they maintain the cohesiveness and control of membrane permeability. Divalent metal ions such as Ca^{2+} and Mg^{2+} bridge neighbouring carboxylate groups of lipoprotein, thus conferring rigidity to the lipoprotein of the membrane. In this way, Ca^{2+} or Mg^{2+} can in fact control the conformation of biological membranes (Brown and Melling 1975). Thus, some chemical agents possess the ability of interacting with biological membranes in such a way as to behave like either Mg^{2+} or Ca^{2+} and this may result in membrane stabilization. Others, however, may interact with biological membrane, in such a way that these ions are lost leading to cation deprivation, and cause an increase in membrane permeability (Fuller and Denyer, 1985).

At cell surface, the binding of phosphates to other components of the membrane molecule such as protein and phospholipids requires the participation of metal ions (Brown and Melling, 1969), acting as bridge inbetween phosphate and other surface components. Increase in sensitivity to both polymyxin and EDTA of cultures of low content of magnesium have been observed (Brown and Melling, 1969). Treatment with ethylene diamino tetraacetic acid (EDTA)

releases these cations, as well as complex proteins and lipopolysaccharides (Brown and Melling, 1975). (Mutoh and Frukawa, 1978) also reported the importance of cations in the structure of both phospholipids and lipopolysaccharides. Inhibition of the growth of E.coli by tetracyclines is reversed by addition of high concentrations of Mg^{2+} (Jogun and Stezowski, 1976). Finally, treatment with EDTA displaces ions from cells resulting in the production of osmotically fragile species, but on addition of divalent ions, brought about the production of osmotically stable forms (Brown and Melling, 1975).

1.2.3 Aim of the Project

The aim of this project was to consider various mechanisms by which β -blockers interact with different biological systems. From the current understanding of the structure and functions of biological surfaces and membranes, it was intended to investigate how the interactions of β -blockers were likely to affect the biochemical and physiological properties of the cell. Previous studies of the membrane activities of β -blockers have been limited to eukaryotic membrane systems, and it was therefore intended to investigate whether these activities are applicable to the bacterial (prokaryotic) systems. For comparative purposes some studies of local anaesthetics and cationic surfactants were included.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO

MATERIALS AND METHOD

SECTION ONE - MATERIALS

2.1.1 Organisms

The organisms used in this project were obtained from The National Collection of Type Culture (NCTC), London. They include:

<u>Escherichia coli</u>	(<u>E.coli</u>)	NCTC 9001
<u>Pseudomonas aeruginosa</u>	(<u>P.aeruginosa</u>)	NCTC 7244
<u>Bacillus cereus</u>	(<u>B.cereus</u>)	NCTC 11755
<u>Candida albicans</u>	(<u>C.albicans</u>)	A39 (Lab strain)

2.1.2 Media and Buffers

Synthetic growth medium was determined prior to the investigations of this project by shaken culture experiments using the organisms listed in 2.1.1. In addition, various conditions such as the pH of the buffer and its ionic strength was chosen to suit the microbial growth in the presence and absence of β blockers. From these, the growth medium (2.1.2b) was chosen.

(a) Nutrient Agar (NA)

Nutrient broth (NB) solidified by the addition of Agar (Oxoid No. 3) 2.0% w/v.
Sterilized by autoclave.

(b) Defined Medium (DM)

Mineral salt solution	410 ml
Carbohydrate solution	400 ml
Casamino acids (vitamin free solution)	190 ml
Thiamine solution	1 ml

When appropriate this medium was solidified by the addition of Agar (Oxoid No. 3) 2% w/v to give a maintenance medium (DMA).

(1) Mineral Salt Solution

Di-sodium hydrogen orthophosphate (Na_2HPO_4) 7.1g
Potassium di-hydrogen orthophosphate (KH_2PO_4) 1.3g
Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) 0.25g
Ferrous sulphate (FeSO_4) 0.1g
Manganese sulphate ($\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$) 0.1g
Distilled water to 410 ml.

(2) Carbohydrate Solution

D-glucose 5 g
Distilled water to 1000 ml.

(3) Casamino Acids (Vitamin free) Solution

Casamino acids 1 g
Distilled water 190 ml.

(4) Vitamin Solution

Thiamine 10 $\mu\text{g/ml}$.

Where appropriate solutions were autoclaved separately (121°C , 15 min). Thiamine was sterilized by filtration. Solutions were aseptically mixed. The final pH of the medium was 6.8.

(c) Carbohydrate Free Medium (CFM)

Medium (DM) from which the carbohydrate solution had been omitted.

(d) Lactose Medium (LDM)

Medium (DM) with glucose replaced by lactose, (5 mg.ml^{-1}).

(e) Low Phosphate Medium (LPM)

Growth medium in which phosphate buffer had been replaced by:

Potassium Chloride 0.074 g.l^{-1}
Sodium Citrate 12.57 g.l^{-1}) Citrate buffer
Citric Acid 1.51 g.l^{-1}) pH 6.8

The medium contained low phosphate as impurities.

(f) Medium of Variable Mineral Content (MVM)

Growth medium (DM) with or without added carbohydrate in which the mineral content (magnesium chloride, calcium chloride or zinc chloride) was varied.

(g) Other Buffers

Tris-HCL	0.1 M	pH 7.4
Tris-HCL	0.2 M	pH 6.8
Tris-Acetate	0.02M	pH 5.8
Tris-Acetate	0.04 M	pH 5.8

(h) Sodium Citrate Buffer

Citric acid	(0.1 M)	50 ml)	0.1 M sodium acetate
Sodium acetate	(0.1 M)	41 ml)	buffer pH 4.8

(i) Glycine Buffer

Glycine	(0.4 M)	10 ml)	glycl
Sodium chloride	(0.4 M)	)	glycine
Glycine	(0.4 M)	10 ml)	buffer pH 4.8
Sodium hudroxide	(0.4 M)		

2.1.3 Reagents

- (a) Dye Solubilization
Sudan Black B.
- (b) Pentose Reagent (Mejbaum, 1939)
FeCl₃ 0.10 g
Orcinol 0.30 g
Concentrated HCL to 100 ml.
- (c) Dehydrogenase Reagent
Triphenyl tetrazolium Chloride (TTC).
(Fisons, Loughborough, England.)
- (d) Reagent C
1 volume of 6 N sulphuric acid.
2 volumes of distilled water.
1 volume of 2.5% ammonium molybdate.
1 volume of 10% ascorbic acid.
- (e) Acid Phosphatase Reagent
P- nitrophenyl phosphate disodium pentahydrate.
(Sigma Chemicals Ltd., Poole, Dorset, England).
- (f) Aryl Sulfatase Reagent
P- nitrocatechol sulphate.
(Sigma Chemicals Ltd., Poole, Dorset, England).

2.1.4 Solvents for Extractions

Chloroform: All of spectroscopic.
Toluene: grade
Methanol:

2.1.5 Miscellaneous

(a) Radiotracers and Associated Materials

³² P- orthophosphate	100 µci
¹⁴ C- glucose	250 µci
¹⁴ C- mannose	250 µci
³ H- propranolol	100 µci

liquid scintillator (LPS). A complete phase combining system for liquid scintillation counting of radioactive aqueous samples. (Radio Chemical Centre, Amersham, Buckinghamshire, England).

(b) Electron Microscopy

Glutaraldehyde (E. M. grade).

(B.D.H. Chemicals Ltd., Poole, England).

Round glass cover slips, 13 mm.

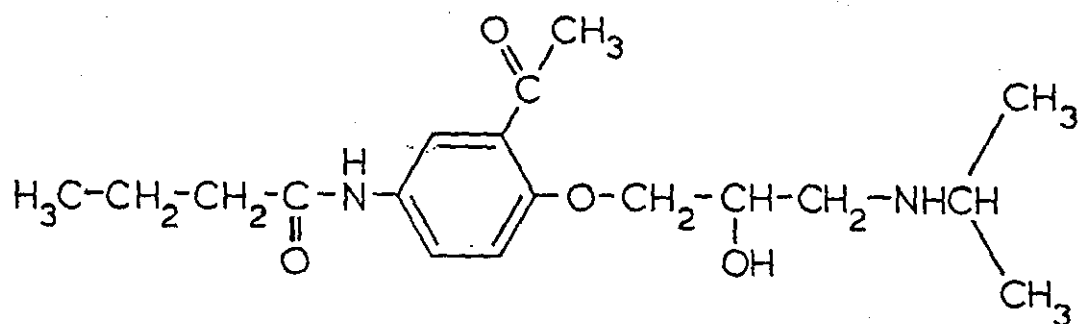
(Chance Propper Ltd., Smethwick, Warley, England).

Gold palladium, 10 mm

2.1.6 Beta-adrenergic blocking agents (β -blockers)

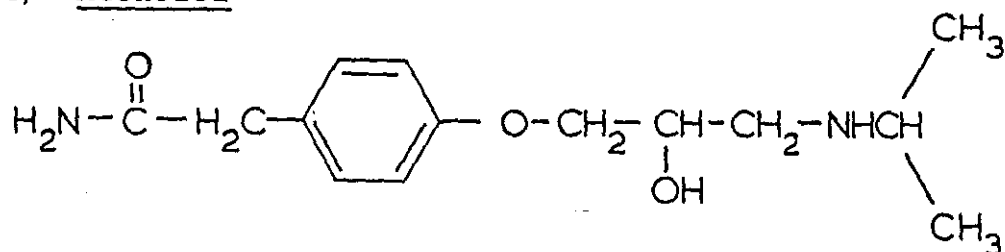
Fig. 2.1:

(i) Acebutolol



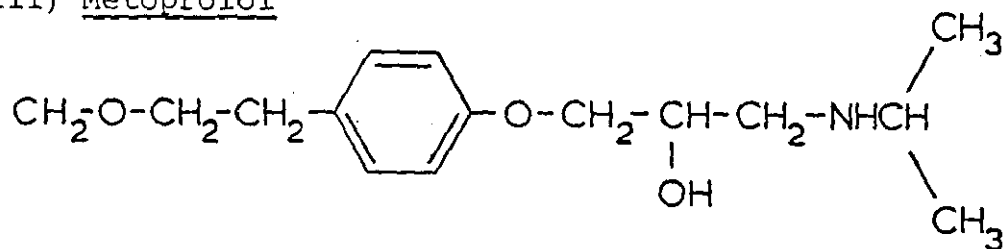
A gift from May and Baker Ltd., Dagenham, England.

(ii) Atenolol



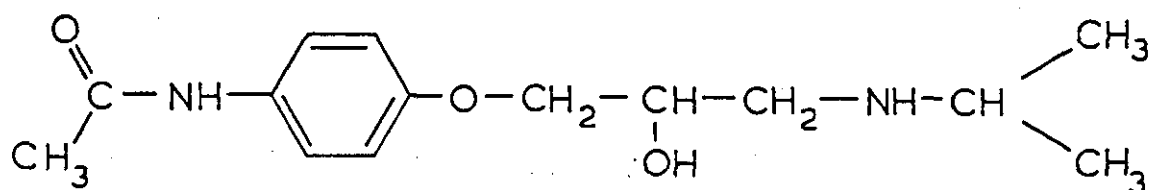
A gift from Imperial Chemical Industry (ICI) Alderley Park, Macclesfield, England.

(iii) Metoprolol



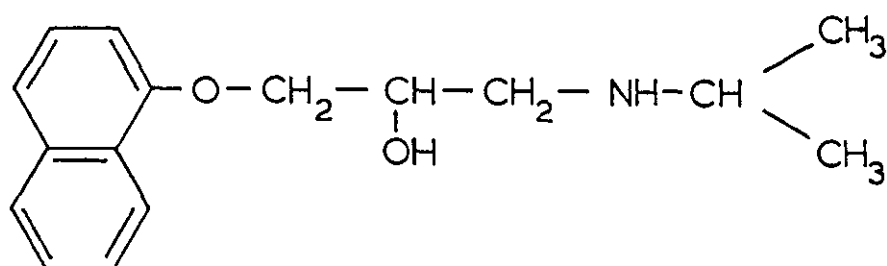
A gift from Geigy, Pharmaceuticals, Horsham, W. Sussex and Astra, England.

(iv) Practolol



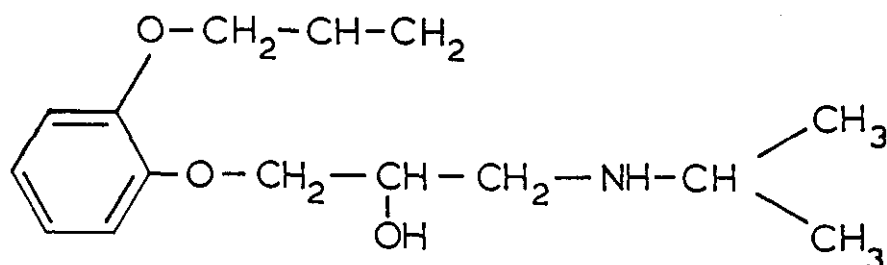
A gift from ICI, Alderly Park, Macclesfield, England.

(v) Propranolol



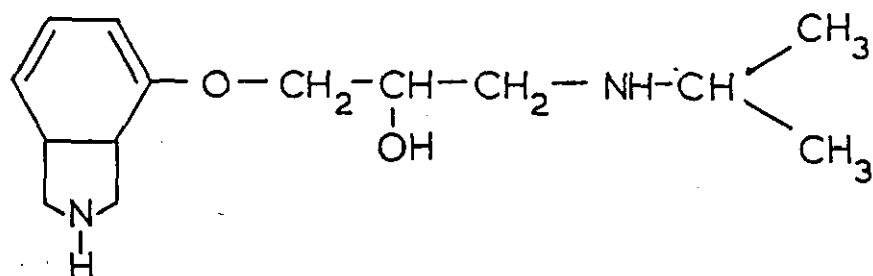
A gift from ICI, Alderly Park, Macclesfield, England.

(vi) Oxprenolol



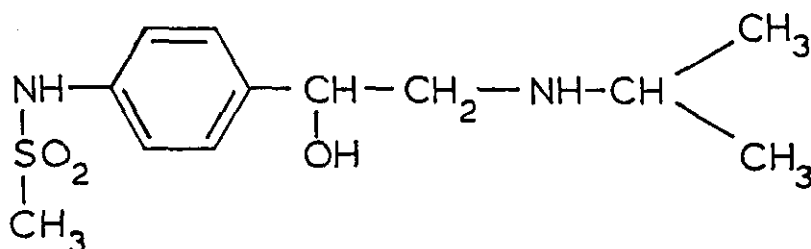
A gift from Ciba Laboratory, Horsham, West Sussex, England.

(vii) Pindolol



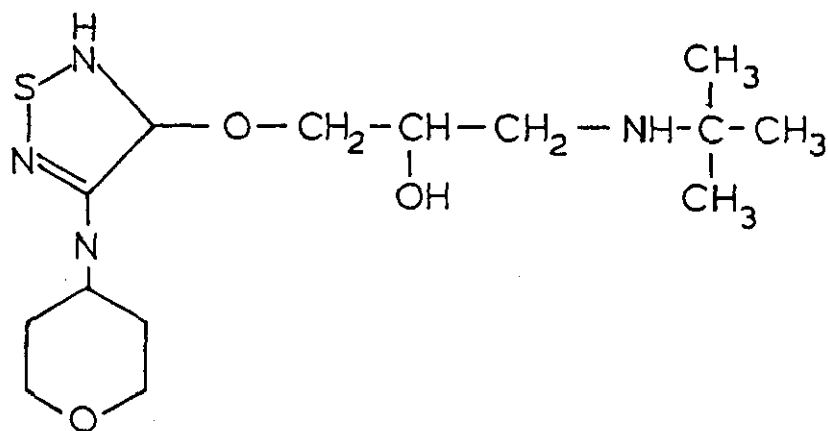
A gift from Sandoz, Feltham, Middlesex England

(viii) Sotalol



A gift from Bristol-Myers Company, Evansville, Indiana, USA.

(ix) Timolol



A gift from Sandoz Products Ltd., Feltham, Middlesex, England.

(i) Tetracaine

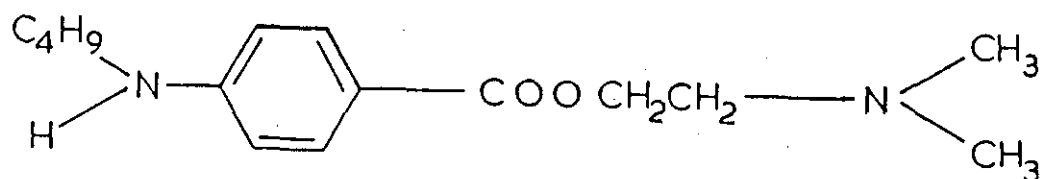


Fig. 2.1j: Sigma Chemical Co. Ltd., Poole, Dorset, England.

(ii) Procaine

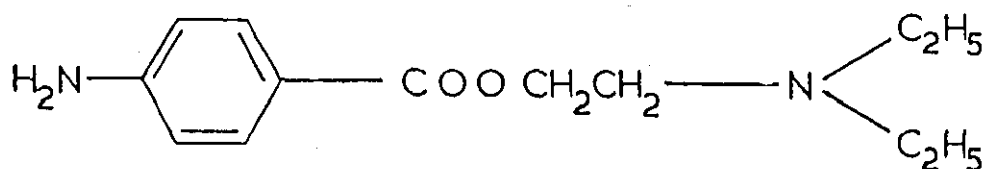


Fig. 2.1k: Sigma Chemical Co. Ltd., Poole, Dorset, England.

(iii) Procainamide

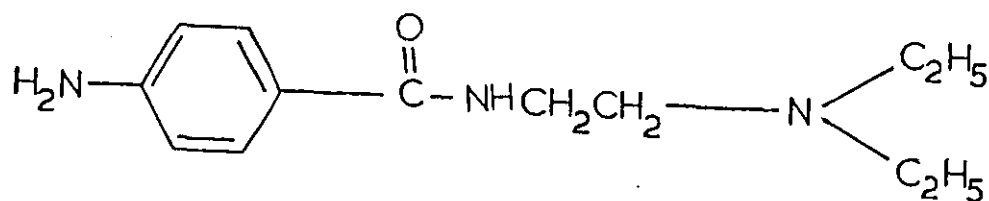
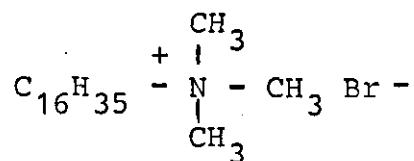


Fig. 2.1 : Sigma Chemical Co. Ltd., Poole, Dorset, England.

Surfactants

- (i) Tween 80 (polyoxyethylene sorbitan monooleate)
- a complex of polyoxyethylene ethers of mixed partial oleic esters of sorbitol anhydrides, purchased from Sigma Chemical Co. Ltd., London, England.

- (ii) HTAB (Hexadecyl trimethyl ammonium bromide)



Purchased from Sigma Chemical Co. Ltd.

All chemicals were of analar standard unless otherwise stated.

SECTION TWO - GENERAL PROCEDURES

2.2.1 Sterilization of Solutions and Equipment

Sterilization of all solutions and some equipment was carried out by standard autoclaving (121°C , 15 min) or by membrane filtration using Nuflow 0.45 millipore filters. Equipment such as pipettes was sterilized in a dry air oven at 150°C for 2 hr.

2.2.2 Cultivation of Organisms

Freeze dried specimens were supplied from standard collections and organisms recovered by the method recommended by the individual collection.

(i) Maintenance of Cultures

Master cultures were maintained on both nutrient agar (NA) and growth medium (DM) slopes, stored in the dark at $2-4^{\circ}\text{C}$, and subcultured at two month intervals. Cell submasters were stored in the dark at $15-20^{\circ}\text{C}$ as DMA slopes, and subcultured at seven day intervals. Cultures were routinely examined microscopically and by streak-plating.

(ii) Cultivation and Preparation of Cell Suspensions

Cultures from sub-master slopes were used for the preparation of cell suspensions. 2 ml (DM) medium was added to the daily slope cultures, shaken and transferred to cleated flask (500 ml) containing approximately 150 ml sterilized (DM) medium and incubated overnight at 37°C in an orbital incubator at 120 rpm. More DM medium (100 ml) was then added to the cleated flask containing the overnight culture and incubation continued as before for two hr. The cells were harvested by centrifugation at 5000 rpm (4500 g) in a MSE mistral 6L centrifuge for 30 minutes.

The supernatant fluid was decanted off and cells washed with, and then resuspended in fresh carbohydrate free medium (CFM). The optical

density of this suspension was adjusted to the required value (typically 0.6; 650 nm, 1 cm path), using a Pye Unicam SP 500 Series II UV/Visible Spectrophotometer.

Such cell suspensions were held at room temperature and used within 30 min of preparation.

2.2.3 Determination of Dry Weight

500 ml volumes of bacterial or candidal suspensions were filtered through weighed membranes (0.45 μ m porosity) previously washed with distilled water and dried at 95°C for 10 min. The cells were washed on the membrane which was then dried at 95°C to a constant weight. The cell weight was found by difference.

2.2.4 Determination of Total Cell Numbers

Hawksley counting chamber (depth 0.1 mm, $\frac{1}{4}$ 00 mm²) was used in the determination of numbers of bacterial or Candidal cells per ml. One loop full of a suspension of serially diluted cell suspension was transferred to the platform of the Hawksley slide and covered with a coverslip. The liquid was allowed to stand for 5 min, after which it was examined under a microscope with a total magnification of X400. The number of organism in 50-100 small squares as counted (between 2 and 10), and the mean number of organisms per small square was calculated. From these the count per ml of the original suspension was determined.

SECTION THREE- SPECIFIC EXPERIMENTAL METHODS

2.3.1 Assessment of Growth Inhibition

(a) Tube dilution method

Equal volumes (5 ml) of drug solutions and bacterial or candidal cell suspensions in CFM were mixed in sterile capped test tubes and kept at room temperature (22°C) for 10 min. 0.15 ml of 20% glucose solution was then added to each tube, after which, the tubes were incubated at 37°C for 24 hr and the optical density (650, 1 cm) measured.

(b) Direct assessment method

Equal volumes (25 ml) of drug solutions of different concentrations and bacterial or candidal cell suspensions in CFM were incubated at 37°C in 150 ml conical flasks in a Gallenkamp shaking water bath ($150 \text{ throw.min}^{-1}$). Immediately, 0.15 ml of 20% glucose was added to each tube to enable cell growth and incubation continued.

The optical density of samples from cultures was measured (650 nm, 1 cm path) at 20 min or 30 min intervals respectively. Samples were returned to the appropriate flask after each measurement. Optical density measurements were continued for 140 min in case of bacteria and 420 mins for C.albicans.

(c) Reversibility of growth inhibition

25 ml volumes of drug solutions and cell suspended in growth media (DM) were incubated for approximately 60 min and 100 min (E.coli or C.albicans respectively). Cultures were either then centrifuged (4500 g, 15 min, 4°C) washed with and then resuspended in 25 ml fresh growth medium (DM) at 37°C or diluted (25 ml drug/cell plus 25 ml fresh

growth medium (DM) at 37°C). Shake incubation was continued in each case and the optical density (650 nm, 1 cm path) measured at time intervals for about 2 or 8 hours (E.coli or C.albicans respectively).

2.3.2 Viable counting

Equal volumes (5 ml) of drug solutions of different concentrations and bacterial or candidal cell suspensions in CFM or phosphate buffer 0.2 M, (pH 6.8) were mixed in sterile capped test tubes and maintained at room temperature (22°C). After 10 min contact time, samples were serially diluted to obtain countable colonies prior to plating out in medium (NA). (Preliminary experiments showed that the use of Tween 80 as a diluent for the inactivation of β -blockers did not alter appreciably the number of colonies counted).

The experiment was repeated for each different concentration of drug, and varying the contact time. After 24 hr incubation at 37°C, the colonies were counted.

2.3.3 Respiration Studies in the Presence of β -Blockers

(a) Determination of Oxygen Consumption (Oxygen electrode) Preliminary

Oxygen consumption was measured using a Rank Oxygen electrode (Rank Brothers, Bothsham, Cambridge) the use of which provides the principles of a continuous monitoring system for assaying the oxygen concentration in the reaction mixture. *Lesler, 1970*

A method based on that described by Lesler (1970) was employed in the measurement. The reaction vessel is cylindrical (2.8 cm long and 2.1 cm diameter) and consists of an outer water jacket which can be connected to a water bath set at desired temperature, and rested on a magnetic stirrer running at about 300 rev. min⁻¹, the constant stirring rate was maintained by a small, nylon-coated magnetic flea.

The electrode required a polarising voltage

of 0.65V (35 μ A) and bubbles were excluded by adjusting the height of the electrode. The current from the electrode was registered on the recorder with a chart speed of 1 cm min⁻¹. The dial on the recorder was initially set at zero position, when the switch is set on to the calibrated mode of operation, and was ready for use when the sensitivity control was turned until the recorder read full scale deflection. The recorder was calibrated by standardising the electrode with distilled water saturated with air (Umbreit, Burns and Stauffer, 1972), which was introduced to the electrode through the vessel base, and raised and lowered by a rack and pinion adjustment.

Oxygen content of the solution was found from standard tables. The rate of utilization of oxygen was determined by the slope of the recorded line.

Method

Cultures of bacterial or candidal cells were grown overnight with glucose, succinate, malate or lactate as described and harvested by centrifugation. The cells were resuspended in substrate free medium at an optical density of 0.3 or 0.6.

The electrode vessel requires a total of 4 ml volume, and therefore the reacting mixtures (cell suspensions, drug solutions, and substrate) were prepared in such a way that 4 ml of reacting mixtures gave the desired concentrations of drug. Since the same amount of cell suspensions and substrate are used each time, different concentration of stock solutions of drug was freshly prepared and stored at 4°C.

The reaction was started by adding 0.1 ml substrate (0.2 M) after a steady baseline had been obtained. Oxygen consumption was usually allowed to proceed for 10-15 minutes. The electrode reactions chamber was washed 3-4 times between each run. Corrections for endogenous respiration was made for all determinations.

(b) Reduction of Triphenyl Tetrazolium Chloride (TTC)

A method based on that described by Hugo (1954) was employed in the determination of dehydrogenase activity of cell suspensions.

Into a series of sterile bottles was placed 2.5 ml of phosphate buffer or CFM containing different concentrations of β -blocker, 1.0 ml of TTC solutions (0.1% w/v) (freshly prepared), 1.0 ml, 0.02 M substrate, and 0.5 ml of cell suspension. The final concentration of cell suspension after addition into the reacting mixture was approximately 4.2×10^9 cells ml^{-1} , or 3.8×10^5 , corresponding to a dry weight of Ca $0.95 \text{ mg} \cdot \text{ml}^{-1}$ or $1.5 \text{ mg} \cdot \text{ml}^{-1}$ for E.coli and C.albicans respectively. Bottles containing neither β -blocker nor substrate were prepared to be used as controls or blanks.

All bottles were shaken in a water bath at 38°C . At various time intervals one control (no β -blocker) one blank (no substrate) and one of the concentrations of β -blocker were removed. Each was acidified with 5 ml glacial acetic acid and the triphenylformazan produced by reduction of TTC (Fig. 2.1) was extracted using several 5 ml quantities of toluene. Any remaining cells were removed by centrifugation and the optical density of the toluene layer at 490 nm was measured. The values were corrected for the actual volume of toluene used.

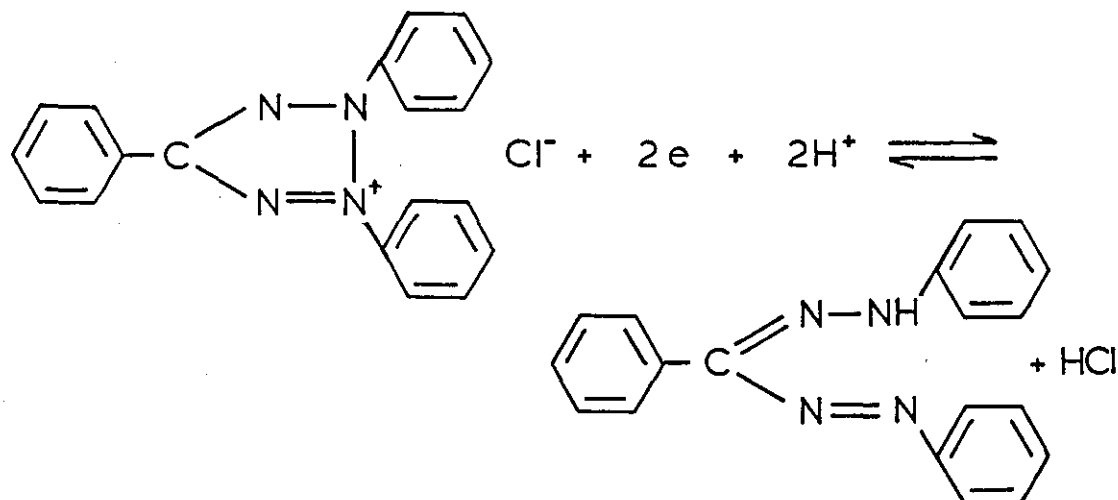


Fig. 2.2: Formation of Triphenylformazon (red) from TTC colourless (Hugo, 1954)

2.3.4 Uptake of ^{14}C -labelled substrates

(a) Cell wall (Dry weight) determination

A calibration curve was constructed in order to determine the dry weight of the cell wall of bacterial and candidal cell suspensions via optical density (650 nm, 1 cm path). Cells were grown in the absence of β -blocker. At time intervals 50 ml of cells was removed, the optical density was measured and resuspended in 5 ml distilled water and frozen overnight in a dialysis tubing. The cell wall was prepared by Hughe's press, placed in a preweighed aluminium plachettes and dried at 55°C . The calibration of dry weight of wall against optical density was constructed.

(b) Incorporation of ^{14}C -glucose or ^{14}C -mannose

Cultures of bacterial or candidal cells were grown overnight in glucose or mannose based (DM) medium, harvested by centrifugation and resuspended in a fresh medium of glucose or mannose, and the optical density adjusted (0.3). The cells were grown in a Gallenkamp orbital incubator with shaking at 37°C until the optical density was 0.6. The cells were harvested and resuspended in CFM and the optical density adjusted by dilution with fresh (DM) medium. 2.5 μCi of ^{14}C -labelled sugar was added to (250 ml) cleated conical flask containing 50 ml cell suspensions, β -blockers were added to the flask by an amount giving the desired concentration, and transferred to a Gallenkamp orbital incubator. The cells were incubated at 37°C with shaking. At time intervals, 5 ml of cells was removed, the optical density measured, centrifuged and the pellet was resuspended in an equal amount of distilled water. 1 ml of cell suspension was monitored for radioactivity using a Microprocessor P.W. 4700 liquid scintillation counter.

(c) Distribution of radioactivity in different fractions of cells

The distribution of radioactivity in different fractions of cells was studied by centrifuging 10 ml cell suspension from above, when the optical densities of treated culture had equalled the optical density attained by the control experiment at the time of sampling.

The supernatant was removed and the pellet was resuspended in 2.5 ml distilled water and frozen overnight in dialysis tubing. The cells were broke open by Hugh's press. The cell envelope or the cell wall was removed by centrifugation and resuspended in 10 ml distilled water. The cytoplasm was diluted to 2.5 ml, and corrections for this amount was taken into account. 1 ml from fractions were monitored for radioactivity.

Preparation of cell wall and cell membrane fraction was carried out by mixing equal volume (5 ml) of suspension of cell envelope and phenol (80%).

After 1 hr the cell membrane, now dissolved in phenol, was separated by centrifugation and the pellet (cell wall) was resuspended in 5 ml distilled water. The radioactivity from each sample (1 ml) was measured. The amount of wall (mg dry weight) was estimated from the previously constructed calibrated curve, from which the percentage of the total was found.

2.3.5 Inhibition of Membrane Associated Enzymes

(a) Inhibition of microbial Mg^{2+} -ATPase

(i) Isolation of the enzyme

Membrane-bound Mg^{2+} -ATPase was isolated from B.cereus and C.albicans by a method based on that described by Abrams (1965). The cells were grown

for 18 hr in growth medium (DM) at 37°C or 30°C. After reaching the stationary phase of growth they were harvested (5000 g for 20 min) and washed three times with cold water. For the preparation of protoplasts, the cells were suspended in 0.001M MgCl₂ and 0.4M glycyl-glycine buffer (pH 7.2) which acts as an osmotic stabilizer. The cells were then incubated at 37°C for 60 min with 90 µg/ml egg white lysozyme (Sigma) to digest the cell wall. In order to lyse the protoplasts and produce membrane ghosts, 0.01M glucose was added. The membrane ghosts were sedimented at 35,000 x g for 15 min at 4°C and washed twice with ice-cold 0.001M MgCl₂.

(ii) Standard Curve for the determination of inorganic phosphate Pi

0.5 ml of phosphorus standards (0.5 - 15 µmole) of potassium dihydrogen monophosphate (KH₂PO₄) was added to 2 ml of 10% trichloroacetic acid in a series of test tubes. The volume was adjusted to 4 ml with distilled water. The reagent blank consists of 4 ml of distilled water. 4 ml of reagent C was pipetted into each tube, capped, mixed and placed back with all tubes in a 37°C water bath for 2 hours. Tubes were removed from the water bath, allowed to cool to room temperature and the absorbance in a spectrophotometer at 820nm against the blank assessed.

(iii) Assay of Mg²⁺-ATPase Activity

Assays of Mg²⁺-ATPase activity was carried out by incubating 1.0 ml of enzyme preparation with 4 ml of assay buffer (0.1M Tris-HCl pH 7.5, 0.005M disodium ATP, 0.005M MgCl₂) and drugs at 37°C for 30 min. Controls containing no drugs were set up. The reaction was stopped by the addition of 5 ml of ice-cold 5% perchloric acid. The (Pi) in the supernatant was determined by the method of Chen, Toribara

and Warner (1965), which is based on the colour formed from the reduction of phosphomolybdate complex. The optical densities at (820 nm) were converted into μ mole of (Pi) present in the supernatant from the previously constructed calibrated curve.

(iv) Reversibility of Mg^{2+} -ATPase inhibition

The reversibility of the actions of β -blockers and HTAB on the activity of Mg^{2+} -ATPase was investigated by the use of:

(a) Divalent Cations

The membrane ghosts of C.albicans, prepared as described above, were resuspended in solutions containing 0.001M EDTA to displace ions. After 30 min incubation at room temperature, the cell suspensions in EDTA were centrifuged and resuspended in fresh distilled water.

The experiment was carried out varying the concentrations of either Mg^{2+} , or Ca^{2+} with fixed concentration of β -blockers, and membrane ghost suspensions. Control experiment was set up in which no ion was present. The activity of Mg^{2+} -ATPase was assayed as described in (iii).

(b) Dilution

The partially purified enzyme from C.albicans was incubated for 10 min at room temperature in the presence of different concentration of drugs, in a final volume of 2.5 ml. An aliquot (1.0 ml) was removed, diluted to (2.5 ml), incubated for a further 10 min and assayed for the remaining activity towards ATPase as described in Section (2.3.5).

Expected inhibition was calculated from the percentage inhibition of enzyme before dilution, and the percentage inhibition remaining after dilution was related to it.

(V) Kinetics of inhibition

The steady states were measured at various divalent ion concentrations in the absence of inhibitor and in the presence of fixed concentrations of β -blockers and fixed amount of membrane ghosts suspensions in Tris buffer pH 7.5, 0.1M with 0.005M $MgCl_2$ and 0.005M ATP in a final volume of 2.5 ml. The reacting mixtures were incubated at 37°C for 30 min, after which the reaction was stopped by 2.5 ml of ice-cold 5% perchloric acid. The (Pi) was assayed as described in (iii).

(b) The effect of β -blockers on β -galactosidase

(i) The effect of β -blockers on purified enzyme

The studies were carried out in a final volume of 4 ml (0.02M phosphate buffer, ONPG (3 mM), 2.0 units of β -galactosidase and different concentrations of β -blockers). Two controls were set up, one contained no enzyme, and the other contained the enzyme, but no β -blocker.

The assay medium was warmed for 10 min at 30°C before adding 1 ml of enzyme. The reaction was stopped by adding 1 ml of 1M potassium bicarbonate, before reading with S.P. 500 spectrophotometer at 430 nm (1 cm path).

(ii) The effect of β -blockers on partially purified enzymes

Harvested cells of E.coli grown overnight were suspended in lactose medium (DM) and the optical density adjusted to (0.6). Three flasks were used, one contained propranolol and one

atenolol; both at a final concentration of 4 mg.ml^{-1} . The other served as control and contained no drug. The flasks were shaken at 37°C in a Gallenkamp orbital water bath. After time intervals of 15 min, 5 ml sample of cells from each flask were centrifuged, then resuspended in phosphate buffer pH 6.8, toluenized and assayed for β -galactosidase activity as described in Section 2.3.5 b(iii).

(iii) The effect of divalent ions

The cells were grown overnight in fresh medium (DM) harvested and resuspended in distilled water containing 0.1M EDTA. After 30 min cells were centrifuged and resuspended in fresh phosphate buffer, the optical density adjusted to 0.6. Three cleaned 150 ml flasks were used, one contained propranolol, one atenolol (both at final concentration of 4 mg.ml^{-1} and divalent ions at final concentration of 0.005M). The other flask served as control, and contained only divalent ions at final concentration of 0.005M but no drug. The flasks were shaken at 37°C in a water bath. After time intervals of 15 min, 5 ml sample of cells from each flask was removed, centrifuged, resuspended in phosphate buffer pH 6.8, toluenized and assayed for β -galactosidase as described in Section 2.3.5b (i).

(iv) Kinetics of inhibition

The steady states were measured at varied OMPG concentrations ($0.3\text{M} - 12 \text{ mM}$) in the absence and presence of 4 mg.ml^{-1} of either propranolol or atenolol and 2.0 units of pure β -galactosidase and 0.02M phosphate buffer in a final 4 ml volume. The reaction was stopped by the addition of 2.0 ml of $1.0\text{M K}_2\text{CO}_3$, and the activity of the enzyme assayed at 430 nm as described above, 2.3.5 b (i).

(C) The effect of β -blockers on lysosomal enzyme

(i) Preparation of lysosomal enzyme fraction

Isolation of lysosomal membranes^s from rat liver was achieved by differential centrifugation. The liver from a freshly killed rat was collected in a glass tube containing 2.0 ml of 0.25 M sucrose in Tris-HCl buffer (0.02M, pH 7.4) and homogenised.

The homogenate was diluted by making up to 40 ml with the same buffer and centrifuged at 600 g for 10 min. The nuclei and intact cells were removed. The supernatant was further diluted with 40 ml of the same buffer and centrifuged at 1500 g for 20 min to isolate mitochondria, lysosomes and microbodies.

For each preparation, the rat liver was weighed and in each case the same amount was used. The pellets containing lysosomes were suspended in 4 ml of 0.4% glycogen, 0.45 sucrose in 0.02M Tris-HCl buffer pH 7.4.

(ii) Assay of enzyme activity

Drugs were dissolved in 0.04M Tris buffer (pH 7.4) containing 0.18M sucrose. A series of concentrations in the test tubes were prepared as stock solutions such that when 1.25 ml of each stock solution and 0.1 ml aliquots are mixed together in a Starstedt tube, the desired concentration is obtained. Mixtures with or without drugs were incubated for 30 min at 37°C. 1 ml from each tube was assayed for enzyme activity.

The experiment was repeated with partially isolated enzyme, obtained by incubating 0.5 ml of lysosomal membrane suspension with hypotonic sucrose solution (0.18M). The partially isolated enzyme was treated with blockers as above. Total activity was achieved by treating the lysosomal membrane suspension with Triton X100. The experiment was repeated with pure enzyme.

(iii) Kinetics of inhibition with pure lysosomal enzymes

The steady states were measured at varied substrate concentrations for each pure lysosomal enzyme in the absence and presence of fixed concentrations of either propranolol or atenolol and various pure lysosomal enzymes (acid phosphatase, aryl sulfatase and β -glucuronidase) in a final volume of 1.35 ml. Mixtures were incubated for 30 min at 37°C and 1 ml from each tube assayed for enzyme activity.

(iv) Determination of enzyme activity

Assay procedures for each enzyme activity were as follows:

(a) Acid phosphatase

1 ml aliquots in a standard cuvette containing 2.0 ml of 0.1M citrate buffer pH 4.8 was incubated at 37°C for 25 min. The reaction was started by adding 50 μ l p-nitrophenyl phosphate disodium pentahydrate, and stopped by the addition of 0.2 ml of cold 4N sodium hydroxide. Extinction values were determined at 405 nm.

(b) Aryl sulfatase

1 ml aliquots in a standard cuvette containing 2.0 ml of 0.2M acetate buffer pH 5.8. was incubated at 37°C with 100 μ l of P-nitrocatechol sulphate. The reaction was terminated by cold 4N NaOH. Extinction values were determined at 510 nm.

(c) β -glucuronidase

1 ml aliquot in a standard cuvette containing 2.0 ml of 0.1M citrate buffer pH 4.8 was incubated.

After incubation at 37°C for 25 min, reaction was initiated by addition of 50 µl of phenolphthalein glucuronic acid. The reaction was terminated with 0.4 ml of 2.2M glycine in 10N sodium hydroxide buffer pH 12.5. Extinction values were determined at 540 nm.

2.3.6. Leakage of Cellular Constituents

(a) Determination of Pentoses

Equal volumes (2 ml) of β -blocker solutions and of bacterial or candidal suspensions in (CFM) were mixed to give a final cell concentration of 4.2×10^9 or 3.8×10^5 cells.ml⁻¹. The drug-cell mixtures were incubated at room temperature (22°C) for 15 min, after which the cells were removed by centrifuging at 7000g for 15 min at 22°C. The experiment was repeated varying the contact time, and at 0°C.

Determination of pentoses was carried out as described by Mejbaum (1939). It is based on the formation of a blue-green colour, as a result of condensation of furfural (formed when pentoses are heated with concentrated HCL) with orcinol in the presence of ferric ions.

1.0 ml samples of the supernatants from drug-cell mixture were used for the estimation of pentoses, and were added to 1.0 ml of reagent for pentose estimation in test tubes. The tubes were heated in a boiling water bath for 20 min. The contents of each tube were then diluted with an equal volume of distilled water and the optical density (520 nm, 1 cm) was measured. D-ribose was used as the standard for a calibration curve.

(b) Release of 260 nm absorbing material

Equal volumes (10 ml) of β -blocker solutions and bacterial or candidal suspensions were mixed to give final cell concentrations of 4.2×10^9 or 3.8×10^5 cells.ml⁻¹. The drug-cell mixtures were allowed to stand at room temperature (22°C) for 30 min, after which the cells were removed by centrifugation (7000g, 15 min, 4°C).

β -blockers absorb in the region (260-289 nm) and therefore the direct measurement of leaked

cellular constituents at 260 nm would result in inaccurate results. The method of Beckett, Patki and Robinson (1954) was therefore employed, involving liquid/liquid extraction of drugs from the assay sample. The supernatant solution was shaken with portions of chloroform (spectroscopic grade) to remove the β -blockers from the aqueous phase. Extraction was assumed to be complete when the optical density (260 nm) reading for each tube remained constant.

(c) Release of K^+

Release of K^+ was determined using a K^+ -sensitive electrode connected to a measuring unit with output to a potentiometric recorder as described by Gale and Johnson (1974). Cultures of organisms were harvested, and washed twice with 0.2M Tris-HCl (pH 6.8) buffer made with deionized water and resuspended in this buffer.

Equal volumes of β -blocker solution (10 ml) and bacterial or candidal cell suspensions in Tris-HCl buffer 0.2M, pH 6.8 were mixed to give the final cell concentration of 4.2×10^9 or 3.8×10^5 cells.ml⁻¹. Release of K^+ from each sample at 22°C was followed at 30 min time intervals. The potentiometre reading in millivolts were converted to μ moles of K^+ released from bacterial or candidal cells using a previously constructed calibration curve.

(d) Leakage of ^{32}P from Pre-labelled cells

Release of ^{32}P from cells pulse labelled with ^{32}P orthophosphate was followed using a method based on that described by Rye and Wiseman (1966). Harvested cells were resuspended in a low phosphate medium (LPM) at 37°C and 1 μ Ci of ^{32}P orthophosphate added to the growing cells. After 90 seconds, 5 ml of 0.1M potassium dihydrogen orthophosphate was added to the growing culture and the ^{32}P labelled cells were harvested by centrifuging (10 min, 7000g), washed

with and then resuspended in CFM. The optical density was adjusted to 0.60 with more CFM.

Equal volume (5.0 ml) of β -blockers at different concentrations in CFM and of labelled bacterial or candidal cell suspensions were mixed and maintained at 22°C for 10 min. The cells were removed by centrifuging at 4500g for 15 min and 1 ml of the supernatant (duplicate) was added to 5 ml of liquid scintillator. Samples were monitored for ^{32}P content of the supernatant by counting for 5 min using a Microprocessor P. W. 4700 liquid scintillation counter.

Separation of the cold (4°C) acid-soluble fractions was determined using 5% trichloroacetic acid (TCA). Equal volumes (5 ml) of bacterial or candidal cell suspension and 10% TCA were mixed and left at 4°C for 30 min. The pellet was removed by centrifugation, and the ^{32}P content of the cold TCA (metabolic pool) was determined in a Microprocessor P.W. 4700 liquid scintillation counter.

2.3.7 Physicochemical Properties of β -blockers

(a) Cellular Uptake of β -blockers

The uptake of selected β -blockers by microbial cells was studied by two different approaches:

(i) Uptake of [^3H] Propranolol

2.5 μCi [^3H] propranolol was added to 10 ml of 100 $\text{mg}\cdot\text{ml}^{-1}$ propranolol in distilled water. 1 ml of this was added to 150 ml cleaned conical flask containing suspension of cells of approximately 4.2×10^9 or 3.8×10^8 cells per ml in CFM. At zero time 2.0 ml of cell suspension was filtered through membranes (0.45 μm porosity). 1 ml of supernatant was transferred into scintillation vial inserts and monitored for radioactivity.

The radioactivity of further samples from these [^3H] propranolol cell mixtures maintained at 37°C in a Gallenkamp shaking water bath was assessed at 2 min intervals for 15 min as above.

Equal volumes of (5 ml) of propranolol solutions, each containing $2.0\ \mu\text{Ci}$ [^3H]-propranolol and bacterial or candidal cell suspensions in CFM were mixed in sterile capped test-tubes and maintained at 37°C or 30°C appropriately in a Gallenkamp shaking water bath ($150\ \text{throw min}^{-1}$). After 15 min contact, the cells were centrifuged at 5000 rpm for 30 min. 1 ml of cell suspension was monitored for radioactivity using micro-processor, P.W. 4700 liquid scintillation counter.

(b) Uptake of Dansyl derivatives of propranolol and atenolol

(i) Derivatisation

Derivatisation of propranolol or atenolol with dansyl chloride was carried out by a method based on that described by Bayer and Gom (1976). 10 ml of $200\ \text{mg.ml}^{-1}$ β -blocker solution was transferred to a 50ml conical flask containing 10 ml, 0.2 M sodium bicarbonate pH 4.0 followed by an equal volume of dansyl chloride dissolved in acetone.

The reaction was allowed to proceed for 24 hours at 37°C (Fig. 2.2). Ratio recording spectrofluorimeter (Model SFR 100) connected to a microprocessor and recording data systems monitored the progress of formation of derivatized β -blockers. Equal amounts were analysed at time intervals until constant peak height was obtained.

(ii) Uptake

A series of tubes (2 sets) were set up. Tubes of one set, each contained 4 ml of different concentrations of β -blockers and 4 ml of CFM. The other set contained 4 ml of the same range of concentrations plus 4 ml of cell suspensions of either E.coli or C.albicans suspended in CFM at 37°C or 30°C respectively. After 30 minutes, cells were removed by centrifuging (6000g, 20 min) and the fluorescence of the supernatant liquid was measured using the ratio recording spectrofluorimeter and the peak height obtained from the data systems.

A calibration curve was constructed by comparing the difference in peak heights of dansyl propranolol or atenolol in the presence and absence of cell suspension, from which the amount of dansyl propranolol or atenolol taken up by cells was estimated.

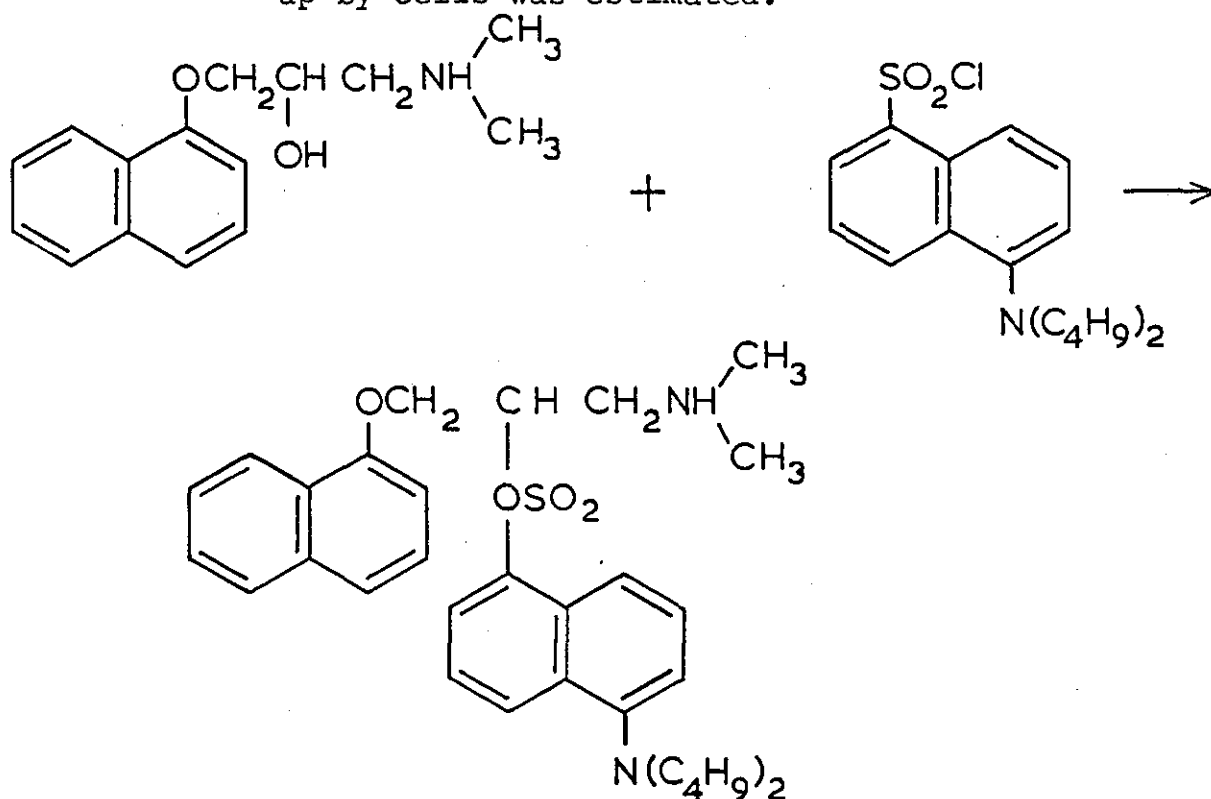


Fig. 2.3: Synthesis of Dansyl Propranolol

(c) Critical Micelle Concentration (CMC) Dye Solubilization

The solubilization of Sudan black B by solutions of β -blockers in CFM was studied by equilibrating solutions of different concentrations with excess of solid dye (10 mg dye in 10 ml solution). The drug-dye mixture was allowed to stand for 1 hr at 22°C with shaking after which the mixture was centrifuged at 4500g for 40 min to remove the undissolved dye. The maximum absorption wavelength of one of the coloured supernatant was assessed using an S.P. 800 Ultraviolet Spectrophotometer (300 - 700 nm) and then the optical density of all the supernatants was measured at that maximum wavelength.

(d) β -blocker Partition Coefficients (K o/w)

The partition coefficients (K o/w) of β -blockers was determined by the method described by Lang and Rye (1982). Equal volumes (20 ml) of known concentrations of β -blockers in phosphate buffer and either chloroform, n-heptane or octanol were mixed and continuously shaken at 22°C for 6 hr. Each container was then allowed to stand for $\frac{1}{2}$ hr. In each case two phases were separated and the concentration of β -blockers remaining in the aqueous phase determined by measuring the optical density at the wavelength of maximum absorbance. From these measurements, the partition coefficient of the drug between these organic solvents and the aqueous medium was calculated.

(e) Surface Tension Measurement

Surface tension was measured with a tensiometer by the method based on that described by Harkins and Jordan, (1930). The drugs were dissolved in CFM phosphate buffer and the surface tension measured at 22°C.

2.3.8. Erythrocyte Stabilization and Lysis in the Presence of β -blockers

(a) Assay for membrane activity

Protection and lysis of erythrocytes in hypotonic solutions was determined by measuring the release of haemoglobin. The procedure of Seeman and Weinstein (1966) was used. The stock suspension of heparinised rabbit erythrocytes (RBC) contained approximately 4.5×10^7 cells.ml. The isotonic salt solution was composed of 154 mM NaCl in 10 mM sodium phosphate buffer pH 7.0. The hypotonic solution contained 55 mM NaCl in the same buffer. Rabbit erythrocytes were used throughout. Blood samples were obtained from the ear vein.

Stock erythrocyte suspension (1.5 ml) was mixed with an equal volume of the hypotonic solution containing β -blockers at different concentrations. A control containing no drug was also prepared. The mixture was held at room temperature for 10 min after which the erythrocytes were removed by centrifuging for 5 min at 800g. The haemoglobin content of the supernatant was measured by recording the extinction at 543 (nm). The experiment was repeated in the presence of divalent metal ions at different concentrations of 0.5 mM, 2.5 mM, 5.0 mM and 10.0 mM.

(b) Release of K^+ from erythrocytes

The experiment was repeated as described above, however, the release of K^+ was measured by K^+ -sensitive electrode as described in Section 2.3.6.C).

(c) Influence of β -blockers on HTAB induced Stabilisation and Lysis of erythrocyte membrane

Protection and lysis of erythrocytes in hypotonic solutions was used as a test for membrane stabilizing activity of drugs. This experiment is

designed to show the influence of β -blockers on the stabilization and lysis caused by HTAB. A fixed concentration of β -blocker which does not alone cause lysis was used.

The experiment was initially carried out as described in Section 2.3.8 using HTAB instead of β -blockers. Later it was repeated in the presence of 0.2 mg.ml^{-1} propranolol: 0.2 mg.ml^{-1} propranolol was initially left for 5 min at 22°C with suspension of erythrocyte before adding HTAB at different concentrations. The drugs - erythrocyte suspensions were allowed to stand for a further 10 min, after which the release of haemoglobin was estimated at 543 nm as previously.

2.3.9 Measurement of Induced Turbidity Changes

(a) General Procedure for the Determination of Changes in Turbidity in Non-growing Microbial Cells

Changes in the turbidity (optical density) of non-growing microbial cell suspensions treated with various β -blocker and local anaesthetics were assessed using a method based on that described by Salt and Wiseman (1970). Equal volumes (5 ml) of different concentrations of β -blockers and bacterial or candidal cells in various media were mixed together in a series of tubes. After 10 min contact at 22°C , the optical density of samples for each tube was measured (650 nm; 1 cm).

The experiment was repeated varying the contact time and the medium composition. Similar experiments were used to assess the effects of HTAB on culture turbidity.

(b) Effect of β -blockers on the turbidity of isolated cell envelope and cytoplasmic constituents preparations

(i) Preparation of isolated cell envelope and

Bacterial or candidal cell suspensions in CFM adjusted to optical density of 0.6 (650 nm) were centrifuged (4,500g for 15 min) and resuspended in CFM.

The cell suspensions in 5 ml lots of CFM were sonicated for a total of about 8 min in an ice-cold vessel (Salton, 1960) at a rate of 9 μ m (peak to peak) in a MSE ultrasonic disintegrator (Model 60W). Intact cells were removed from the 'sonicated' by centrifugation (2,000 x g for 10 min). The supernatant was collected and recentrifuged (10,000 x g for 15 min). The pellet, consisting of isolated cell envelopes (Rogers, 1963) was washed three times with 1% w/v sodium chloride to remove adhering cytoplasmic contents and finally resuspended in CFM to give a cell envelope preparation. The amount of CFM needed to give the original OD of 0.6 was related to the initial amount and the amount used for sonication. The supernatant was a suspension of intracellular material diluted with CFM.

(ii) Effect of β -blockers

Equal volumes (5 ml) of different concentrations of β -blockers were mixed with either cell envelope preparation or dilute cytoplasmic constituents in a set of test tubes, and maintained at 22°C for 10 min. The optical density of samples from each tube was measured at 650 nm (1 cm).

- (c) Effect of β -blockers on the turbidity of lipid depleted cells and cell free lipid dispersions
- (a) Preparation of lipid extracted cells and isolation of total lipids

Large volumes of microbial cell suspensions, in mid log phase, were isolated by centrifuging (4,500 x g for 15 min). Extraction procedures was based on the method of Shaw and Dinglinger (1969). The pellet of cells were freeze dried and then suspended in chloroform: methanol (2:1). The suspension of cells were shaken for 48 hr at 22°C. After separation, the pellet of lipid-depleted cells was washed with and resuspended in CFM to the required density.

The solvent was removed from the extract under vacuum in a rotary evaporator (Rotavapor-R.Buchi, Switzerland), and the remaining extracted lipids were dispersed in CFM using ultrasonics (MSE ultrasonic disintegrator model 60W).

- (ii) Effect of β -blockers

Equal volumes of different concentrations of β -blockers were mixed with either lipid extracted cells (O.D. 0.6) or lipid emulsions in a set of test-tubes, and maintained at 22°C for 10 min. The optical density of samples from each tube was measured at 650 nm (1 cm).

2.4.1 Electron Microscopy

Scanning electron microscopy (SCM)

Harvested cells of either bacterial or candidal suspensions were mixed with different concentrations of β -blockers and shake incubated at 37°C for 15 min, after which glutaraldehyde (E.M. grade) was added to give

final concentration of 1.5% (w/v). The mixture was left to stand for 2 min, and the cells were centrifuged at 3000g for 15 min at 4°C, then resuspended in 2 ml of glutaraldehyde (5% v/v) for 16 hr at 4°C.

The cells were centrifuged, and washed 3 times with and resuspended in distilled water to a faint opalescence. One drop from each of these suspensions was placed on a 13 mm round microscope cover slip and allowed to dry in air, after which they were dehydrated over CaCl_2 under partial vacuum for 12 - 18 hr. The cover slips were fixed to 15 mm aluminium stubs using quick drying silver cement (DAG) and coated in an Edward high vacuum unit, with gold palladium to give a coating film of Ca 10 nm thickness.

The samples were examined in a Cambridge Stereoscan Electron Microscope with a beam angle of 45° and a voltage of (10 - 15 kv) magnification ranged from X30 to X30,000. Images of typical cell groups were produced on the screen of a cathode ray tube and photographed on 35 mm film (Ilford FP4 or HP5).

CHAPTER THREE

RESULTS

CHAPTER THREE - RESULTS

3.1.1 Growth inhibition

(i) Tube dilution and direct assessment procedures

The MIC values of two local anaesthetics and ten β -blockers determined by tube dilution method or via growth curves are shown in Tables 3.1a and 3.1b respectively. C.albicans and B.cereus were generally more sensitive than E.coli and P.acruginosa. Propranolol, a β -blocker, and tetracaine, a local anaesthetic are more potent drugs compared to the rest of the drugs evaluated. Tables 3.1a and 3.1b represent a decreasing order of effectiveness of drugs evaluated within each group.

Table 3.2 illustrates the effect of increasing the Mg^{2+} content of the medium on MICs. Concentrations ranging from 0.1 to 1.0 mM magnesium ion content brought about no change in the values of MIC from those initially obtained by tube dilution, but as the concentration of Mg^{2+} was increased from 5.0 to 10 mM, the MIC values for propranolol against E.coli and C.albicans increased from 0.75 and 0.45 $mg.ml^{-1}$ to 2.15 and 1.45 $mg.ml^{-1}$ respectively.

The growth curves for all the organisms are shown in Figs. 3.1 - 3.24 and represent the direct assessment method for the determination of MIC. The overall growth pattern show similarity in profile. The exponential growth rate is drug concentration dependent and with higher concentration of drugs diminishes, eventually becoming zero.

The lowest concentration of drug required to cause zero growth (i.e. growth maintained at approximately optical density of 0.3) was taken to be the MIC value for the particular compound. For each drug, the MIC value is approximately twice that obtained using the tube dilution method (Table 3.1b).

Table 3.1a: Minimum inhibitory concentrations (MICs) in
mg.ml⁻¹ of β -blockers and local anaesthetics
(Tube dilution)

MINIMUM INHIBITORY CONCENTRATIONS (mg.ml ⁻¹)	
Organisms	
Drug	<u>E.coli</u> <u>P.aeruginosa</u> <u>B.cereus</u> <u>C.albicans</u>
	β -BLOCKERS
Propranolol	0.75 $\pm$ 0.11 1.25 $\pm$ 0.03 0.58 $\pm$ 0.21 0.45 $\pm$ 0.44
Oxprenolol	1.65 $\pm$ 0.07 1.85 $\pm$ 0.13 0.75 $\pm$ 0.28 0.65 $\pm$ 0.31
Metoprolol	7.00 $\pm$ 0.05 5.50 $\pm$ 0.14 3.55 $\pm$ 0.31 2.00 $\pm$ 0.42
Acebutolol	8.00 $\pm$ 0.07 9.00 $\pm$ 0.09 4.00 $\pm$ 0.09 3.00 $\pm$ 0.46
Timolol	8.50 $\pm$ 0.04 9.50 $\pm$ 0.09 7.50 $\pm$ 0.18 6.00 $\pm$ 0.39
Sotalol	9.35 $\pm$ 0.044 10.75 $\pm$ 0.08 8.25 $\pm$ 0.21 6.75 $\pm$ 0.35
Nadalol	9.50 $\pm$ 0.05 11.00 $\pm$ 0.37 9.50 $\pm$ 0.45 7.15 $\pm$ 0.40
Pindolol	12.50 $\pm$ 0.16 13.50 $\pm$ 0.28 8.50 $\pm$ 0.42 8.00 $\pm$ 0.36
Atenolol	12.85 $\pm$ 0.07 14.75 $\pm$ 0.41 9.00 $\pm$ 0.32 8.50 $\pm$ 0.35
Practolol	13.25 $\pm$ 0.12 15.55 $\pm$ 0.47 9.25 $\pm$ 0.42 8.75 $\pm$ 0.38
	LOCAL ANAESTHETICS
Tetracaine	0.84 $\pm$ 0.02 1.35 $\pm$ 0.06 9.57 $\pm$ 0.26 9.80 $\pm$ 0.39
Procaine	13.52 $\pm$ 0.06 14.45 $\pm$ 0.05 9.75 $\pm$ 0.31 10.15 $\pm$ 0.43

Table 3.1b: Minimum inhibitory concentrations (MICs) of β -blockers and local anaesthetics (Shaken Culture - estimated values)

Organisms	MINIMUM INHIBITORY CONCENTRATION (mg.ml ⁻¹)			
	<u>E.coli</u>	<u>Ps.aeruginosa</u>	<u>B.cereus</u>	<u>C.albicans</u>
Drugs				
	β -BLOCKERS			
Propranolol	1.25 ~ 1.5	2.0	1.0	1.0
Oxprenolol	2.5 ~ 3.0	>3.0	2.0	1.5 ~ 2.5
Atenolol	>30.0	>35.0	>25.0	25.0
Practolol	>30.0	>35.0	>25.0	25.0
	LOCAL ANAESTHETICS			
Tetracaine	3.0	>2.0	1.0	0.75 ~ 1.0
Procaine	>30	>30	25.0	25.0

Table 3.2: The minimum inhibitory concentrations (MICs)
of propranolol mg.ml⁻¹ against E.coli and
C.albicans suspended in growth medium (DM)
containing different concentrations of Mg²⁺
(mM) (Tube dilution)

Mg ²⁺ (mM)	MIC (mg.ml ⁻¹ for Propranolol				
	0.1	0.5	1.0	5.0	10.0
<u>E.coli</u>	0.75 ± 0.22	0.75 ± 0.16	0.800 ± 0.09	1.75 ± 0.06	2.15 ± 0.09
<u>C.albicans</u>	0.45 ± 0.31	0.45 ± 0.39	0.500 ± 0.42	0.85 ± 0.29	1.45 ± 0.43

(ii) Reversibility of growth inhibition

Figures 3.25 - 3.36 show the reversibility of growth inhibition caused by either local anaesthetics or β -blockers on E.coli and C.albicans. After partial inhibition, which for E.coli is 40 min and C.albicans is 100 min, the cell suspensions were centrifuged and resuspended in fresh growth medium. Effects of concentrations up to the MIC (tube dilution) is reversible and show growth patterns which are linear over much of their range and have approximately equal growth rate as the control. Levels of drug up to the MIC (shaken culture method) or less show an initial lag phase, or convex pattern becoming linear with time; the rate is slower than that of the control culture. For example, culture of E.coli treated with 1.0 mg.ml^{-1} propranolol brought about an initial growth rate which was slow, lasting for 20 min, after which growth rate became linear.

With higher concentrations of either local anaesthetics or β -blockers, the initial lag phase or convex pattern, increased with time and in some instance growth rate was approximately zero, for example, 1.5 mg.ml^{-1} propranolol (Fig. 3.25a), the growth rate was approximately zero and in another case, growth proceeded at even much slower rate compared to that of control, as is the case of either atenolol or practolol or procaine treated cultures of E.coli and C.albicans (Fig. 3.27, 3.28, 3.30).

3.1.2 Cell Viability

Figure 3.37 illustrates the effect of different concentrations of propranolol and atenolol on culture viability after 15 min contact time at 22°C . Exposure of non-growing cultures of E.coli to levels of propranolol up to 0.5 mg.ml^{-1} resulted in no loss of viability. With 1.0 mg.ml^{-1} propranolol, only about 20 per cent loss in cell viability was observed. Concentrations of approximately twice the MIC value (tube dilution) and

over brought about significant loss of culture viability. This exposure of cultures of E.coli to levels of 1.5, 2.5 and 5.0 mg.ml⁻¹ propranolol resulted in approximately 75, 90 and 99.5 per cent loss of cell viability respectively.

Levels of atenolol, 5.0, 10.0, 15.0 and 20.0 mg.ml⁻¹ did not cause any loss in culture viability of cells of E.coli (Fig. 3.37). Little effect was brought about by levels of 25.0, 30.0 and 35.0 mg.ml⁻¹ atenolol. Approximately 2.5, 3.0 and 5.0 per cent loss of cell viability respectively was estimated.

Fig 3.38 illustrates the effect of different concentrations of propranolol and atenolol on culture viability after 15 min. Exposure of non-growing cultures of C.albicans to 0.1, 0.25 and 0.5 mg.ml⁻¹ propranolol resulted in no loss in viability of cells of C.albicans. Exposure to 0.75 and 1.0 mg.ml⁻¹ caused approximately 10 and 35 per cent respectively loss of culture viability of cells of C.albicans. Significant loss of about 95 and 99 per cent resulted on exposure of cells of C.albicans to 1.5 and 2.5 mg.ml⁻¹.

Loss of viability was not observed with levels of 5.0, 10.0, 15.0 and 20.0 mg.ml⁻¹ atenolol. Small changes, approximately 5.0, 10.0 and 15 per cent resulted from the exposure of cells of C.albicans to 25, 30 and 35 mg.ml⁻¹ atenolol.

The effect of contact time on the viability of cells of E.coli treated with different concentrations of propranolol is shown in Fig. 3.39. 0.5 mg.ml⁻¹ propranolol caused no loss in culture viability for contact times from 10 to 40 min. With 1.0 mg.ml⁻¹ propranolol loss of culture viability was not significant and after 40 min contact time, approximately 24 per cent loss of culture viability was estimated. However with concentrations greater than 1 mg.ml⁻¹ and over brought about loss of viability, the extent of which is time dependent. Thus with 2.5 and 5.0 mg.ml⁻¹, propranolol approximately, 15 and 24 per cent loss in cell viability were detected after 5 min contact and as the contact time increased (10 min)

approximately 70 and 99.5 per cent loss of cell viability occurred. Further increase in contact times resulted to complete loss in cell viability.

Fig. 3.40 illustrates the effect of increasing the contact time on the loss of viability due to atenolol. Concentrations of 25.0 and 35.0 mg.ml⁻¹ atenolol did not cause loss of viability on cells of E.coli after 30 min contact time. However, increasing the contact time (1 hr) caused approximately 20 and 40 per cent loss of viability. Further increases in contact time (2 to 3 hr) brought about little or no change, as approximately 25 per cent loss of viability was observed with 25 mg.ml⁻¹ atenolol and 50 per cent loss in viability was observed with 35.0 mg.ml⁻¹ atenolol.

Fig. 3.41 represent the effect of increasing the contact times on the percentage culture viability of C.albicans treated with propranolol. The pattern of the curve is similar to that for E.coli treated with propranolol (Fig. 3.39) but a lower concentration of propranolol was required for to cause viability loss. Thus 0.25 mg.ml⁻¹ propranolol had no effect on the loss of viability. 0.5 mg.ml⁻¹ propranolol caused 25 per cent loss of culture viability after 40 min contact time. However, exposure to 1.0 and 2.5 mg.ml⁻¹ brought about significant loss of culture viability (98 - 100%) after 10 to 15 min. Initially, 5 min exposure time only brought about 15 and 40 per cent loss of cell viability respectively with these concentrations, but after 10 - 15 min complete loss of culture viability occurred.

Levels of 25 and 30 mg.ml⁻¹ atenolol did not result in any loss of viability of C.albicans after 30 min contact time (Fig. 3.42). After 1 hr exposure time, however, approximately 22 and 40 per cent loss of cell viability was respectively detected. Further increase in contact times (2 to 3 hr) resulted in further loss of cell viability, the extent of which are small compared to the initial drop. Thus at the end of 3 hr, 40 and 50 per cent loss of cell viability was brought about by 25 and 30 mg.ml⁻¹ respectively compared to 35 and 45 per cent loss of cell

viability caused after 2 hr.

The effect of propranolol and atenolol on the loss of viability of B.cereus, P.aeruginosa was also investigated, this is given in Table 3.3a,b. At each contact time percentage loss in cell viability was estimated and in each case propranolol was more effective than atenolol in causing loss of viable cells.

3.1.3 The effect of β -blockers on Respiration

(i) Reduction of triphenyl tetrazolium chloride (TTC)

The effect of β -blockers on the reduction of TTC by growing cells is shown in Figs. 3.43 - 3.50. The profiles of the curves are essentially similar for all β -blockers and substrates evaluated. It is in each case concentration dependent (either E.coli, Fig. 3.43 - 3.46 or C.albicans, Fig. 3.47 - 3.50). Various concentration ranges of each drug were employed, except for atenolol and practolol where the same concentration range was employed.

Propranolol is more effective than oxprenolol, the latter being more effective than either atenolol or practolol. Only propranolol and oxprenolol reduced respiration to endogenous levels. Thus at a concentration of 7.5 and 12.0 mg.ml⁻¹ of propranolol and oxprenolol respectively, endogenous respiration was completely inhibited where as at a concentration of 30 mg.ml⁻¹ atenolol or practolol endogenous respiration was not affected (Figs. 3.43-3.47).

The extent of TTC reduction in the presence of β -blockers is affected by different substrates. Thus in the presence of malate and lactate as substrates, the extent of TTC reduction was greater compared to either glucose or succinate.

Figures 3.47 - 3.50 illustrates the reduction of TTC by cells of C.albicans by β -blockers. The overall profile of the curve is similar to that for E.coli (represented above). Similarly, TTC reduction by β -blockers is drug concentration dependent but lesser concentrations of either propranolol or oxprenolol were

Table 3.3: Percentage loss of viability after different times in the presence of:

(a) 2.5 mg.ml^{-1} Propranolol

Time (min)	Loss of viability(%) after time intervals (min)		
	5	10	15
<u>E.coli</u>	15.0	70.0	99.5
<u>Ps.aeruginosa</u>	13.0	61.5	84.5
<u>B.cereus</u>	33.5	91.5	100
<u>C.albicans</u>	40.0	100	100

(b) 25 mg.ml^{-1} Atenolol

Time (min)	Loss of viability (%) after time intervals (min)			
	30	60	120	180
<u>E.coli</u>	0	40.0	48.0	50.0
<u>Ps.aeruginosa</u>	0	22.0	34.5	44.5
<u>B.cereus</u>	0	45.0	49.5	52.0
<u>C.albicans</u>	0	44.0	49.0	50.0

required. Thus 5.0 and 7.5 mg.ml⁻¹ of propranolol and oxprenolol respectively completely inhibited endogenous respiration. Similarly, the same concentrations of either atenolol or practolol were used as was the case for E.coli. Endogenous respiration was not inhibited by either of these drugs, although the extent of TTC reduction was greater with C.albicans compared to E.coli at all concentrations of atenolol and practolol.

The extent of substrates on the influence of TTC reduction is also similar to that observed for E.coli. Thus in the presence of either malate or lactate as substrates, the extent of TTC reduction was greater compared to either glucose or succinate as substrates.

(ii) The effect of β -blockers on oxygen consumption by cells of E.coli and C.albicans

The effect of β -blockers on oxygen consumption of cell suspensions of E.coli is shown in Figs. 3.51 - 3.59. Succinate, glucose, malate and lactate were used as substrates.

There is an initial fall in oxygen consumption with subinhibitory concentrations of propranolol, metoprolol and atenolol, after which it remains constant or shows either a small increase or small decrease in oxygen consumption. An exception to this is the effect of propranolol with glucose as substrate (Fig. 3.51). The initial steep fall in oxygen consumption is followed by a gradual fall as the propranolol concentration was further increased. The extent of the initial fall in oxygen consumption with subinhibitory concentration of β -blockers was more influenced compared to either metoprolol or atenolol, the effect is more pronounced with succinate as substrate. Atenolol is least effective. The extent of slight increase in oxygen consumption following the initial fall in oxygen consumption was influenced by all the β -blockers, approximately to the same extent but the effect was more pronounced with lactate as substrate in the presence of propranolol.

The effects of β -blockers on oxygen consumption of

cell suspension of C.albicans are shown in Fig. 3.55 - 3.58. The pattern of the curve show much similarity with those for E.coli (represented above).

The initial fall in oxygen consumption was similarly more pronounced in the presence of propranolol (Fig. 3.51) with succinate and glucose as substrates. Similarly atenolol is the least effective, but atenolol is particularly effective in the presence of malate as substrate. In contrast to the observations made with E.coli metoprolol (cf. Figs. 3.52 and 3.56) is relatively ineffective with succinate, glucose and lactate in reducing oxygen consumption, and did not attained an overall inhibition of 50% oxygen consumption, except in the presence of glucose and succinate where the inhibition was greater than 50%. On the other hand, propranolol did not exceed 50% inhibition, except in the presence of lactate. Overall the inhibition of oxygen consumption is lower compared to metoprolol (Fig. 3.55). Atenolol within the concentration range studied, did not cause inhibition of oxygen consumption in excess of 50% (Fig. 3.57).

DNP was used as a comparison for the activities of β -blockers (Fig. 3.54; 3.58). The initial inhibition of oxygen consumption observed for β -blockers is not observed with DNP, rather it caused an increase in oxygen consumption in excess of 100% in both organism. The next phase was the inhibition of oxygen consumption, but never attained an overall of 50% inhibition in the presence of all the substrates evaluated (Figs. 3.54; 3.58). In both organisms DNP is particularly effective in inhibiting oxygen consumption in the presence of succinate as substrate.

The effect of time on the influence of oxygen consumption of E.coli and C.albicans treated with either β -blockers and DNP is shown in Fig. 3.59. Higher concentrations of drugs were chosen where necessary to avoid any stimulation. Apart from DNP, propranolol is very effective, thus completely inhibiting oxygen consumption of both organisms, which for E.coli is 80 min and C.albicans is 100 min. Metoprolol and atenolol did not completely — (cont) p. 92

Figure 3.120 show that the inhibition caused by Propranolol on Succinate dehydrogenase is reduced in the presence of divalent cations, the extent of which is greater in the presence of Zn^{2+} , followed by Ca^{2+} , and then Mg^{2+} .

inhibit oxygen consumption, but the former is more effective than the latter. The overall pattern of the curve is similar for both β -blockers and DNP and for both organisms.

3.1.4 Uptake of ^{14}C -glucose and mannose by cells of E.coli and C.albicans

The effects of β -blockers on the uptake of ^{14}C - glucose and mannose by cells of E.coli and C.albicans are illustrated in Fig. 3.60 - 3.61 and in Table 3.4 - 3.6.

The pattern of the curve is similar to that obtained in the study of growth curves (Fig.3.1), irrespective of the type of the carbon source. Propranolol is more effective than atenolol. (Definitions in legend).

T_1 for E.coli is 150 min, and that for C.albicans is 240 min. At T_1 the radioactivity count per minute per ml cells of either E.coli and C.albicans is lower in treated cultures, the extent of reduction in the radioactivity count is greater with propranolol than practolol, irrespective of the carbon source. At T_2 , the radioactivity count is approximately equal in both treated and untreated cultures. T_2 for the treated cultures is dependent on the type of β -blocker, and the organisms under study, thus T_2 for propranolol (240 min) is greater than the T_2 for practolol (180 min) and this value increases with C.albicans irrespective of the carbon source (Fig. 3.61). However, uptake of carbon source is more enhanced in the presence of ^{14}C -glucose.

The distribution of radioactivity in fractions of cells of E.coli with glucose as a carbon source is shown in Table 3.4. The percentage recovery (estimated from the radioactivity count obtained at T_1 and T_2) was typically 90%.

As shown in Table 3.4, much of the radioactivity is recovered from the cytoplasm compared to the cell envelope in both treated and untreated cultures (at T_2). Comparing the distribution of radioactivity in cell membrane and cell wall, it is observed that more of

Table 3.4: Distribution of radioactivity in fractions of cells of E.coli with glucose as substrate

Cell Fractions	Drug Concentrations mg/ml				
	Control	Propranolol (0.15)		Practolol (2.0)	
		T1	T2	T1	T2
CPM/ml cells in Cyto + CE	710.35	399.90	669.70	429.55	674.60
% uptake in cytoplasm	55.55 (394.60)	38.55 (154.16)	53.90 (360.97)	37.33 (160.35)	52.37 (353.29)
% uptake in cell envelope	44.72 (317.66)	49.35 (197.35)	34.78 (232.92)	44.58 (191.50)	33.44 (225.59)
% uptake in cell wall	14.82 (105.27)	28.22 (112.85)	10.12 (67.77)	25.18 (108.16)	11.83 (79.81)
% uptake in cell membrane	30.15 (214.17)	20.88 (83.50)	24.55 (171.78)	18.95 (81.39)	21.56 (145.44)

T2 = 180 and 240 min for Practolol and Propranolol respectively.

T1 = 150 min for Control, Practolol and Propranolol.

Values in parenthesis refer to counts per minute (CPM) per ml⁻¹ of cells.

Cyto - cytoplasm, CE - cell envelope

Results are means of duplicate determinations.

Table 3.5: Distribution of radioactivity in fractions of cells of C.albicans with glucose as substrate.

Cell fractions	Drug Concentrations mg/ml				
	Control	Propranolol (0.15)		Practolol (2.0)	
		T1	T2	T1	T2
CPM/ml cells in Cyto + CE	497.90	247.15	495.20	358.20	498.25
% uptake in cytoplasm	57.90 (288.28)	36.88 (91.15)	59.55 294.89	41.32 (148.00)	58.88 (293.37)
% uptake in cell envelope	36.06 (179.54)	53.17 (131.40)	37.87 (187.53)	48.58 (174.01)	39.17 (195.16)
% uptake in cell wall	9.36 (46.60)	31.32 (77.40)	10.17 (50.36)	30.55 (109.43)	12.66 (63.08)
% uptake in cell membrane	26.53 (132.09)	21.95 (54.25)	27.55 (136.42)	17.65 (63.22)	26.35 (131.29)

T2 = 360 and 420 min for Practolol and Propranolol respectively.

T1 = 240 min for Control, Practolol, and Propranolol.

Values in parenthesis refer to counts per minute per ml of cells.

Cyto - cytoplasm, CE - cell envelope.

Results are means of duplicate determinations.

it is recovered from the cell membrane of the untreated sample. In contrast, more of it is recovered from the cell wall when the cell is treated, the extent of which is greater for propranolol than atenolol.

As treated cultures are allowed to grow until T_2 , the radioactivity count in various fractions approximates to the same value to that of the untreated culture. For example, the radioactivity in the cytoplasm of untreated culture relative to the treated cultures at T_2 is in the ratio of approximately 1:1 for both. The distribution of radioactivity in fractions of cells in C.albicans with glucose as the carbon source is shown in Table 3.5. There was no wide variations in the recovery of radioactivity in both treated and untreated cultures. Similarly, as in E.coli, much of the radioactivity is recovered from the cytoplasm and approximately, in a 1:1.3 ratio relative to that in the cell envelope. However, in treated cultures, approximately 1:1.6 and 1.1 ratio may be observed for propranolol and practolol respectively. Similarly as in E.coli, most of the radioactivity is recovered from the cell membrane of the untreated sample compared to the cell wall, where as in treated samples more is recovered from the cell wall compared to the cell membrane, the extent of which is greater for propranolol compared to atenolol.

The relation between uptake of ^{14}C -glucose and growth of E.coli and C.albicans are shown in Table 36a,b. At T_1 and T_2 , the growth of these organisms were monitored by noting the optical density and estimating the dry weight from previously constructed calibration curve.

The optical density and weight of cells of treated cultures are comparatively smaller than that of the control or untreated culture. The effect is greatly influenced in the presence of propranolol. For example, the optical density, mg.dry wt/ml^{-1} of cells, and the CPM/ml^{-1} of cells of E.coli treated with propranolol are 0.410, 0.129 and 289.30, and that for practolol are 0.498, 0.169 and 631.25. These values are lower than that of the control. This is also the case with C.albicans. At T_2 , however, the radioactivity count, optical density and weight of cells are

Table 3.6: Relation between the uptake of ^{14}C - glucose and growth of:

(a) E.coli

	Drug Concentration mg.ml^{-1}				
	Control	Propranolol (0.25)		Practolol (3.5)	
		T1	T2	T1	T2
CPM/ ml^{-1} of cells	710.35	289.30	619.25	631.25	787.80
Optical Density	0.602	0.410	0.595	0.498	0.612
mg.dry wt/ ml^{-1} of cells	0.210	0.129	0.199	0.169	0.216

T2 = 180 min for Practolol and propranolol respectively

T1 = 150 min for control, Practolol and Propranolol.

(b) C.albicans

	Drug concentrations mg.ml^{-1}				
	Control	Propranolol (0.15)		Practolol (0.2)	
		T1	T2	T1	T2
CPM/ ml^{-1} of cells	464.80	236.70	474.60	323	485.40
Optical Density	0.615	0.425	0.618	0.565	0.620
mg.dry wt/ ml^{-1} of cells	0.192	0.155	0.205	0.175	0.215

T2 = 360 and 420 min for practolol and propranolol respectively

T2 = 240 min for Control, Practolol and Propranolol.

approximately equal to that of the control. For example, at T_2 , the CPM/ml⁻¹ of cell, optical density and Mg.dry wt/ml⁻¹ of cells of E.coli treated with propranolol are 619.25, 0.595 and 0.199. These values are approximately equal to that of the control, T_2 in the presence of practolol, and is also the case with C.albicans (Table 3.6b).

3.1.5 Leakage of Cellular Constituents

(i) Pentose estimation

The leakage of pentose from cells of E.coli and C.albicans exposed to different concentrations of β -blockers are shown in Figs. 3.62 to 3.67. Propranolol (Figs. 3.62b and 3.63b) and oxprenolol (Figs. 3.62a and 3.63a) caused release of pentose from both organisms in a dose dependent manner. The effect is greater with propranolol compared to oxprenolol. Maximum release occurred with 15 and 10.0 mg.ml⁻¹ oxprenolol and propranolol respectively. Atenolol and practolol (Fig. 3.62a,b) caused no pentose release within the concentration range studied.

The effect of time on the release of pentoses from cells suspended in CFM at 22°C containing either propranolol, oxprenolol, practolol or atenolol is shown in Figs. 3.64, 3.65. With propranolol and oxprenolol, there is an initial rapid release of pentose which occurred within 15 min, followed by a second slower phase. Thus the release of pentose from cells of E.coli and C.albicans was at a maximum with 10.0 and 20.0 mg.ml⁻¹ propranolol, 20.0 and 25.0 mg.ml⁻¹ oxprenolol. After a contact time of 1 hr, atenolol and practolol caused small release of pentoses from both organisms.

Further experiments were carried out in an attempt to characterise the initial and the later release phases of primary and secondary release. This is illustrated in Figs. 3.66 - 3.67, and shows the release of pentose from cells of E.coli and C.albicans at 0°C. Similar curve profiles were obtained for both organisms. The

initial or primary release was still apparent, but the later or secondary phase was much reduced and release remained constant after 15 min exposure time.

Further investigations were carried out in which release of pentose was initially induced at 0°C and later reincubated at 37°C. This is illustrated in Fig. 3.67. Secondary phases of pentoses release is now apparent at this elevated temperature.

(ii) Release of K^+

The release of K^+ from cells of E.coli and C.albicans incubated in the presence of different concentrations of propranolol and atenolol at 22°C is illustrated in Figs. 3.68-3.69. Propranolol caused a linear increase in the release of K^+ from cells of E.coli and C.albicans as the concentration increases from 1.0 to 20.0 mg.ml⁻¹. Atenolol did not cause the release of K^+ from cells of either organism within the concentration range studied.

The time dependence of the release of K^+ from cells of E.coli and C.albicans in the presence of propranolol is shown in Fig. 3.69. The release of K^+ in each case increases as the exposure time increases. With 20.0 mg.ml⁻¹ propranolol exposed to E.coli, maximum release was not attained after 4 hr contact time. With 5.0 and 15.0 mg.ml⁻¹ pentose release was at maximum, at 1 and 2 hr respectively, after which further release was slower. In contrast to C.albicans, maximum release with 5.0 and 15.0 mg.ml⁻¹ propranolol occurred at 2 and 1 hr respectively. Very little K^+ was released from cells of E.coli and C.albicans with 0.5 and 1.0 mg.ml⁻¹ propranolol.

(iii) Leakage of 260 nm absorbing materials

The effect of propranolol, practolol and atenolol on the release (after 15 min contact) of 260 nm absorbing materials from cells of E.coli and C.albicans are shown in Fig. 3.70a,b. Release of 260 nm absorbing materials from both cells increases as the concentration of propranolol

increases, reaching a maximum at about 7.5 mg.ml^{-1} . At higher concentrations of propranolol, the release of 260 nm absorbing materials from both cell types either remains unchanged or declines. Neither atenolol or practolol caused the release of 260 nm absorbing materials from the organisms within the concentration range studied.

(iv) Release of ^{32}P -labelled cell suspensions

The leakage from ^{32}P -labelled cell suspensions of E.coli and C.albicans in the presence of β -blockers is shown in Fig. 3.71-3.74. The percentage release of ^{32}P -labelled cell suspensions from both organisms in the presence of β -blockers was estimated and expressed as a percentage of that obtained by treating cells with cold 5% trichloroacetic acid.

There was very little leakage of ^{32}P -labelled materials from cells (i.e. in the absence of drug). Leakage of ^{32}P -labelled cells of E.coli increased as the concentration of propranolol and oxprenolol increased, reaching a maximum (equivalent to approximately 80% to that caused by TCA) at approximately $5.0 - 7.5 \text{ mg.ml}^{-1}$ propranolol and 15.0 mg.ml^{-1} oxprenolol (Fig. 3.71a,b). Leakage from ^{32}P -labelled cells of C.albicans also increased as the concentration of propranolol and oxprenolol increased, reaching a maximum (equivalent to 100% of that caused by TCA) at 7.5 mg.ml^{-1} propranolol and oxprenolol (Fig. 3.72a,b). Higher concentration either resulted in a slight change or decline of the release of ^{32}P -labelled cells of both organisms. Atenolol and practolol did not cause the release of ^{32}P -labelled materials from either organisms within the range of concentrations used (Fig. 3.72a,b).

The time course for the release of ^{32}P -labelled cells of E.coli and C.albicans in the presence of fixed concentrations of propranolol and oxprenolol are shown in Figs. 3.73 - 3.74. Release of ^{32}P -labelled cells from both organisms was initially rapid, reaching a maximum within 15 min approximately, causing 90 per cent loss of ^{32}P -labelled cells from both organisms in the presence of

propranolol (2.5 mg.ml^{-1}) and oxprenalol (5.0 mg.ml^{-1}).

3.1.6 Physiochemical Properties of β -blockers

(i) Uptake of dansyl propranolol and atenolol by cells of *E.coli* and *C.albicans*

The uptake of dansyl propranolol and atenolol by cells of *E.coli* and *C.albicans* are shown in Fig. 3.75a,b. The amount of dansyl β -blockers taken up by cells was expressed as mg.ml^{-1} determined by previously constructed calibrated curve from which percentage uptake was expressed. Uptake of dansyl propranolol increases linearly with increase in dansyl propranolol concentrations up to 12.0 and 7.5 mg.ml^{-1} of dansyl propranolol treated *E.coli* and *C.albicans* respectively. Above these maximum concentrations, the isotherm curve flattens out, as little change of uptake of dansyl propranolol was observed. In contrast, the uptake of dansyl atenolol was linear and did not attain a maximum within the concentration range studied.

(ii) Uptake of ^3H -propranolol by cells of *E.coli* and *C.albicans*

The uptake of ^3H -propranolol by cells of *E.coli* or *C.albicans* maintained at 37°C or 30°C respectively is illustrated in Figs. 3.76a,b. The uptake isotherm with time (Fig. 3.76 a) for both *E.coli* and *C.albicans* is linear over short range of time (2 min) reaching a maximum within 4 min, after which, uptake of ^3H propranolol by both organisms brought about no change. In contrast, the uptake isotherm at different concentrations of ^3H propranolol (Fig. 3.76b) is linear over the concentration studied for both organisms.

(iii) β -blockers partition coefficient

The partition coefficients of β -blockers between chloroform or octanol or n-heptane and phosphate buffer

at 22°C are shown in Table 3.7. Partition coefficient increases with the pH of the buffer, and the order of β -blockers solubility in these organic solvents is propranolol > oxprenolol > atenolol $\approx$ practolol.

Table 3.7: The partition coefficients of β -blockers in chloroform-phosphate (0.2M), n-octanol - phosphate (0.2M) and n-heptane phosphate buffer (0.2M). (pH 7.0 and 8.0)

Drug	Chloroform		n-Octanol		n-Heptane	
	pH 7.0	pH 8.0	pH 7.0	pH 8.0	pH 7.0	pH 8.0
Propranolol	53.45	150.15	5.45	54.22	0.31	3.22
Oxprenolol	10.75	110.12	1.25	5.65	0.067	0.59
Atenolol	0.62	5.89	0.012	0.12	0.022	0.175
Practolol	0.55	5.68	0.010	0.11	0.0185	0.170

(iv) Determination of critical micelle concentration (CMC) by dye solubilization

A critical micelle concentration (CMC) for propranolol, tetracaine, practolol, atenolol and procaine was established from the data obtained from the solubilization of Sudan Black B at 22°C, after agitation. The results (Fig. 3.77) show a distinctive change in slope at approximately 5.0 mg.ml⁻¹ for propranolol and tetracaine, and this value was taken as the CMC for propranolol and tetracaine. Such distinctive change was not observed with either practol, or atenolol or procaine in the presence of Sudan B Black up to 30.0 mg.ml⁻¹. In the presence of cell (Fig. 3.77b) the distinctive change occurred at approximately the same amount of propranolol and tetracaine. As in Fig.3.77a practolol, atenolol and procaine did not show any distinctive change in the presence of cells.

(v) Surface tension measurement

Figure 3.78 shows the surface tension measurements in the presence of propranolol or atenolol dissolved in CFM. Whereas propranolol lowered the surface tension in a dose dependent manner, atenolol did not up to 50 mg.ml^{-1} . Higher concentrations of atenolol ($60\text{-}100 \text{ mg.ml}^{-1}$) only caused small lowering of surface tension.

3.1.7 Membrane activity of β -blockers

(i) The effect of β -blockers on the stabilization/lysis of erythrocytes membrane

Fig. 3.79 - 3.80 show the stabilizing and lytic effects of β -blockers on rabbit erythrocytes membrane. Other drugs such as local anaesthetic, and HTAB were included as representative of membrane active compounds. It can be seen that propranolol, tetracaine, procaine and HTAB and not atenolol were found at low concentrations to protect erythrocytes from hypotonic haemolysis. At high concentrations these compounds only had the opposite effect, resulting in lysis of erythrocyte. The biphasic pattern of stabilization/lysis is characteristic to all the active compounds. At a concentration range of $0.2 - 0.4 \text{ mg.ml}^{-1}$ (Fig. 3.79) propranolol brought about maximum stabilization corresponding to approximately 45% protection against osmotic haemolysis. The minimum range of concentration causing lysis is approximately 1.0 mg.ml^{-1} .

The relationship between stabilization of the erythrocyte membrane and the release of K^+ is shown in Fig. 3.81. It shows that both the release of K^+ and haemoglobin were prevented by concentrations of propranolol up to 0.6 mg.ml^{-1} . The solubilization-release profiles for both K^+ and haemoglobin are very similar.

The effect of divalent cations on stabilization-lysis caused by propranolol is represented in Figs. 3.82 - 3.83. Both Mg^{2+} and Ca^{2+} influenced the overall profile of stabilization-lysis curves. In the presence of Mg^{2+} and Ca^{2+} , the erythrocyte membrane is further stabilized, the extent of which is dependent on the concentration of either Mg^{2+} or Ca^{2+} (Fig. 3.82 or 3.83). In addition, Mg^{2+} and Ca^{2+} reduced the lytic effect of propranolol, and the extent of which is dependent on the concentrations of these ions. At 10 mM of either Mg^{2+} or Ca^{2+} , the erythrocytes membrane is completely protected from the lytic effects of propranolol. Further experiments were carried out to investigate the importance of these ions in

erythrocyte membrane stability to propranolol. The result of such investigation is illustrated in Fig. 3.84 and shows that Ca^{2+} protects the erythrocyte membrane from the release of haemoglobin in the presence of saline.

Fig. 3.85 shows the effect of 0.2 mg.ml^{-1} propranolol on the stabilization-lysis caused by various concentrations of HTAB. In the presence of propranolol, the stabilizing effect of HTAB was potentiated, and the lysis of erythrocyte membrane occurred at a higher concentration of HTAB compared to the curve in which 0.2 mg.ml^{-1} propranolol was absent. The extent of protection from lysis caused by HTAB is related to the initial concentration of HTAB that caused lysis of erythrocyte membrane in the absence of 0.2 mg.ml^{-1} propranolol ($\sim 8 \text{ mg.ml}^{-1}$) to that which caused the lysis of the erythrocyte in the presence of propranolol (60 mg.ml^{-1}).

(ii) The effect of β -blockers on the stabilization/lysis of lysosomal membrane

The effect of β -blockers on the release of lysosomal enzymes from rat liver lysosome suspensions are illustrated in Figs. 3.86-3.87. The release of lysosomal enzymes are expressed as percentage of total activity of enzyme. The concentration dependent curve shows 2 phases. The initial phase corresponds to protection or stabilization in which the release of lysosomal enzymes are prevented, reaching a maximum at approximately ($0.5 - 1.0 \text{ mg.ml}^{-1}$) propranolol. Approximately 12, 24 and 26 per cent of acid phosphatase, β -glucuronidase and arylsulfatase relative to the control are stabilized against the hypotonic medium. Above this concentration range, release of lysosomal enzyme gradually increases above the maximum range of stabilization. Concentration range of propranolol responsible for this lies within ($1.5 - 4.0 \text{ mg.ml}^{-1}$) for acid phosphatase ($1.5 - 3.5 \text{ mg.ml}^{-1}$) for both arylsulfatase and β -glucuronidase. For acid phosphatase, this concentration range did not cause the release of those enzymes in excess to that of the control (Fig. 3.87). However, further increase in the concentration of

propranolol to 4.0 mg.ml^{-1} resulted in the release of β -glucuronidase and arylsulfatase in excess to the control. Approximately 8% in excess of the control was released. Atenolol (Fig. 3.86 - 3.87) has no effect on the stabilization of lysosomal membrane against hypotonic medium, or on the release of lysosomal enzymes.

(iii) Induced Turbidity changes in β -blockers

Figure 3.88 - 3.93 shows the effect of β -blockers and local anaesthetics on the turbidity of non-growing cells of E.coli, C.albicans, P.aeruginosa and B.cereus. Initial culture absorbance at (650 nm) was approximately 0.3. Propranolol, oxprenolol and tetracaine showed a common pattern of turbidity changes profile.

The curves show three main regions: a primary region, in which little or no change on turbidity was apparent. Approximately, concentration range within $1.0 - 2.5 \text{ mg.ml}^{-1}$ of propranolol and tetracaine, $1.0 - 5.0 \text{ mg.ml}^{-1}$ oxprenolol caused little or no change. The secondary region is one of large increase in turbidity and commences with distinctly a large change in culture turbidity. Thus for propranolol and tetracaine, a distinct change occurred with 5.0 mg.ml^{-1} and for oxprenolol 7.5 mg.ml^{-1} . Increase in turbidity is concentration dependent reaching a maximum at approximately $10-15 \text{ mg.ml}^{-1}$ for propranolol, tetracaine and oxprenolol. As the concentrations of these drugs are increased, turbidity either remains virtually constant or declines. This gives the final region of the pattern of the curve profile. The characteristic pattern is similar with all the organisms studied.

The effect of 7.5 mg.ml^{-1} propranolol and 20.0 mg.ml^{-1} of either atenolol and practolol on changes in turbidity increases of E.coli and C.albicans at varying times are shown in Fig. 3.94. The initial rapid increase in turbidity with propranolol is complete within 15 min. Further increase in exposure time brought about a slight change or decline in turbidity increases. In the

presence of atenolol or practolol, there was no increase in turbidity changes of both organisms, instead a slight decrease was noticeable over the period of 5 hr.

Figures 3.95 - 3.96 shows the effect of propranolol on the turbidity changes of non-growing cells of E.coli and C.albicans suspended in either 0.2M phosphate buffer or distilled water. In the presence of either 0.2M phosphate buffer or water, the initial region is absent, and increase in turbidity changes progressively, increased with increased concentration of propranolol, reaching a maximum at approximately 15 mg.ml^{-1} . The only exception is in the presence of C.albicans, where the maximum increase in turbidity changes occurred with 2.5 mg.ml^{-1} propranolol. Higher concentrations as in E.coli brought about small changes in turbidity change.

Figures 3.97 - 3.98 shows the effect of propranolol in the turbidity increase of cells of E.coli and C.albicans suspended in a medium containing 0.001M of either Mg^{2+} or Ca^{2+} or Zn^{2+} . In the presence of divalent cations (Fig. 3.97) the concentration range giving rise to the initial region increases, thus increasing the threshold concentration corresponding to the distinct change. Furthermore, the overall increases in turbidity changes caused by propranolol in the absence of divalent ion are significantly reduced at all levels of propranolol concentration. The effect is more influenced by Zn^{2+} , followed by Ca^{2+} and then Mg^{2+} .

Figure 3.98 shows the effect of varying the strength of divalent ions on the increase in turbidity of cells of E.coli and C.albicans suspended in CFM at 22°C in the presence of 7.5 mg.ml^{-1} propranolol. The tube containing 7.5 mg.ml^{-1} propranolol in the absence of cations served as control. Increasing the concentration of divalent ion from (1 - 20 mM) resulted in an increase in the reduction of the turbidity increases of both cell suspensions. The increasing order of effectiveness in reducing turbidity increases of cell suspension is Mg^{2+} , Ca^{2+} and Zn^{2+} ,

The effect of propranolol and atenolol on the

turbidity changes of cell envelope and intracellular material preparations of E.coli and C.albicans are illustrated in Fig. 3.99. The increase in turbidity of cell envelope in the presence of propranolol is very slight. The increase in turbidity merely reached an optical density of 0.1 in cells of E.coli with 30 mg.ml^{-1} of propranolol relative to 0.025 of the control. The effect is greater in C.albicans reaching an optical density of 0.2 with 30.0 mg.ml^{-1} propranolol. In contrast, propranolol caused a larger increase in the turbidity of intracellular material preparations of both organisms. The characteristic regions are apparent, thus the initial phase lies in the range of $1.0 - 2.5 \text{ mg.ml}^{-1}$ of propranolol, the distinct change occurring with a concentration of 5.0 mg.ml^{-1} propranolol. Maximum increase in turbidity changes occurred with approximately 10.0 mg.ml^{-1} propranolol. Higher concentrations of propranolol brought about no change in turbidity increases in both organisms. Although the curve profile is much smaller in cell envelope, these regions are still apparent. Atenolol brought about no increase in turbidity changes in either cell envelope and intracellular preparations.

Figure 3.100 shows the effect of propranolol and atenolol on the turbidity changes of lipid extracted cells and lipid dispersed preparations of E.coli and C.albicans. Propranolol caused the increase in turbidity changes of both lipid dispersions and lipid depleted cells. The initial phase, characteristic of slight change or no change in turbidity increases, is absent in either lipid dispersions or lipid extracted cell preparations. Thus increase in turbidity changes increases with an initial concentration of 1.0 mg.ml^{-1} propranolol, reaching a maximum at 10.0 mg.ml^{-1} propranolol, on lipid extracted cell and lipid dispersed preparations, the exception is observed in lipid dispersed preparations of C.albicans, where the maximum increase in turbidity changes occurred with a concentration of 5.0 mg.ml^{-1} propranolol. Higher concentrations of propranolol brought a decline in the increase in turbidity changes. The decline in turbidity

changes is more pronounced in lipid dispersed preparations. In E.coli and C.albicans, increase in turbidity changes declined to approximately 0.195 and 0.150 (O.D.) respectively. Atenolol had no effect on the turbidity of lipid extracted cells and lipid dispersed preparations of both organisms.

The effect of propranolol and atenolol on the induced turbidity increase caused by different concentrations of HTAB on cells of E.coli is shown in Fig. 3.101. The experiment was carried out with HTAB in the absence of propranolol or atenolol and with HTAB in the presence of 0.005, 0.5, 1.0, 2.5, 5.0, 20.0 mg.ml^{-1} propranolol and 1.0, 5.0, 10.0 mg.ml^{-1} atenolol.

In the presence of 0.5, 1.0 and 2.5 mg.ml^{-1} propranolol, the increase in turbidity caused by HTAB was significantly reduced. The effect is more pronounced the smaller the concentration of propranolol. In the presence of these concentrations, the initial phase of HTAB is slightly increased, the threshold concentration is also affected, and the maximum increase in turbidity changes with higher concentrations are significantly reduced. In contrast, 5.0 and 20.0 mg.ml^{-1} propranolol, shifted the HTAB induced curve to the left, the higher the concentration of propranolol the greater is this effect. In the presence of these concentrations, the initial phases are lacking, the turbidity increase of cells of E.coli increases as the concentration of HTAB is increased, reaching a maximum with approximately 5.0 mg.ml^{-1} HTAB, and similarly higher concentrations of HTAB brought about no further increase in turbidity changes. With a threshold concentration of 0.005 mg.ml^{-1} propranolol, the increase in turbidity caused by HTAB was not significantly affected, as the overall profile is similar.

In the presence of atenolol (Fig. 3.101b) the overall pattern of the curve of increase in turbidity changes by HTAB was not significantly affected, thus the initial phase is not affected as the concentration of HTAB responsible for distinct change remains unchanged.

However, the maximum increase in turbidity increase was reduced in the presence of 5.0 and 10.0 mg.ml⁻¹ atenolol, but the concentration of HTAB corresponding to the maximum increase remains unchanged.

Further experiments were carried out to investigate the extent to which the initial phase caused by HTAB on cells of E.coli is affected by 0.25 mg.ml⁻¹ propranolol. The result of such an experiment is illustrated in Fig. 3.102). It shows that the initial phase shifted further to the right up to 60 µg.ml⁻¹ HTAB. A distinct change is apparent with 70.0 µg.ml⁻¹ HTAB compared to approximately 5.0 µg.ml⁻¹ HTAB in the absence of propranolol. In Fig. 3.103 the effect of HTAB on the growth of E.coli in minimal growth media at 37°C containing propranolol and atenolol was investigated. While in the presence of propranolol the sensitivity of HTAB to E.coli was reduced, atenolol increased it.

3.1.8 The effect of β-blockers on Membrane and Membrane Associated Cytoplasmic Enzymes

(i) The effect of β-blockers on Mg²⁺-ATPase

The effect of β-blockers on the activity of membrane-bound Mg²⁺-ATPase of B.cereus and C.albicans is represented in Fig. 3.104 - 3.105). The concentration-dependent inhibition curves were roughly linear up to 2.5 mg.ml⁻¹ propranolol or practolol and 5 µg.ml⁻¹ of HTAB. 50% inhibition of Mg²⁺-ATPase was brought about by 3.0 mg.ml⁻¹ propranolol and 2.0 µg.ml⁻¹ HTAB. With practolol, however, 50% inhibition was not achieved.

The inhibition of Mg²⁺-ATPase from both organisms reached a maximum at concentrations of 5.0 mg.ml⁻¹ of propranolol or atenolol and 5.0 µg.ml⁻¹ HTAB are 66, 35, and 80 per cent respectively. These values are similar with enzymes from both organisms.

Figures 3.106 - 3.107 shows the effect of varying the time of exposure of Mg²⁺ ATPase from B.cereus and

C.albicans to fixed concentrations of 1.0 and 3.5 mg.ml⁻¹ of propranolol and practolol respectively. The decrease in the activity of the enzyme continued up to 3 hr. Further increase in the exposure time after 3 hr brought about smaller decrease in the activity of the enzymes. Practolol is less effective, thus after 5 hr exposure time, approximately 30 and 35 per cent of inhibition of the activity of the enzyme by practolol and propranolol, respectively, was detected.

Further experiments were carried out in an attempt to determine the kinetics of inhibition of Mg²⁺-ATPase by β -blockers and HTAB. Figure 3.109 shows that the double reciprocal plot of the kinetics of Mg²⁺-ATPase by fixed β -blockers and HTAB concentrations and varied ATP concentration is competitive. The various kinetic constants are given in Table 3.8a. The increase in Vmax follows the order HTAB > propranolol > practolol. The experiment was repeated in the presence of various concentrations of Mg²⁺ ion with a fixed concentration of 0.5 and 5.0 mg.ml⁻¹ propranolol and practolol respectively, and 0.5 μ g.ml⁻¹ HTAB (Fig. 3.110) and shows that the type of inhibition is competitive towards Mg²⁺ ion. The kinetic constant is shown in Table 3.8b, and the order of increasing Km is HTAB > propranolol > practolol.

The effect of drugs in the presence of divalent ion (Mg²⁺) on the activity of Mg²⁺ ATPase is shown in Fig. 3.108. At a fixed concentration of 1.0 and 5.0 mg.ml⁻¹ propranolol and practolol respectively contained different concentrations of Mg²⁺. The inhibition brought about by 1.0 and 5.0 mg.ml⁻¹ propranolol and practolol respectively, represents 100% inhibition from which the per cent inhibition in the presence of Mg²⁺ ion was determined. The inhibition brought about by various concentrations of either 1.0 mg.ml⁻¹ propranolol and 5.0 mg.ml⁻¹ practolol was reduced in the presence of Mg²⁺ ions, the extent of which is concentration dependent.

The reversible manner of the inhibition of the activity Mg²⁺-ATPase by β -blockers and HTAB was investigated by a dilution technique (Table 3.9). The results

Table 3.8: Kinetic constants for Mg^{2+} ATPase activity treated with β -blockers in the presence of fixed concentration of ATP. The correlation coefficients of the regression R was obtained from 6 data points.

(a) (Fixed concentration of ATP)

Drug Concentration	$V_{max}(\mu g/min^{-1}$ $1\ ml^{-1}\ enzyme$	K_m (mM)	Slope	R
Control $0.0\ mg.ml^{-1}$	3.64	2.44	1.37	0.991
Propranolol $0.5\ mg.ml^{-1}$	6.45	2.55	0.87	0.987
Practolol $5.0\ mg.ml^{-1}$	5.26	2.35	1.1	0.966
HTAB $0.5\ \mu g.ml^{-1}$	8.93	2.62	0.79	0.994

(b) (Fixed concentration of Ca^{2+})

Drug Concentration	$V_{max}(\mu g/min^{-1}$ $1\ ml^{-1}\ enzyme$	K_m (mM)	Slope	R
Control $0.0\ mg.ml^{-1}$	7.15	1.28	1.61	0.995
Propranolol $0.5\ mg.ml^{-1}$	7.08	2.63	0.97	0.990
Practolol $5.0\ mg.ml^{-1}$	7.10	1.8	1.32	0.994
HTAB $0.5\ \mu g.ml^{-1}$	7.0	3.3	0.87	0.987

Table 3.9: Reversibility of β -blockers on HTAB inhibition of Mg^{2+} -ATPase after dilution

Drug Concentrations			Inhibition expected (%)	% Inhibition after dilution
Atenolol (mg.ml ⁻¹)	Propranolol (mg.ml ⁻¹)	HTAB μ g.ml)		
1.0	-	-	8.5	1.22
2.5	-	-	22.0	1.85
5.0	-	-	33.0	2.45
-	0.5	-	10.5	1.33
-	1.0	-	27.0	2.14
-	2.5	-	75.0	55.86
-	-	0.25	9.42	1.31
-	-	0.50	20.0	1.95
-	-	1.00	68.35	47.88

obtained show that the inhibition caused by drugs are reduced at all levels of drug concentrations but the extent is greater with lower concentrations compared to higher concentrations of propranolol and HTAB.

(ii) The effect of β -blockers on β -galactosidase

The effect of different concentrations of β -blockers on the inhibition of pure β -galactosidase enzyme is shown in Fig. 3.111. Both propranolol and atenolol inhibited the activity of the enzyme. The concentration dependent profile of the curve show progressive decrease in enzyme activity up to 6.0 and 5.0 mg.ml^{-1} atenolol and propranolol respectively and also causing 80 and 70% inhibition respectively. Surprisingly, atenolol is more effective than propranolol in this respect. Thus values for 50% inhibition are 3.5 and 5.5 mg.ml^{-1} atenolol and propranolol, respectively.

The double reciprocal plot of the kinetics of inhibition of the enzyme in the presence of fixed concentrations of β -blockers and the enzyme is shown in Fig. 3.114. It shows a single intersection point of treated and untreated enzyme assay in the ordinate, characteristic of competitive type of inhibition. The kinetic constants are shown in Table 3.10. The values of the K_m for the substrate shows that atenolol is more effective inhibitor of the activity of this enzyme compared to propranolol.

The inhibition of β -galactosidase enzyme by β -blockers was investigated with unpurified form of the enzyme from cells of E.coli. A fixed concentration of 4 mg.ml of propranolol and atenolol was used (Fig. 3.112). Propranolol is more effective in this case, thus after 15 min exposure time, propranolol and atenolol caused about 50 and 25 per cent inhibition of the enzyme activity, and after 90 min contact time, 70 and 35 per cent of the inhibition of the enzyme had resulted in the presence of propranolol and atenolol respectively.

The inhibition of unpurified β -galactosidase

Table 3.10: Kinetic constant for β -galactosidase activity treated with β -blockers. The correlation coefficient of the linear regression (R) was obtained from 10 data points.

Drug Concentration (mg.ml ⁻¹)	Vmax mM/min/unit Enzyme	Km (mM)	Slope	R (n)
Control 0.0	3.35	1.0	5.51	0.992
Propranolol 4.0	3.45	3.3	4.42	0.989
Atenolol 4.0	3.5	5.0	1.19	0.993

enzyme by propranolol and atenolol is reduced in the presence of divalent ions (Fig. 3.113). The results show that the effect of β -blockers are reduced by both Mg^{2+} and Ca^{2+} ions, the extent of which is greater for atenolol compared to propranolol.

(iii) The effect of β -blockers on lysosomal enzymes

The effect of β -blockers on lysosomal enzymes are illustrated in Fig. 3.115 - 3.116.. Propranolol and atenolol inhibited the activities of lysosomal enzymes, the order of sensitivity is: arylsulfatase > β -glucuronidase > acid phosphatase. Atenolol did not have any significant effect on these enzymes. Neither propranolol or atenolol caused a 50% inhibition on arylsulfatase (Fig. 3.115a) or β -glucuronidase (Fig. 3.115a) within the range of concentration range studied and on acid phosphatase (Fig. 3.116) only caused approximately 30.0 and 10.0 per cent inhibition of the enzyme activity respectively.

The effect of β -blockers on pure lysosomal enzymes are shown in Fig. 3.117. The concentration dependent curve show that propranolol and atenolol inhibit the activities of arylsulfatase and β -glucuronidase without any detectable effect on acid phosphatase. Similarly as in partially purified enzyme, 50% inhibition was not achieved by either propranolol or atenolol. Maximum inhibition occurred at a concentration of 2.0 mg.ml^{-1} propranolol on arylsulfatase and about 1.0 mg.ml^{-1} of propranolol on β -glucuronidase.

The kinetic inhibition of arylsulfatase and β -glucuronidase are represented in Figs. 3.118 and 3.119 in a double reciprocal plot. Non competitive type of inhibition was observed. The kinetic constants are shown in Table 3.11.

Table 3.11a: Kinetic constants for β -glucuronidase activity treated with β -blockers. The correlation coefficients of the linear regression R was obtained from 6 data points

Drug Concentration (mg.ml ⁻¹)	Vmax/ (mM/min/unit enzyme	Km (mM)	Slope	R
Control 0.0	5.5	9.5	1.23	0.985
Propranolol 0.5	2.8	9.0	1.12	0.988
Atenolol 10.0	4.5	10.22	1.17	0.981

Table 3.11b: Kinetic constants for Arylsulfatase activity treated with β -blockers. The correlation coefficient of the linear regression was obtained from 6 data points

Drug Concentration (mg.ml ⁻¹)	Vmax/ mM/min/unit enzyme	Km (mM)	Slope	R
Control 0.0	9.09	2.5	1.74	0.989
Propranolol 0.5	5.2 5.2	2.55	2.85	0.990
Atenolol 10.0	6.6	2.52	2.42	0.993

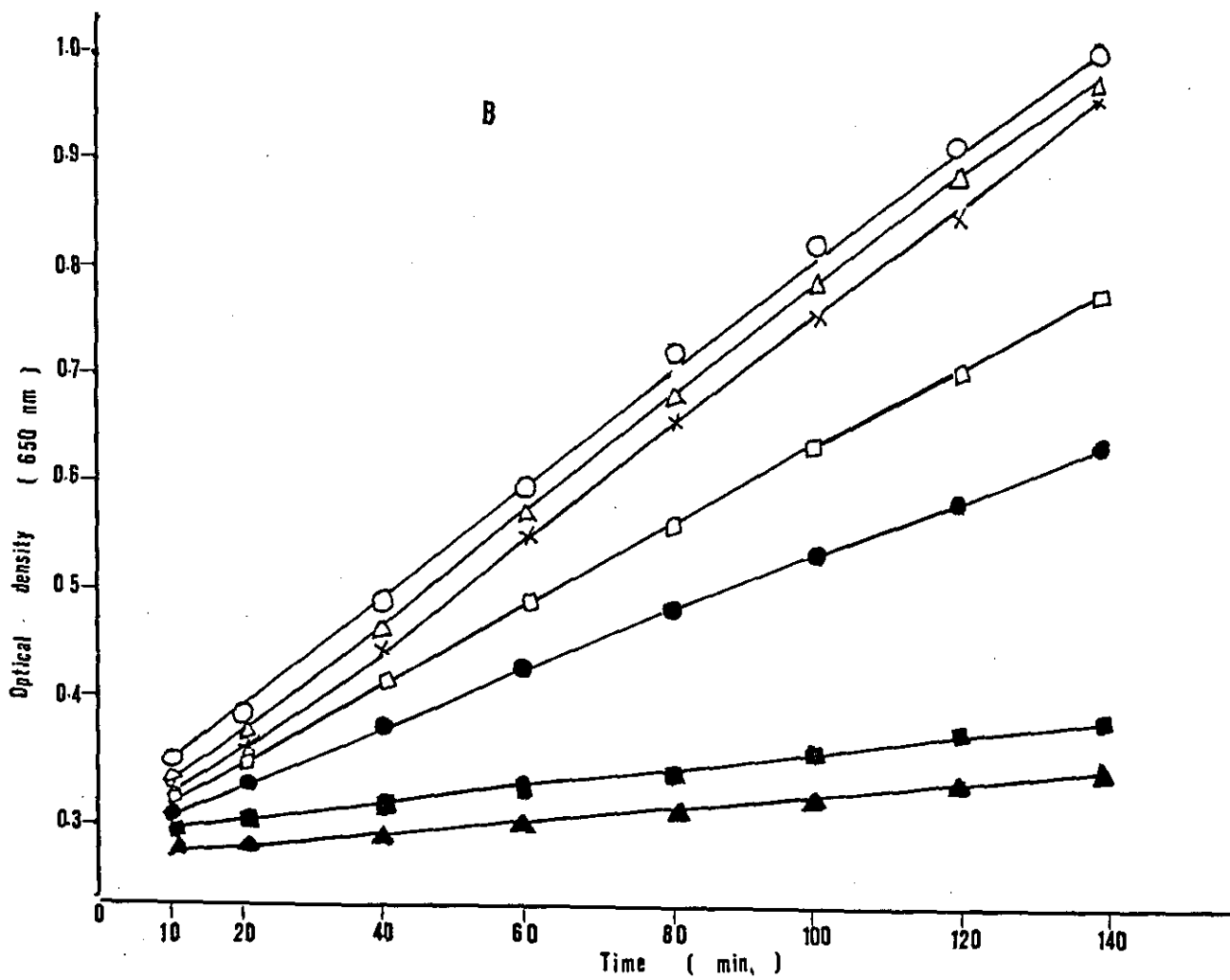
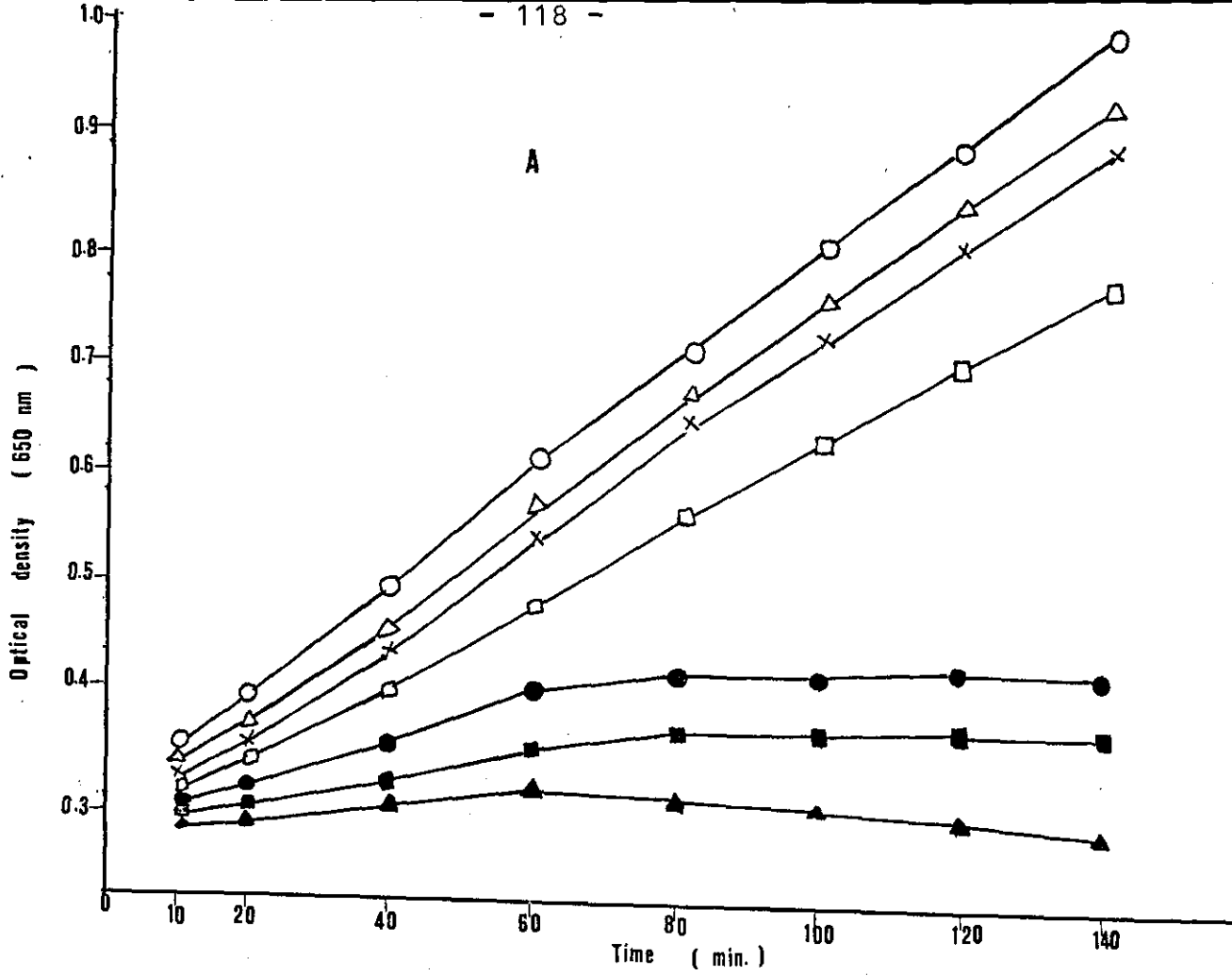
3.1.9 Electron Microscopy

Plates (3.1 - 3.7) are scanning electron micrographs of untreated and β -blocker treated cells of E.coli. Untreated cells appear rod-shaped with smooth surfaces. Cells treated with 0.5 and 30.0 mg.ml⁻¹ propranolol and atenolol respectively (Plate 3.2 and 3.3) also appear rod-shaped with smooth surfaces similar to untreated cells. In the presence of 1.0 mg.ml⁻¹ propranolol (Plate 3.4), most of the cells show similar characteristic appearance to the untreated cells, while some show surface damages. The number of cells showing surface damages increases as the concentration of propranolol are increased from 2.5, 5.0 and 7.5 mg.ml⁻¹.

Plates (3.8 - 3.14) are scanning electron micrographs of untreated and β -blocker treated cells of C.albicans. Untreated cells consists of both yeast (round or oval in shape) and vegetative mycelium (long branched filaments) forms. Plates (3.9, 3.10, 3.13 and 3.14) treated with 0.25, 0.5 mg.ml⁻¹ propranolol and 10.0 and 25.0 mg.ml⁻¹ atenolol are similar in appearance to that of the untreated cells. Both the yeast and vegetative mycelial forms appear not to be affected by these levels of propranolol and atenolol. However, (Plate 3.11) treated with 1.0 mg.ml⁻¹ propranolol the yeast form appear not to be affected, but the vegetative forms show some surface damages. As the concentration is increased to 5.0 mg.ml⁻¹ propranolol both forms of Candidal cells show extensive surface damages.

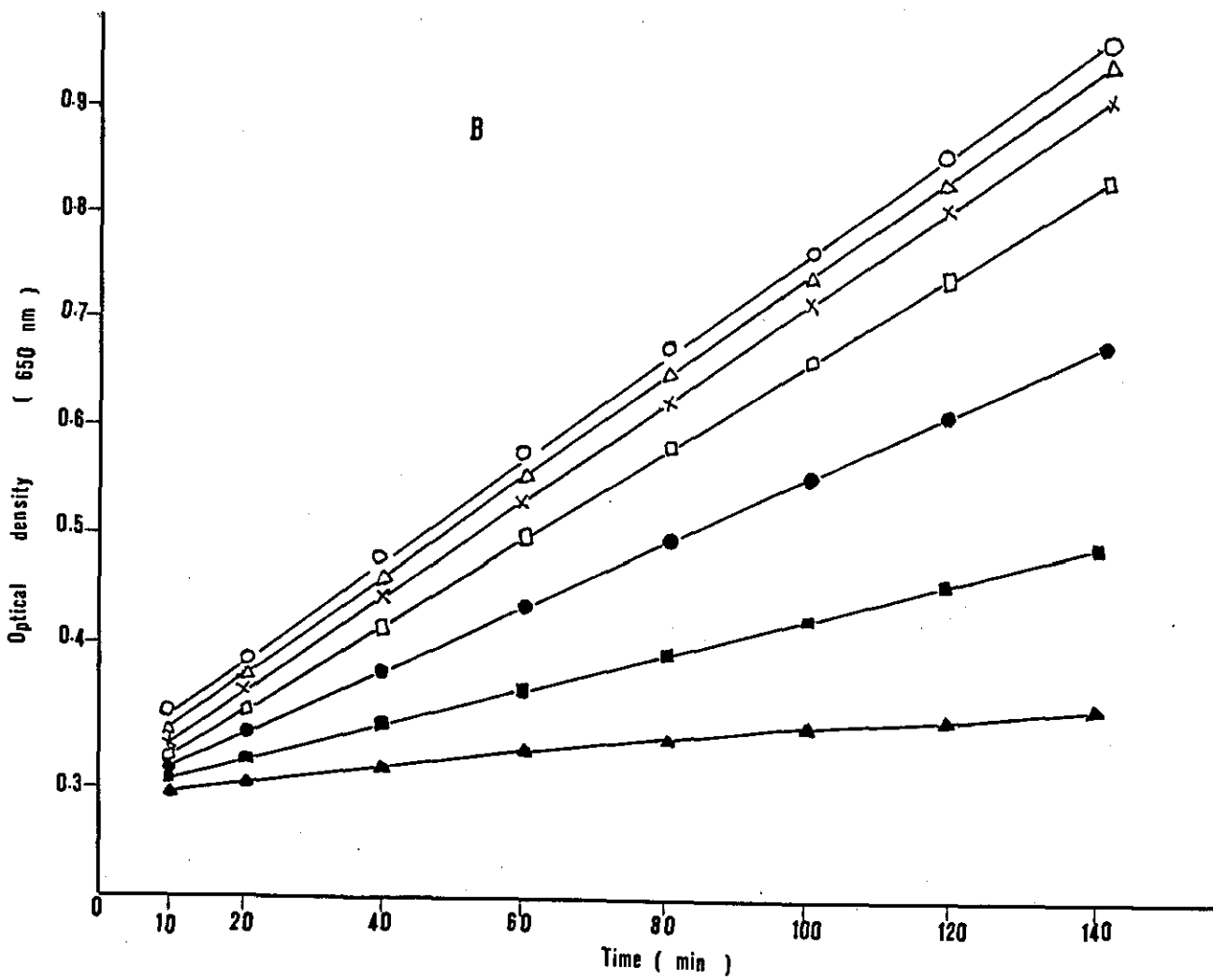
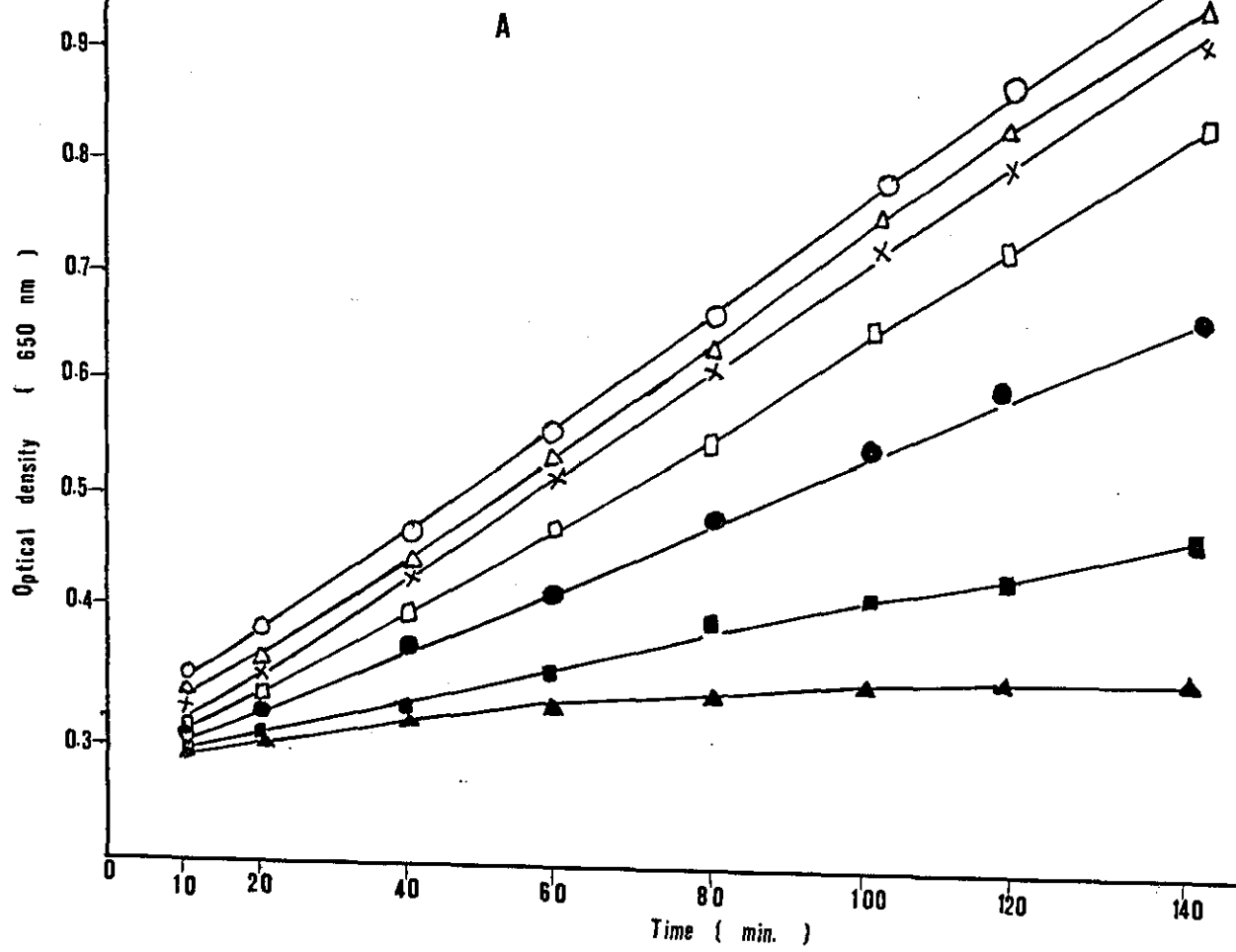
A. Fig. 3.1: Changes in optical density of cells of E.coli initially suspended for 10 min in CFM containing propranolol, following addition of glucose and incubation at 34°C. Cell concentration 42×10^9 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹) 0-0, 0-0; Δ - Δ , 0.25; x-x, 0.50; $\square$ - $\square$, 0.75; $\bullet$ - $\bullet$, 1.0; $\blacksquare$ - $\blacksquare$, 1.25; $\blacktriangle$ - $\blacktriangle$, 1.5.

B. Fig. 3.2: Changes in optical density of cells of E.coli initially suspended for 10 min in CFM containing oxprenolol, following addition of glucose and incubation at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Oxprenolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ - Δ , 0.25; x-x, 0.50; $\square$ - $\square$, 1.0; $\bullet$ - $\bullet$, 1.5; $\blacksquare$ - $\blacksquare$, 2.5; $\blacktriangle$ - $\blacktriangle$, 3.0.



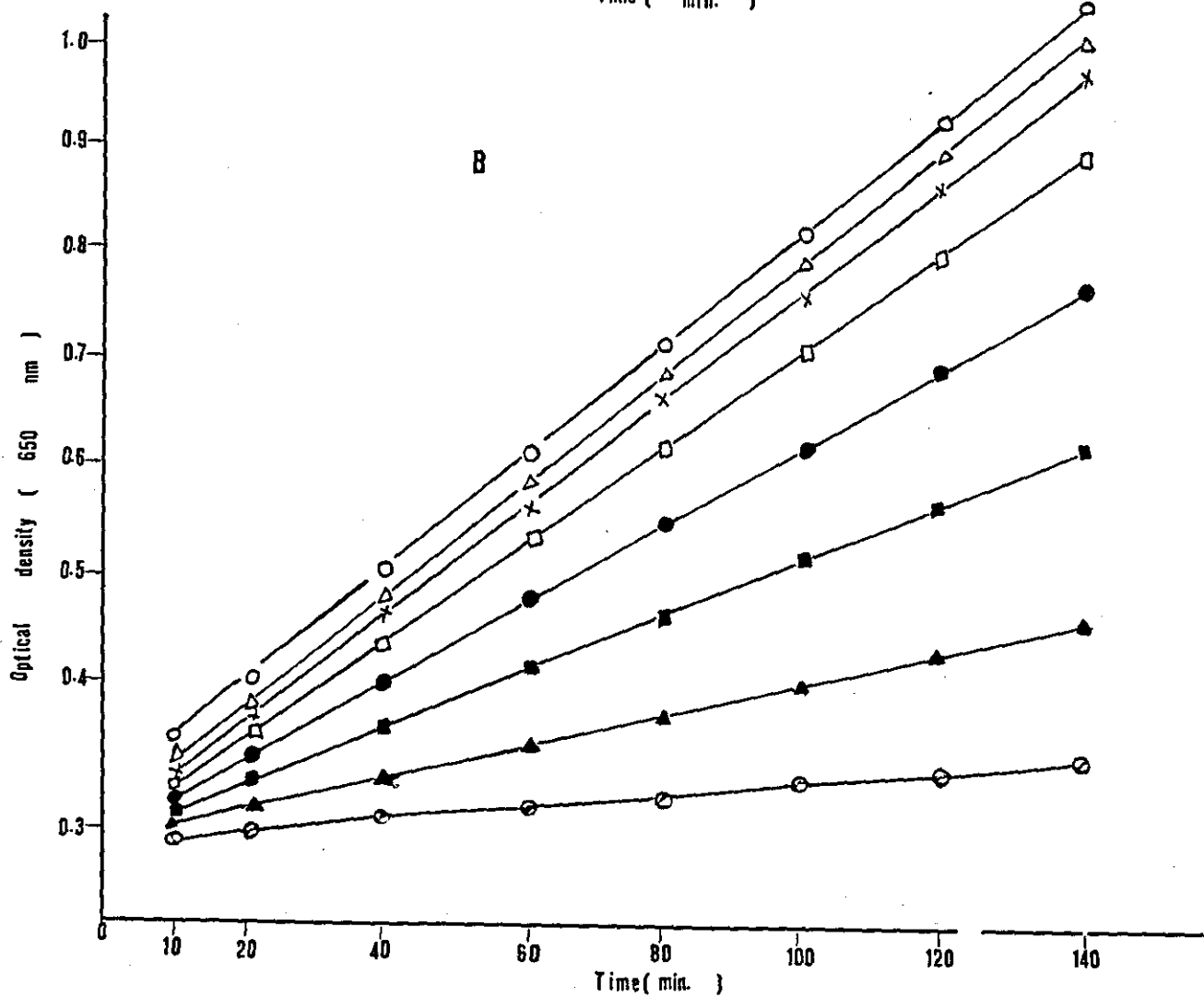
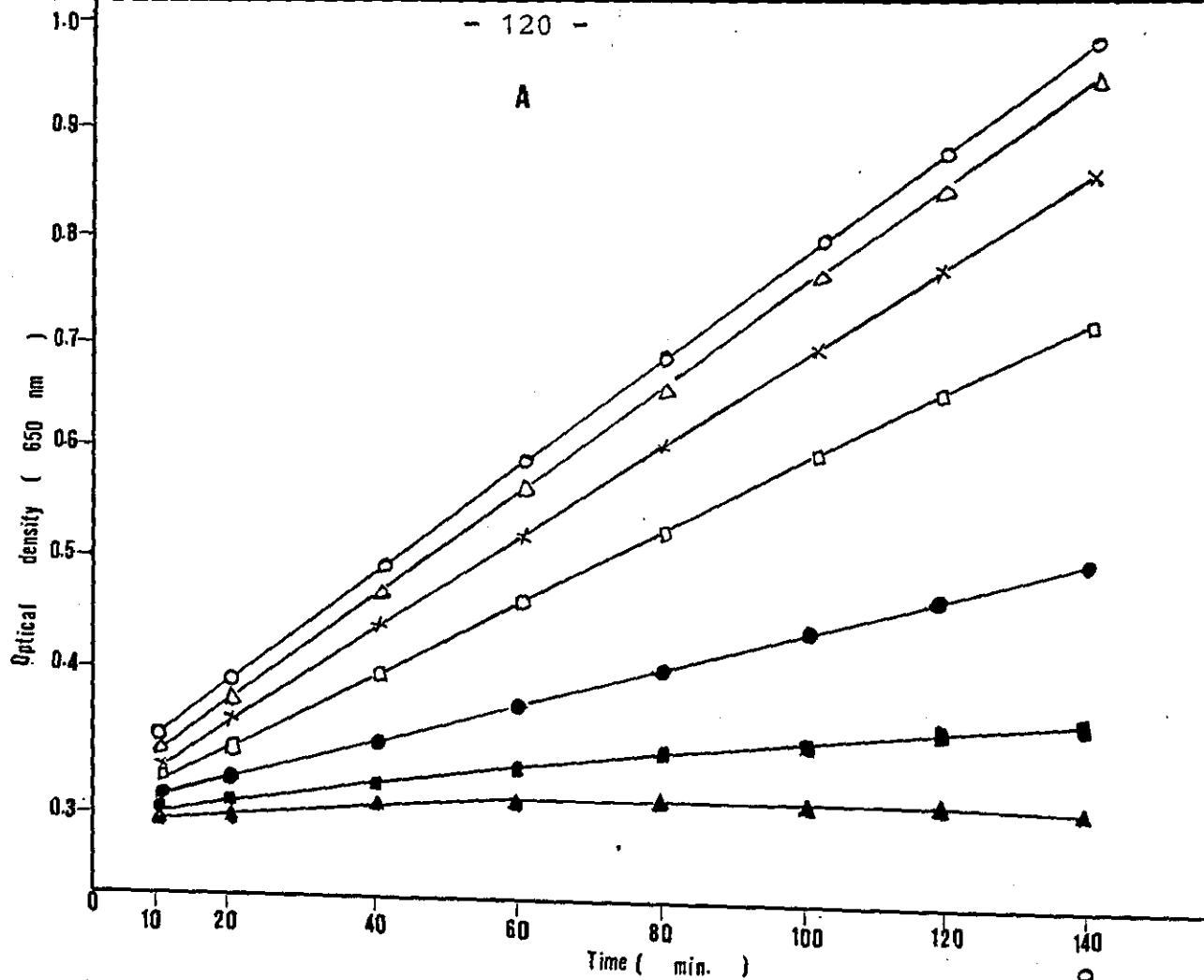
A. Fig. 3.3: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing atenolol following addition of glucose and incubation at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 2.5; x-x, 5.0; □-□, 10.0; ●-●, 20.0; ■-■, 25.0, ▲-▲, 30.0.

B. Fig. 3.4: Changes in optical density of cells of E.coli initially suspended for 10 min in CFM containing practolol following addition of glucose and incubation at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Practolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 2.5; x-x, 5.0; □-□, 10.0; ●-●, 20.0; ■-■, 25.0; ▲-▲, 30.0.



A. Fig. 3.5: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing tetracaine, following addition of glucose and incubation at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Tetracaine concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 0.25; x-x, 0.50; □-□, 0.75; ●-●, 1.0; ■-■, 1.25; ▲-▲, 1.00.

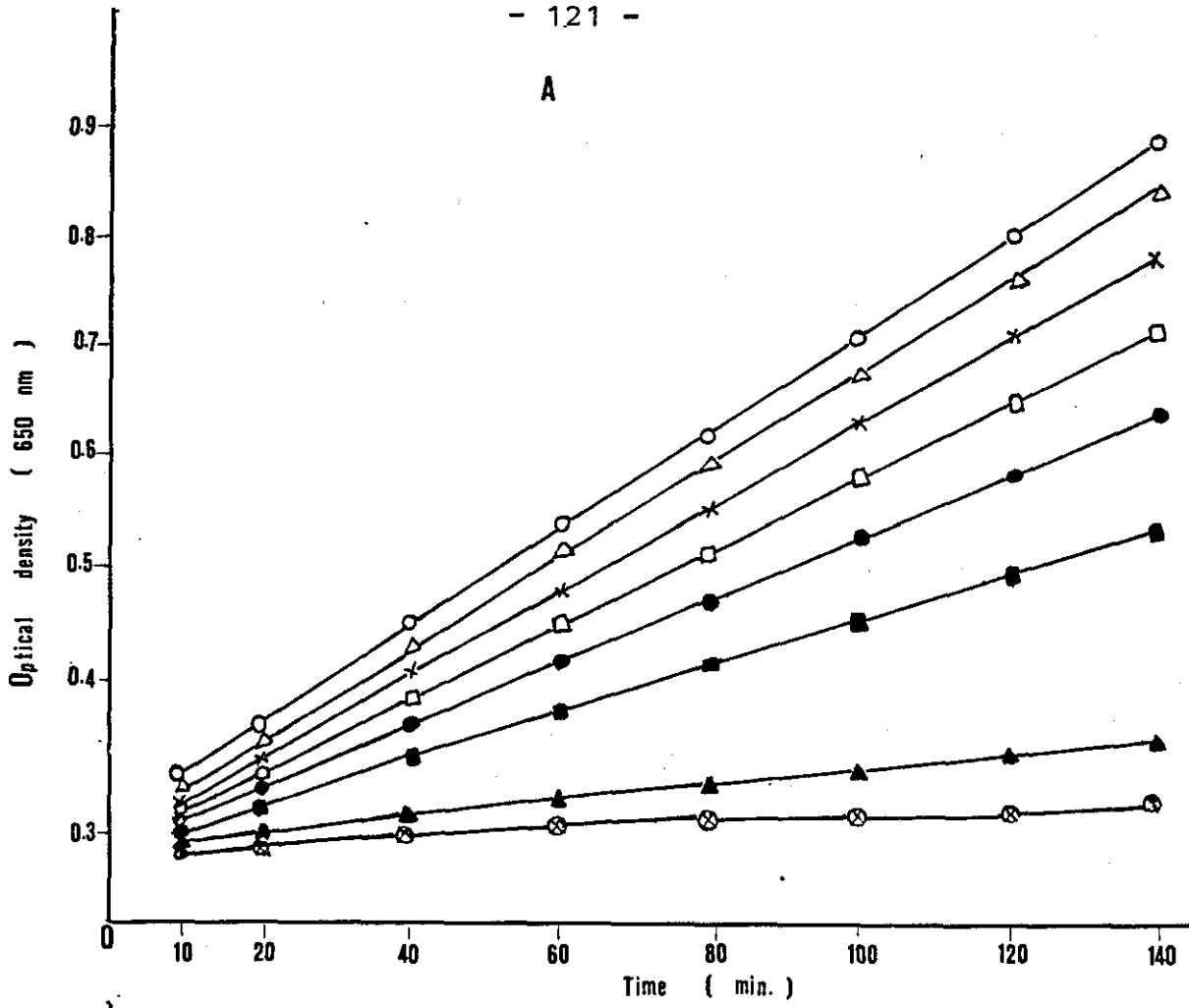
B. Fig. 3.6: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing procaine following addition of glucose and incubation at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Procaine concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 2.5; x-x, 5.0; □-□, 10.0; ●-●, 20.0; ■-■, 25.0; ▲-▲, 30.0; ○-○, 35.0.



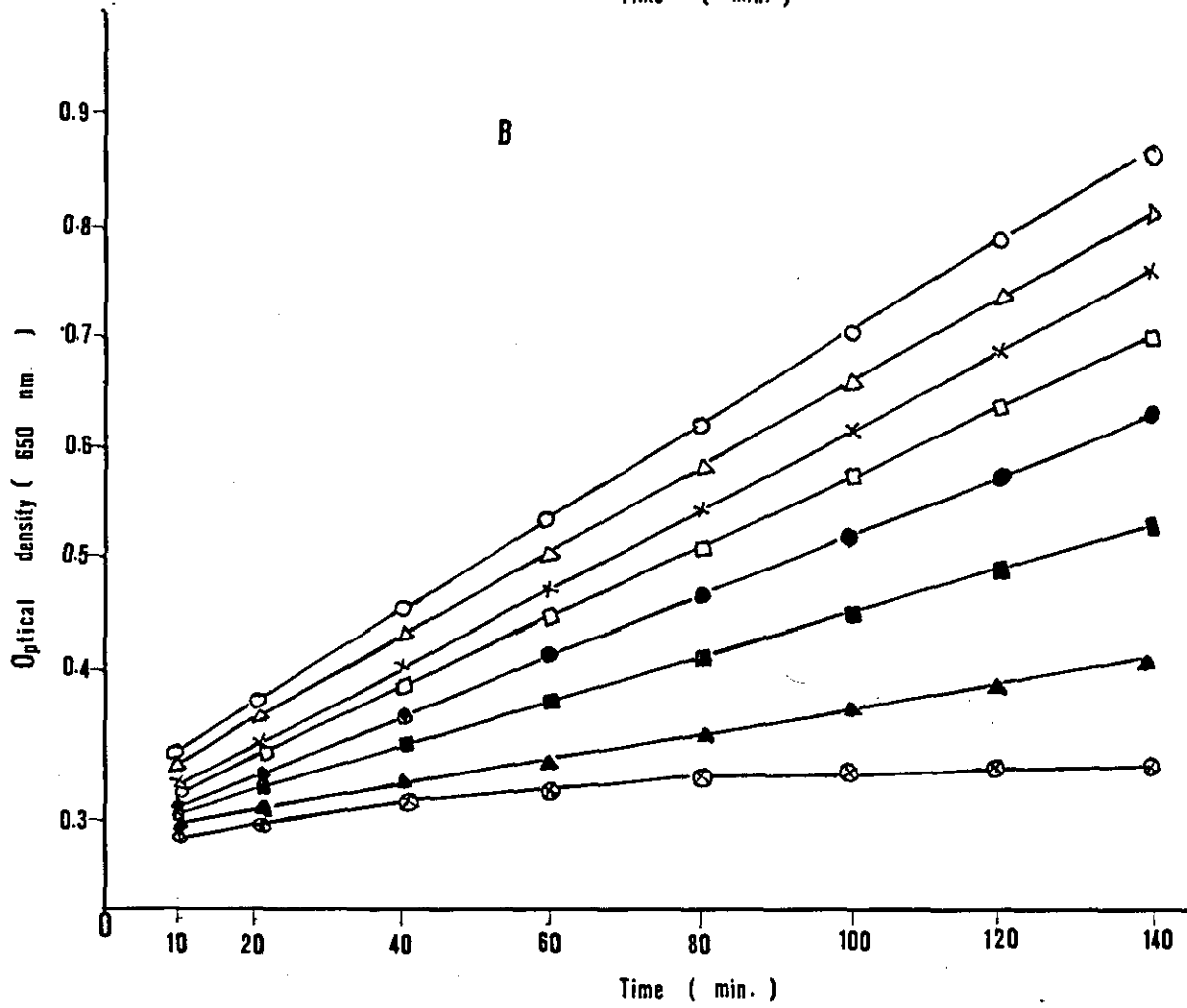
A. Fig. 3.7: Changes in the optical density of cells of Ps.aeruginosa, initially suspended for 10 min in CFM containing propranolol, following addition of glucose and incubation at 37°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 0.50; x-x, 0.75; □-□, 1.0; ●-●, 1.25; ■-■, 1.5; ▲-▲, 2.0, ⊗-⊗, 2.5.

B. Fig. 3.8: Changes in the optical density of cells of Ps.aeruginosa, initially suspended for 10 min in CFM containing oxprenolol, following addition of glucose and incubation at 37°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Oxprenolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 0.75; x-x, 1.0; □-□, 1.5; ●-●, 2.0; ■-■, 2.5; ▲-▲, 3.0, ⊗-⊗, 4.0.

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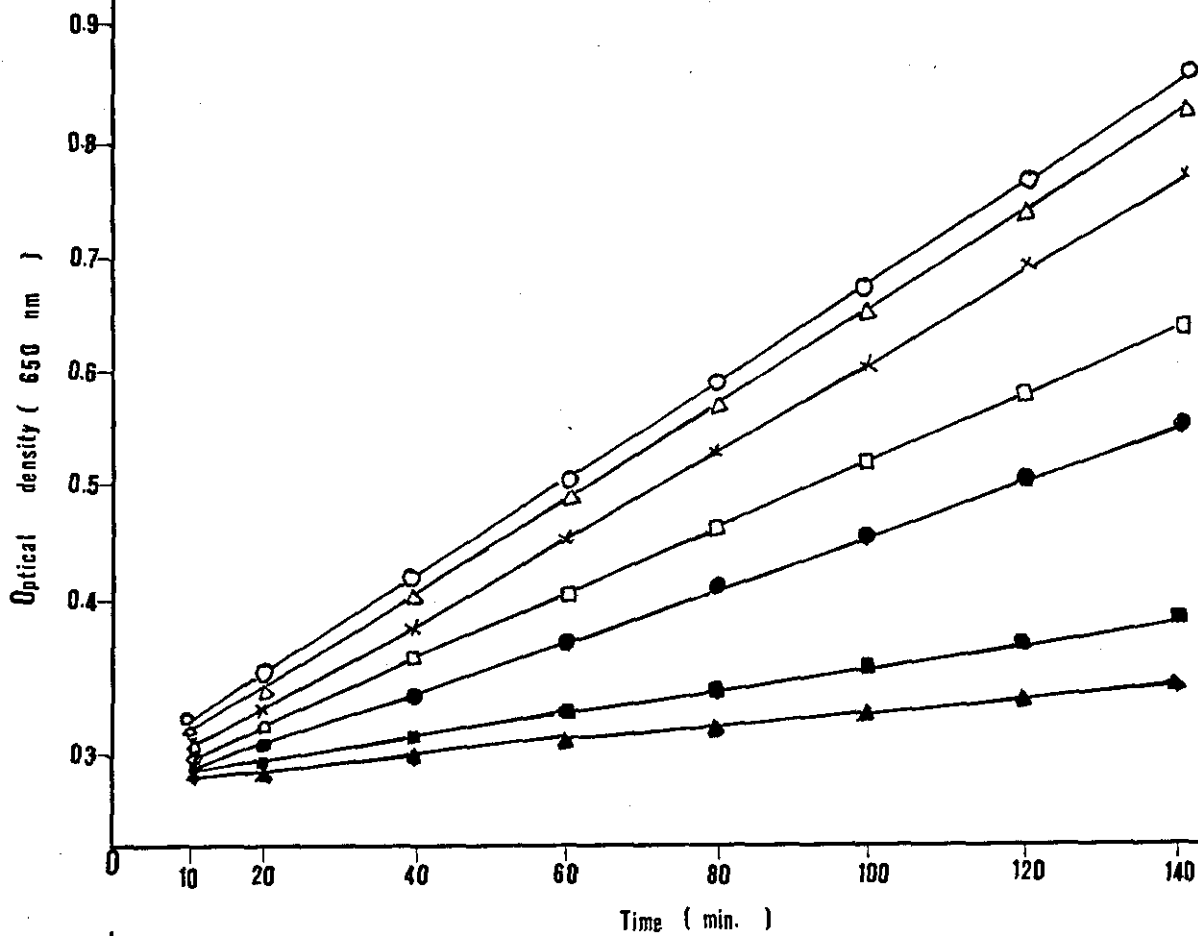
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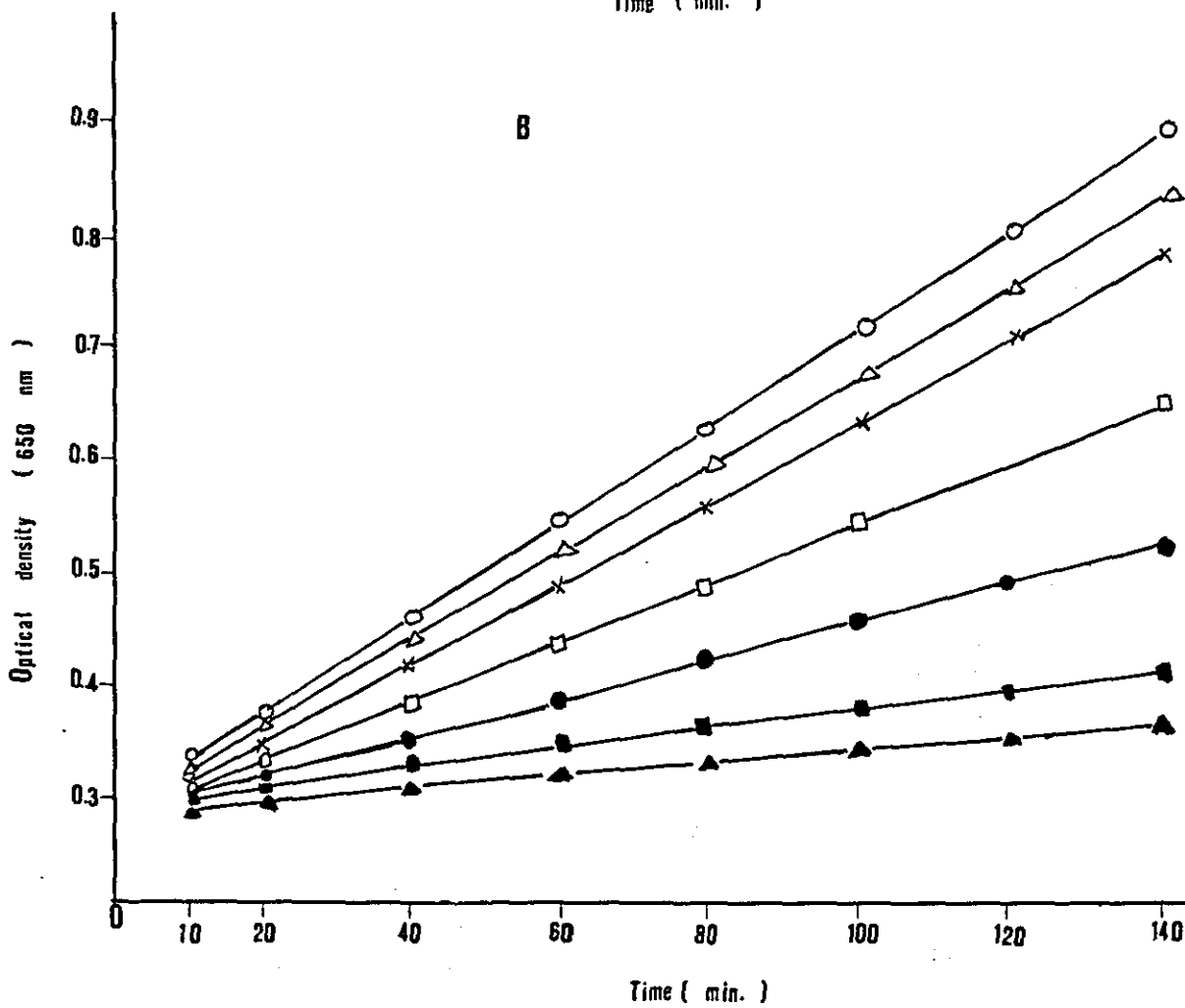
A. Fig. 3.9: Changes in optical density of cells of Ps.aeruginosa, initially suspended for 10 min in CFM containing atenolol, following addition of glucose and incubation at 37°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 5.0; x-x, 10.0; □-□, 20.0; ●-●, 25.0; ■-■, 30.0; ▲-▲, 35.0

B. Fig. 3.10: Changes in optical density of cells of Ps. aeruginosa, initially suspended for 10 min in CFM containing practolol, following addition of glucose and incubation at 37°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Practolol concentration (mg.ml⁻¹) 0-0, 0.0; Δ-Δ, 5.0; x-x, 10.0; □-□, 20.0; ●-●, 25.0; ■-■, 30.0; ▲-▲, 35.0.

A



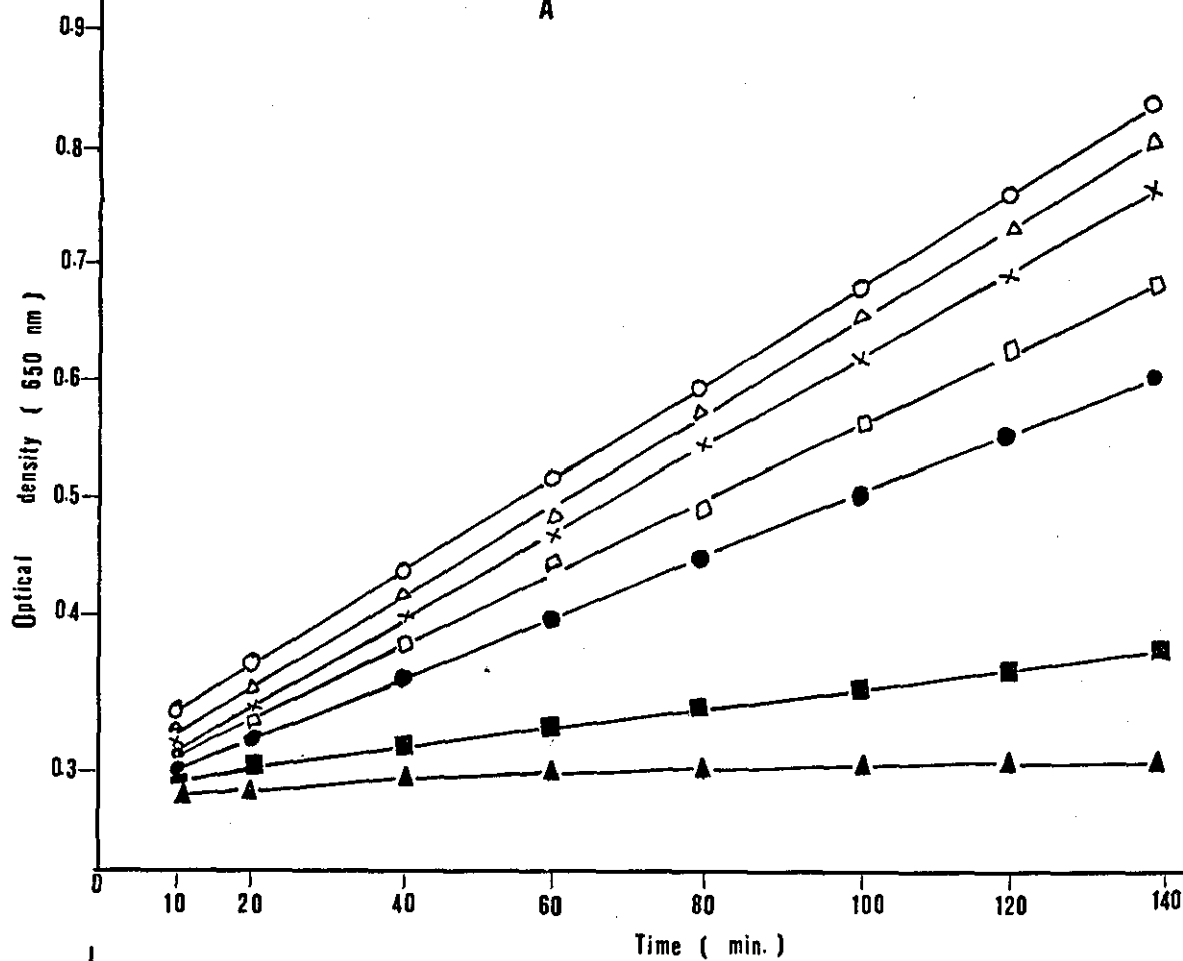
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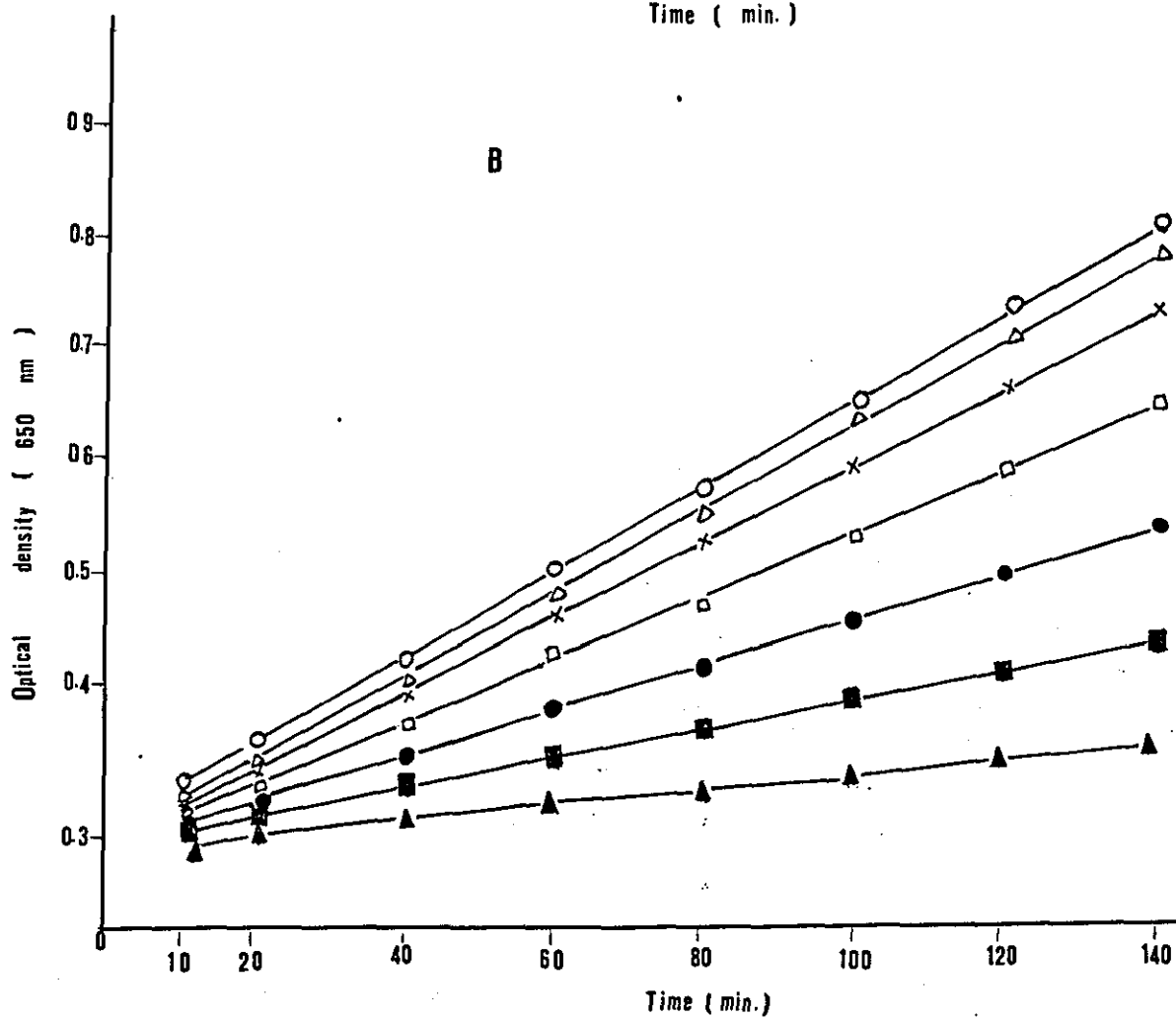
A. Fig. 3.11: Changes in optical density of cells of Ps.aeruginosa, initially suspended for 10 min in CFM containing tetracaine, following addition of glucose and incubation at 37°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Tetracaine concentration (mg.ml⁻¹) O-O, 0.0; Δ-Δ, 0.50; x-x, 0.75, □-□, 1.0; ●-●, 1.25; ■-■, 2.0; ▲-▲, 1.5.

B. Fig. 3.12: Changes in optical density of cells of Ps.aeruginosa, initially suspended for 10 min in CFM containing procaine, following addition of glucose and incubation at 37°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Procaine concentration (mg.ml⁻¹) O-O, 0.0; Δ-Δ, 5.0; x-x, 20.0; □-□, 25.0; ●-●, 25.0; ■-■, 30.0, ▲-▲, 40.0.

A



B

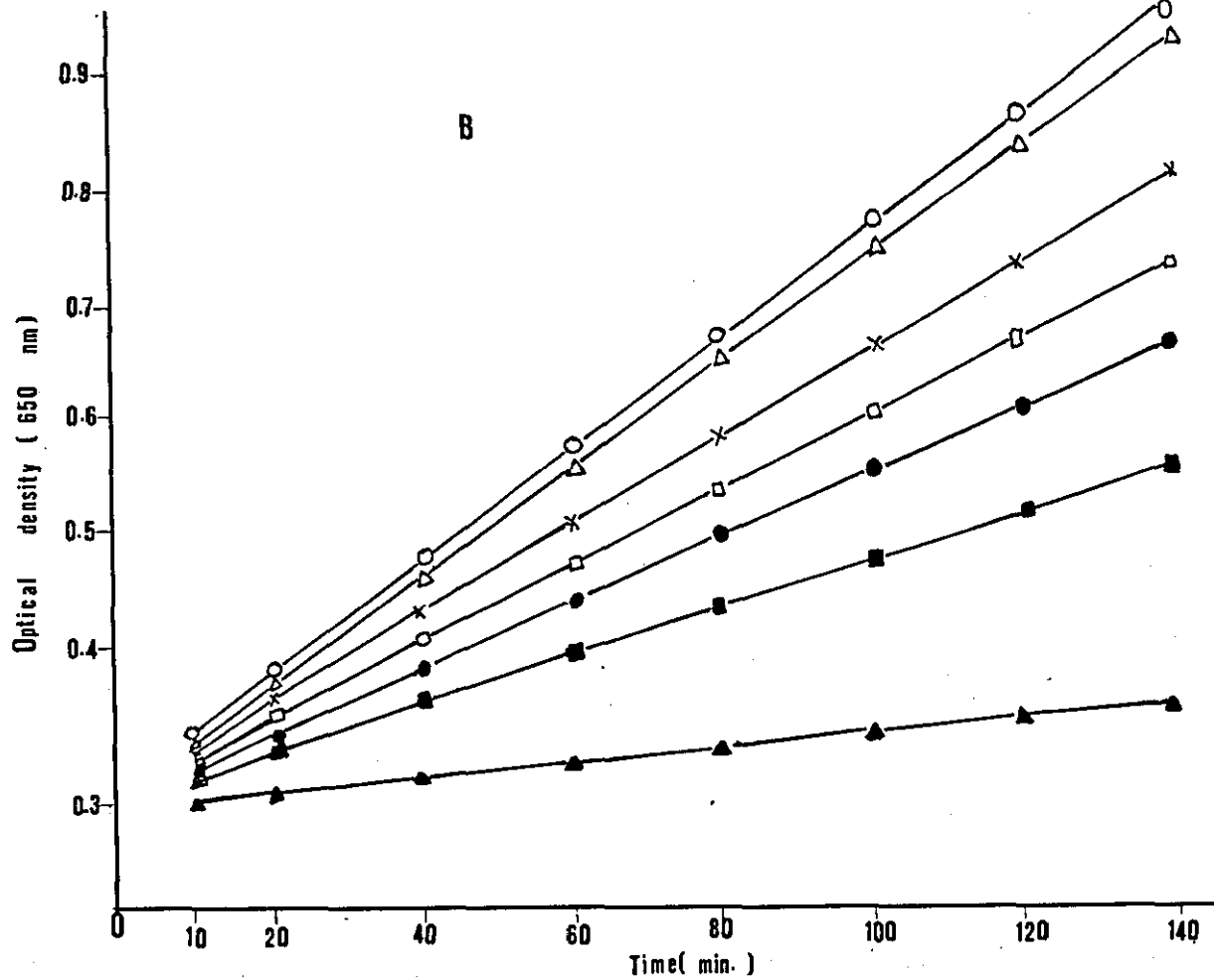
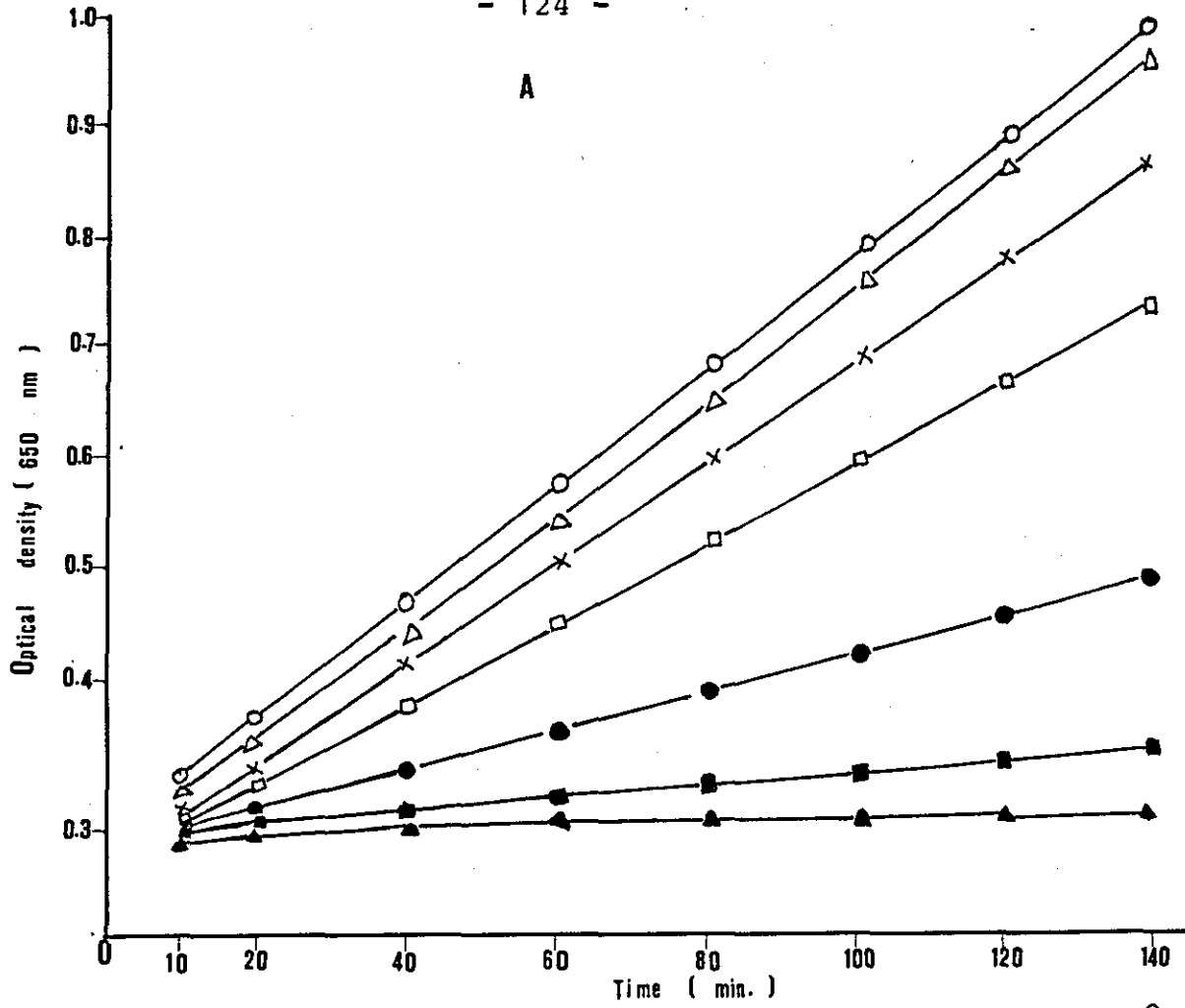


A. Fig. 3.13 Changes in optical density of cells of B.cereus, initially suspended for 10 min in CFM containing propranolol, following addition of glucose and incubation at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹)

O-O, 0.0; Δ-Δ, 0.1; x-x, 0.20; □-□, 0.40; ●-●, 0.50; ■-■, 0.75; ▲-▲, 1.0.

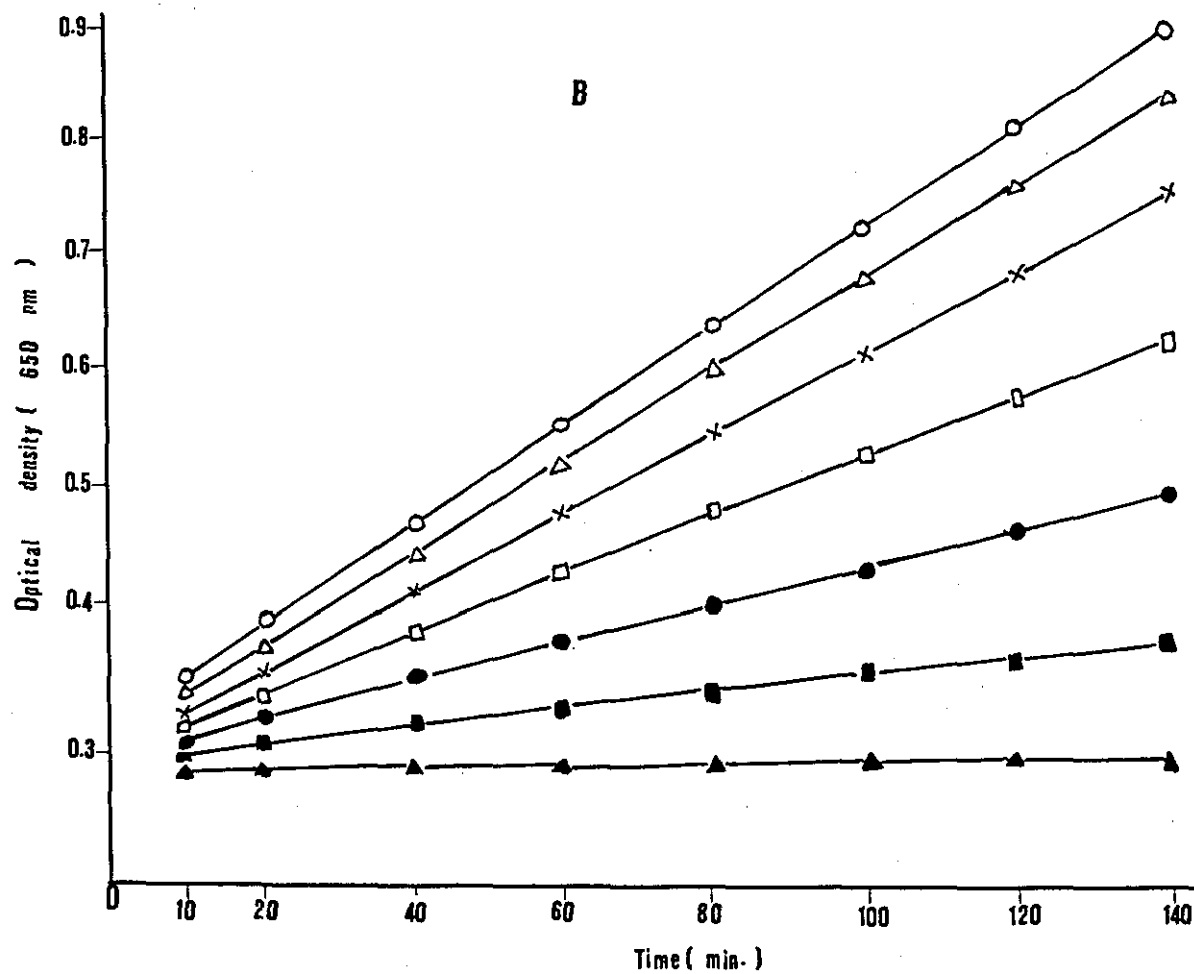
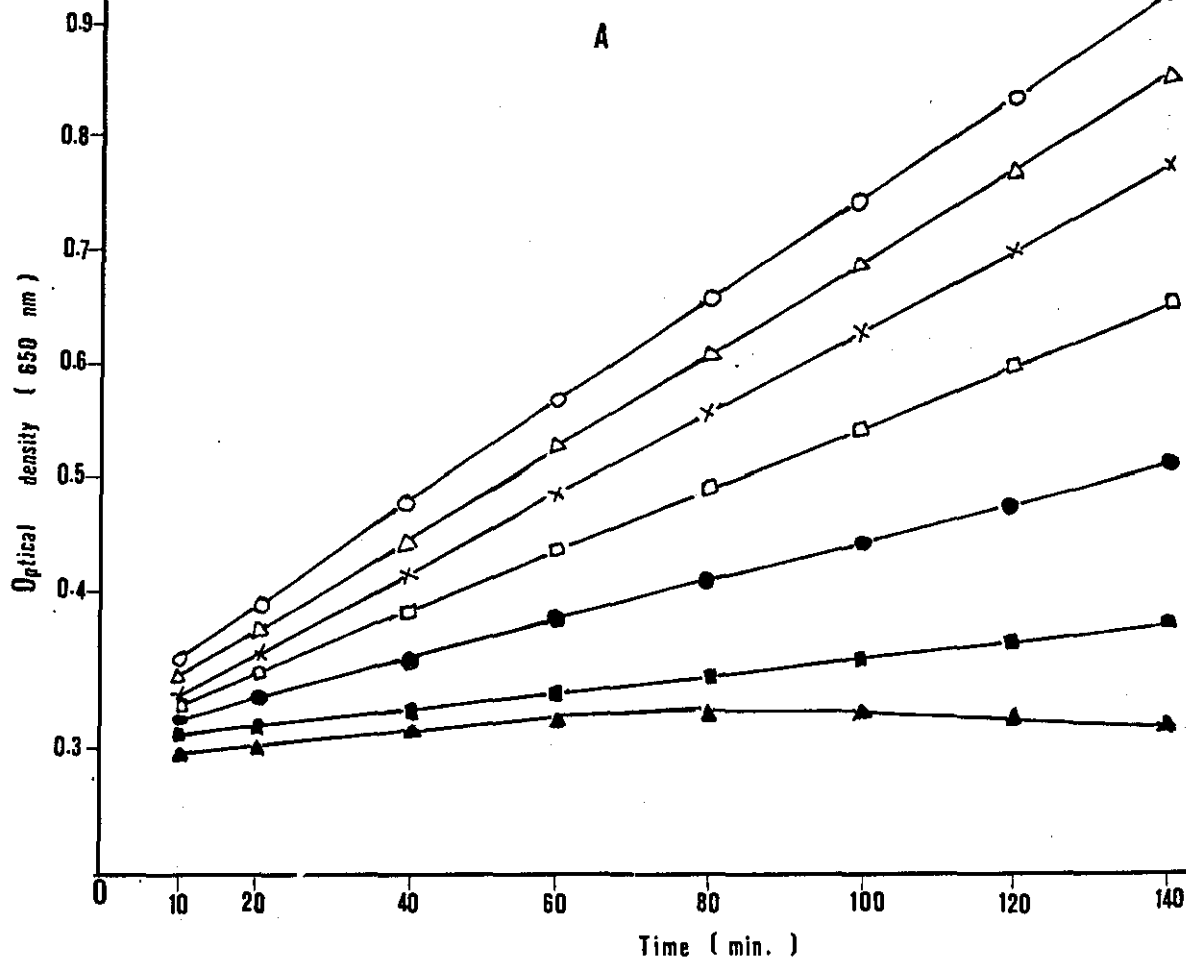
B. Fig. 3.14: Changes in optical density of cells of B.cereus, initially suspended for 10 min in CFM containing oxprenolol, following addition of glucose and incubation at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Oxprenolol concentration (mg.ml⁻¹)

O-O, 0.0; Δ-Δ, 0.25; x-x, 0.50; □-□, 0.75; ●-●, 1.0; ■-■, 1.5; ▲-▲, 2.0.



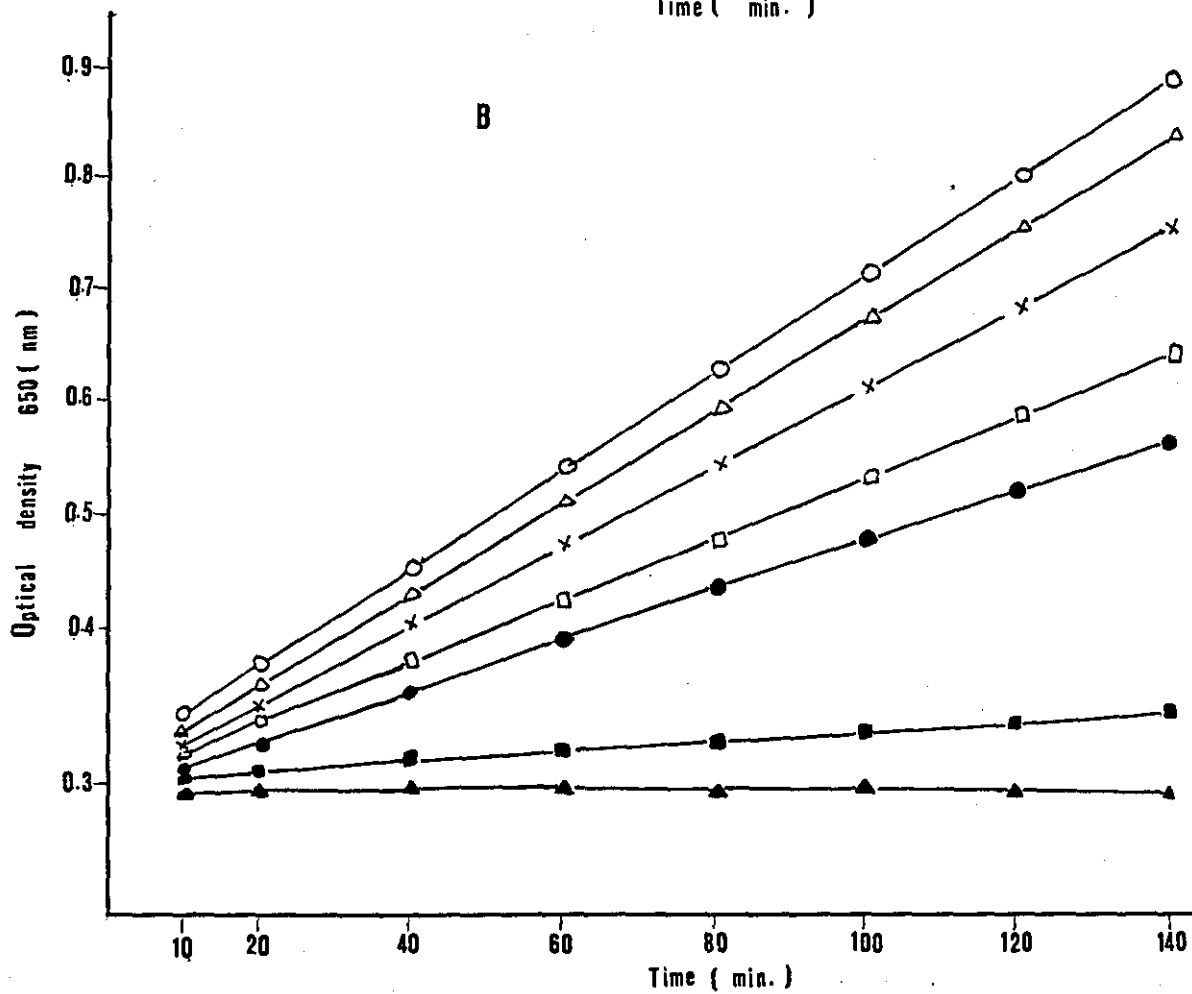
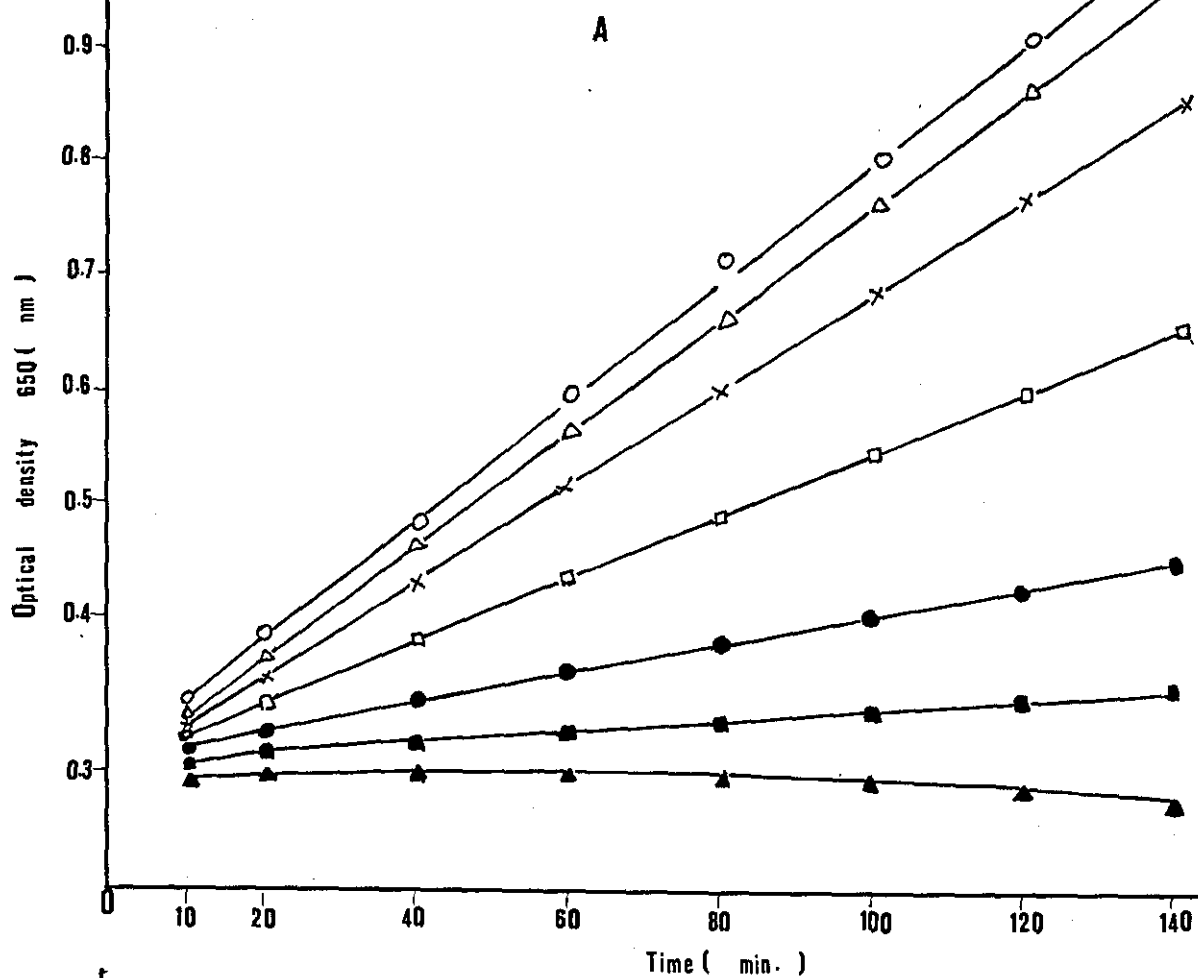
A. Fig. 3.15: Changes in optical density of cells of B.cereus, initially suspended for 10 min in CFM containing atenolol, following addition of glucose and incubation at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹) O-O, 0.0; Δ-Δ, 2.5; x-x, 5.0; □-□, 10.0; ●-●, 15.0; ■-■, 20.0; ▲-▲, 25.0.

B. Fig. 3.16 Changes in optical density of cells of B.cereus, initially suspended for 10 min in CFM containing practolol following addition of glucose and incubation at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Practolol concentration (mg.ml⁻¹) O-O, 0.0; Δ Δ, 2.5; x-x, 5.0; □-□, 10.0, ●-●, 15.0; ■-■, 20.0; ▲-▲, 25.0.



A. Fig. 3.17: Changes in optical density of cells of B.cereus, initially suspended for 10 min in CFM containing Tetracaine, following addition of glucose and incubation at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Tetracaine concentration (mg.ml⁻¹) O-O, 0.0; Δ-Δ, 0.1; x-x, 0.2; □□, 0.4; ●-●, 0.5; ■-■ 1.0; ▲-▲, 0.75.

B. Fig. 3.18: Changes in optical density of cells of B.cereus, initially suspended for 10 min in CFM containing Procaine, following addition of glucose and incubation at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Procaine concentration (mg.ml⁻¹) O-O, 0.0; Δ-Δ, 2.5; x-x, 5.0; □-□, 10.0; ●-●, 20.0; ■-■, 25.0; ▲-▲, 30.0.



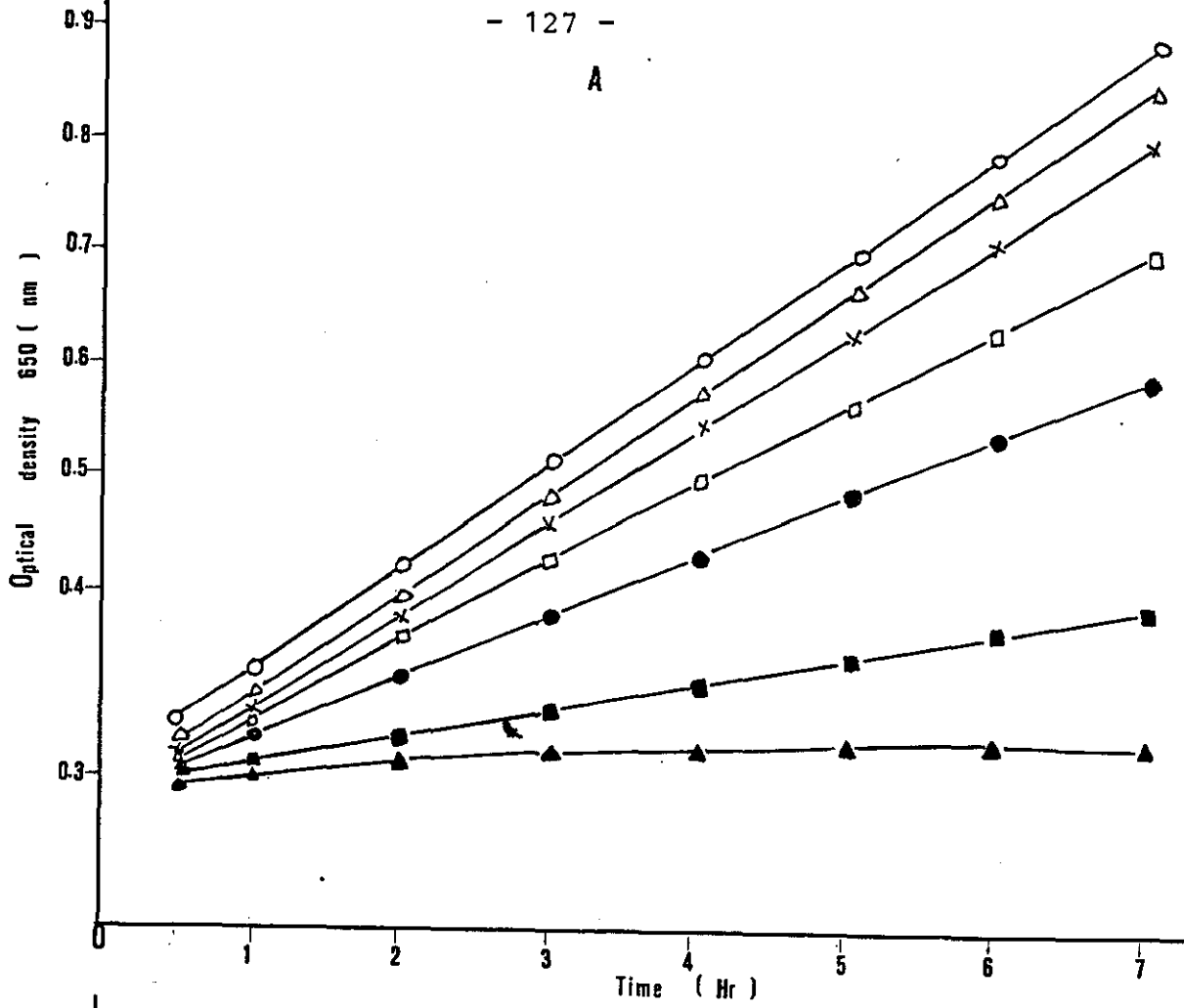
A. Fig. 3.19: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Propranolol, following addition of glucose and incubation at 30°C. Cell concentrations 3.2×10^5 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹)

0-0, 0.0; Δ-Δ, 0.1; x-x, 0.2;
 □-□, 0.3; ●-●, 0.5; ■-■, 0.75;
 ▲-▲, 1.0.

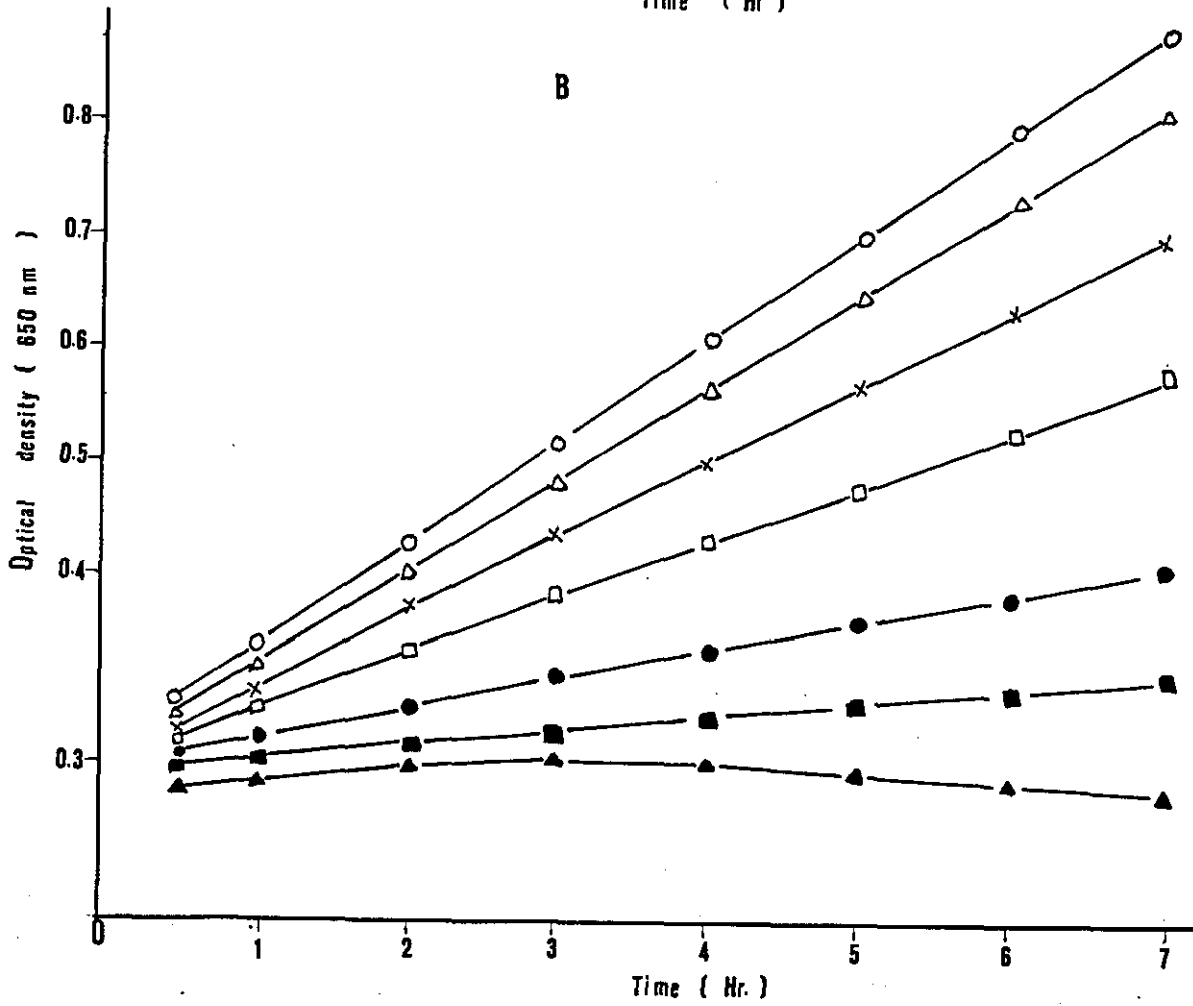
B. Fig. 3.20 Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Oxprenolol, following addition of glucose and incubated at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Oxprenolol concentration (mg.ml⁻¹)

0-0, 0.0; Δ-Δ, 0.25; x-x, 0.5;
 □-□, 0.75; ●-●, 1.0; ■-■, 1.5;
 ▲-▲, 2.5.

A

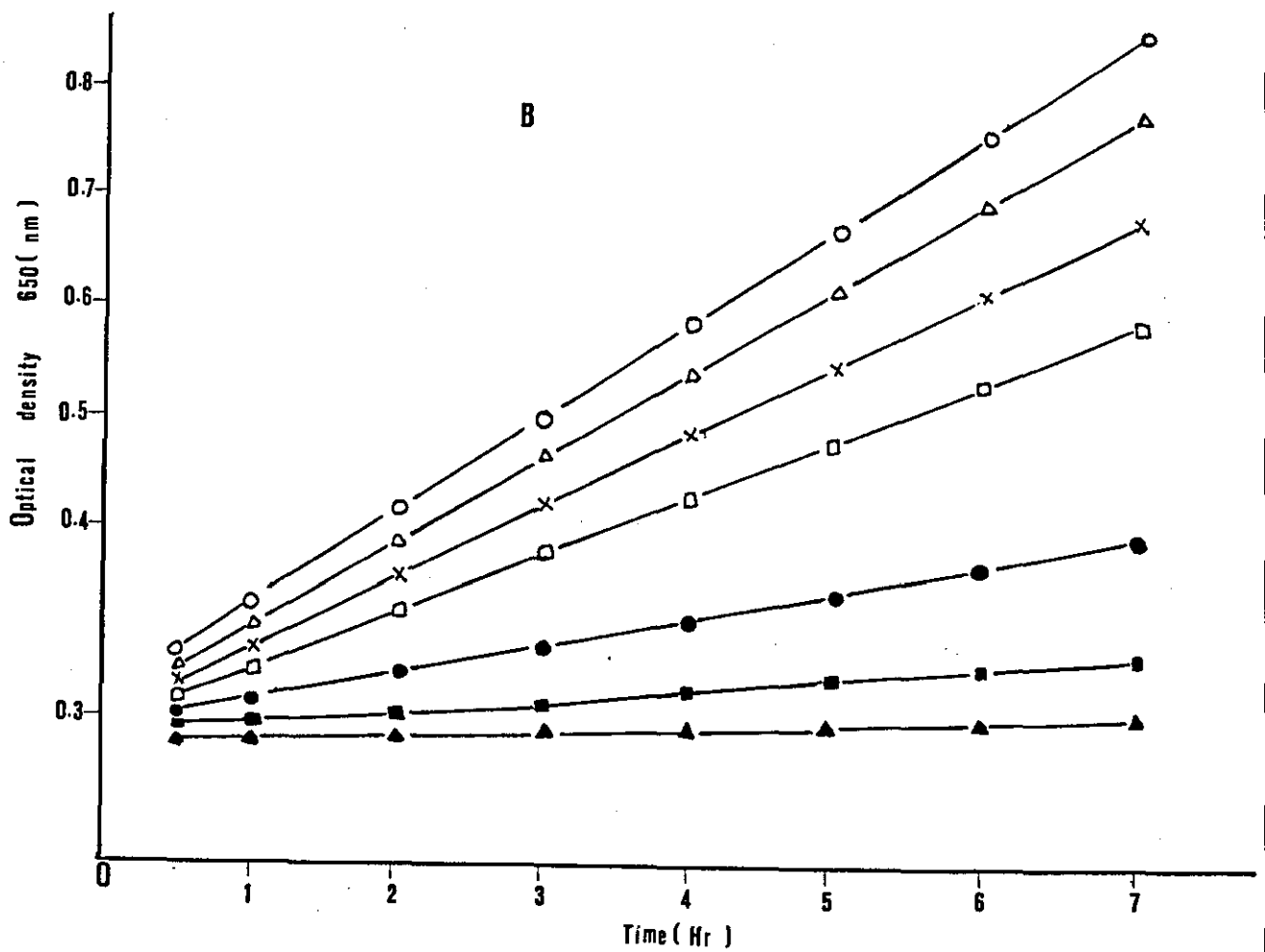
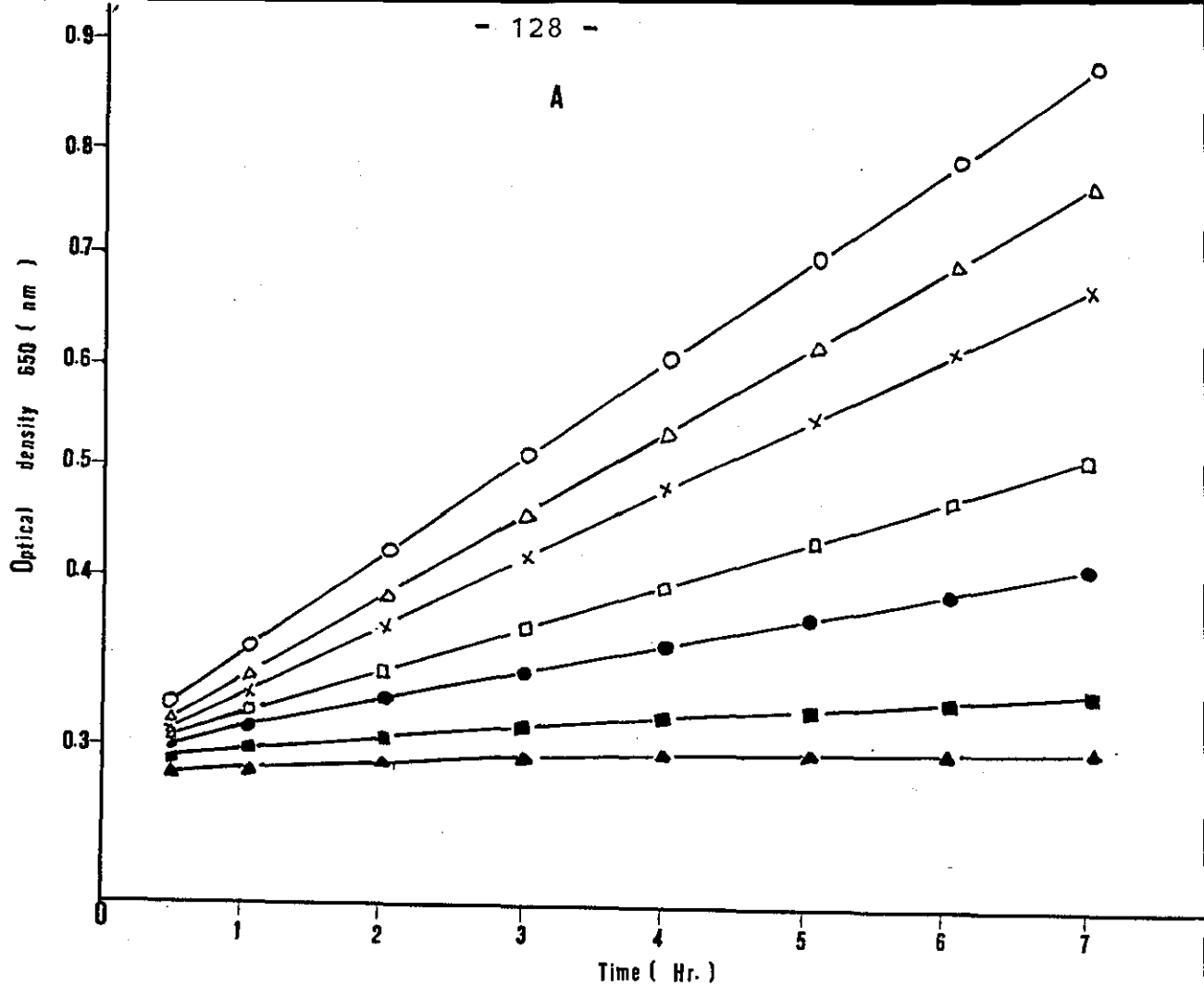


B



A. Fig. 3.21: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Atenolol, following addition of glucose and incubation at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹.
Atenolol concentration (mg.ml⁻¹)
O-O, 0.0; Δ-Δ, 2.5, x-x, 5.0; □-□, 10.0;
●-●, 15.0; ■-■, 20.0; ▲ ▲, 25.0.

B. Fig. 3.22: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Practolol, following addition of glucose and incubation at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹.
Atenolol concentration (mg.ml⁻¹)
O-O, 0.0; Δ Δ, 2.5; x-x, 5.0;
□-□, 10.0; ●-●, 15.0; ■-■, 20.0;
▲-▲, 25.0.

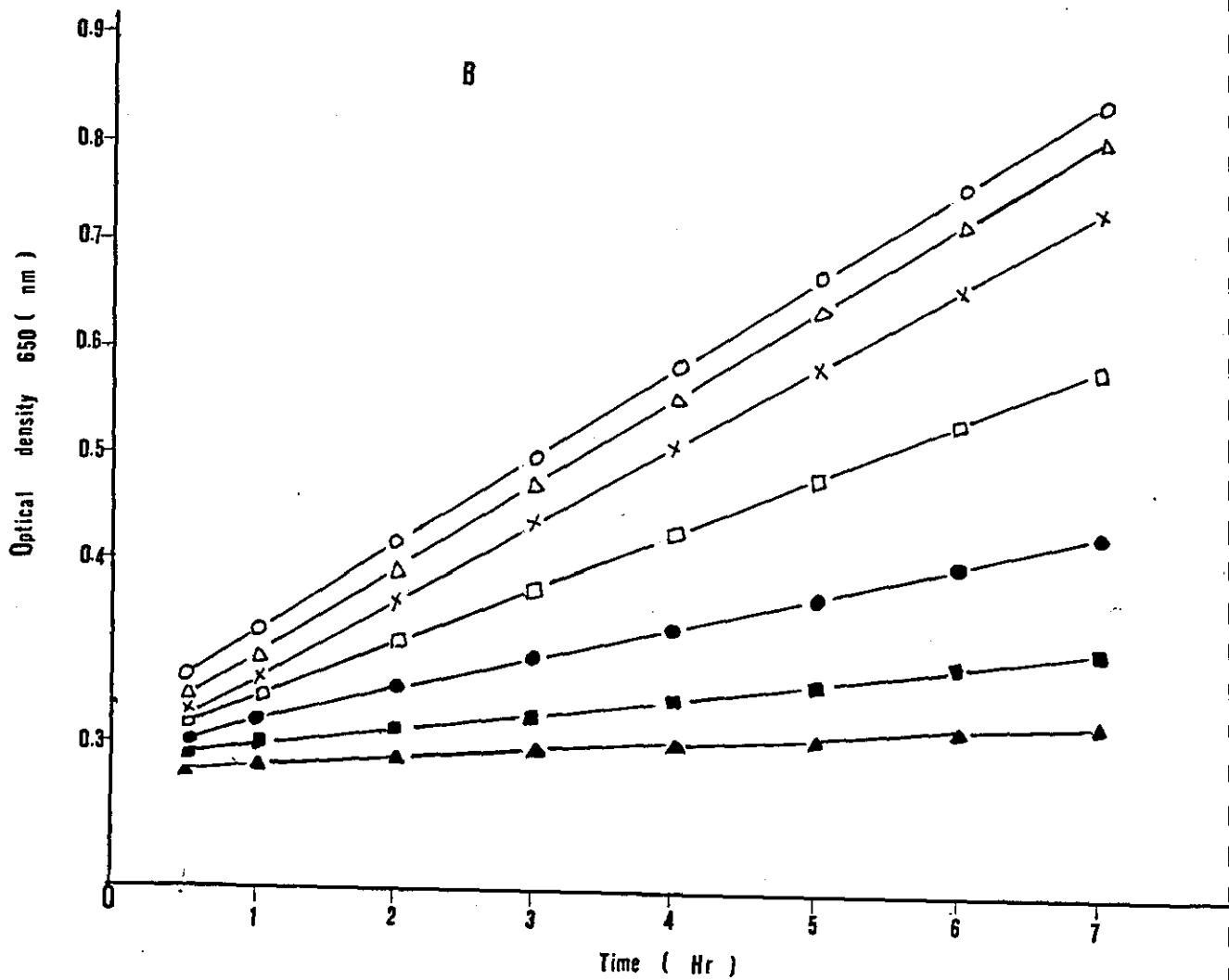
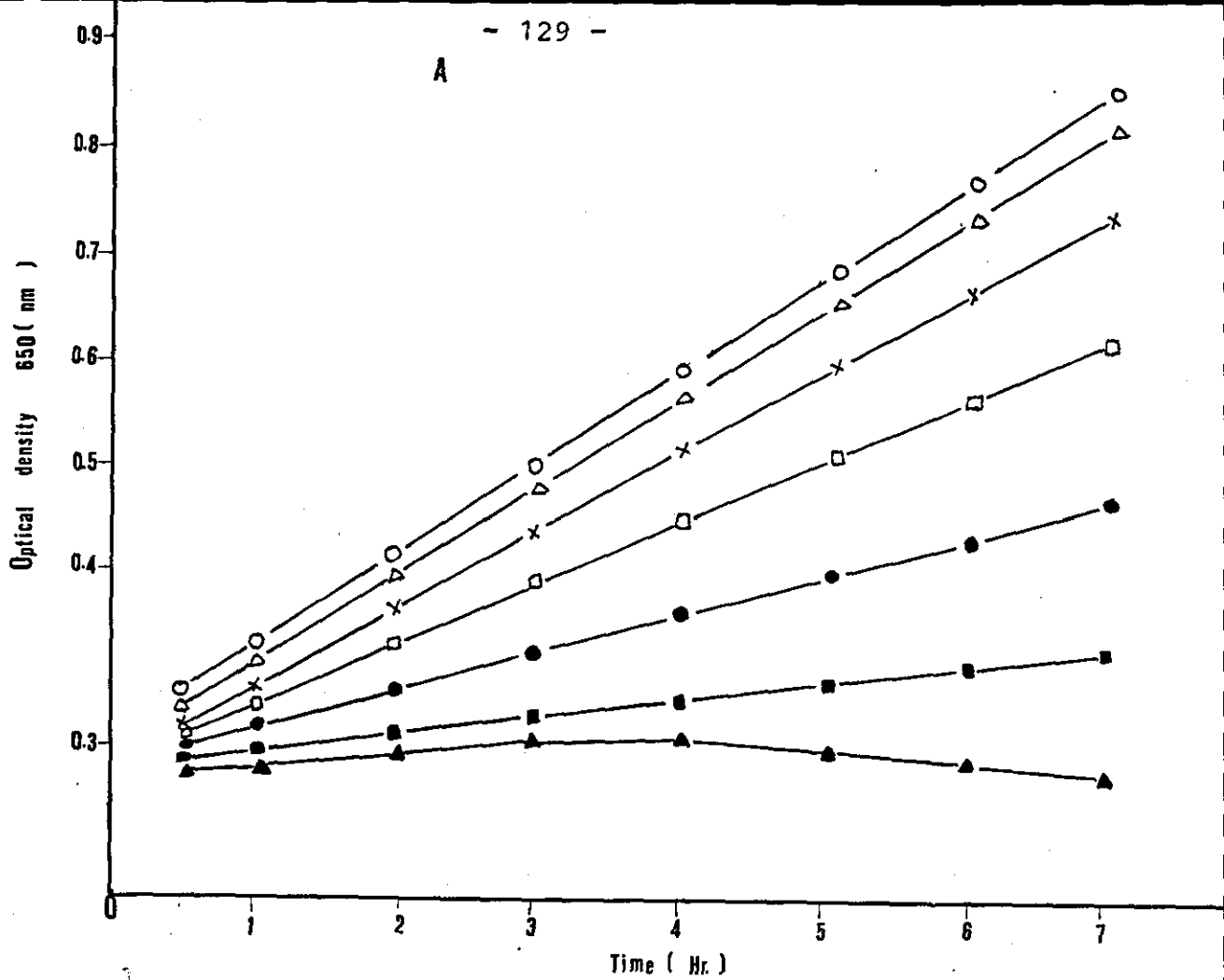


A. Fig. 3.23 Changes in the optical density of cells of C.albicans, initially suspended for 10 min in CFM containing tetracaine, following addition of glucose and incubation at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Tetracaine concentration (mg.ml⁻¹)

O-O, 0.0; Δ-Δ, 0.10; x-x, 0.20;
 □-□, 0.30; ●-●, 0.50; ■-■, 0.75;
 ▲-▲, 1.0.

B. Fig. 3.24: Changes in the optical density of cells of C.albicans, initially suspended for 10 min in CFM containing procaine, following addition of glucose and incubation at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Procaine concentration (mg.ml⁻¹)

O-O, 0.0; Δ-Δ, 2.5; x-x, 5.0; □-□, 10.0;
 ●-●, 15.0; ■-■, 20.0; ▲-▲, 25.0.



A. Fig. 3.25: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing Propranolol, following addition of glucose and after resuspension in the same volume of growth medium at (40 Min). Incubation temperature 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹.

Propranolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 0.25;

○-○, 0.0; △-△, 0.25;

■-■, 0.5; x-x, 1.0; ∇-∇, 1.5;

□-□, 0.5; ⊗-⊗, 1.0; ⊠-⊠, 1.5.

B. Fig. 3.26: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing Oxprenolol, following addition of glucose and after resuspension in the same volume of growth medium at (40 min). Incubation temperature 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹.

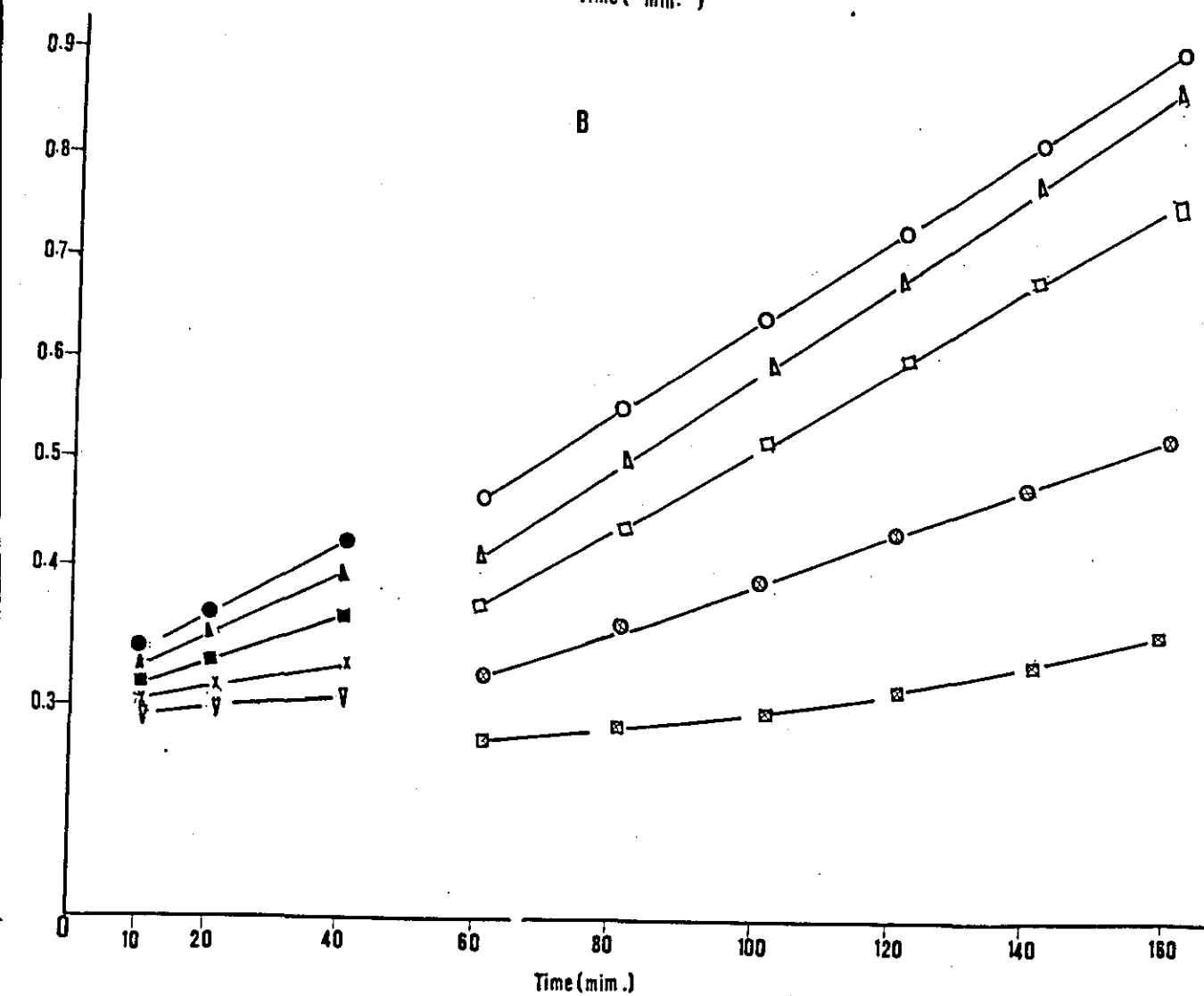
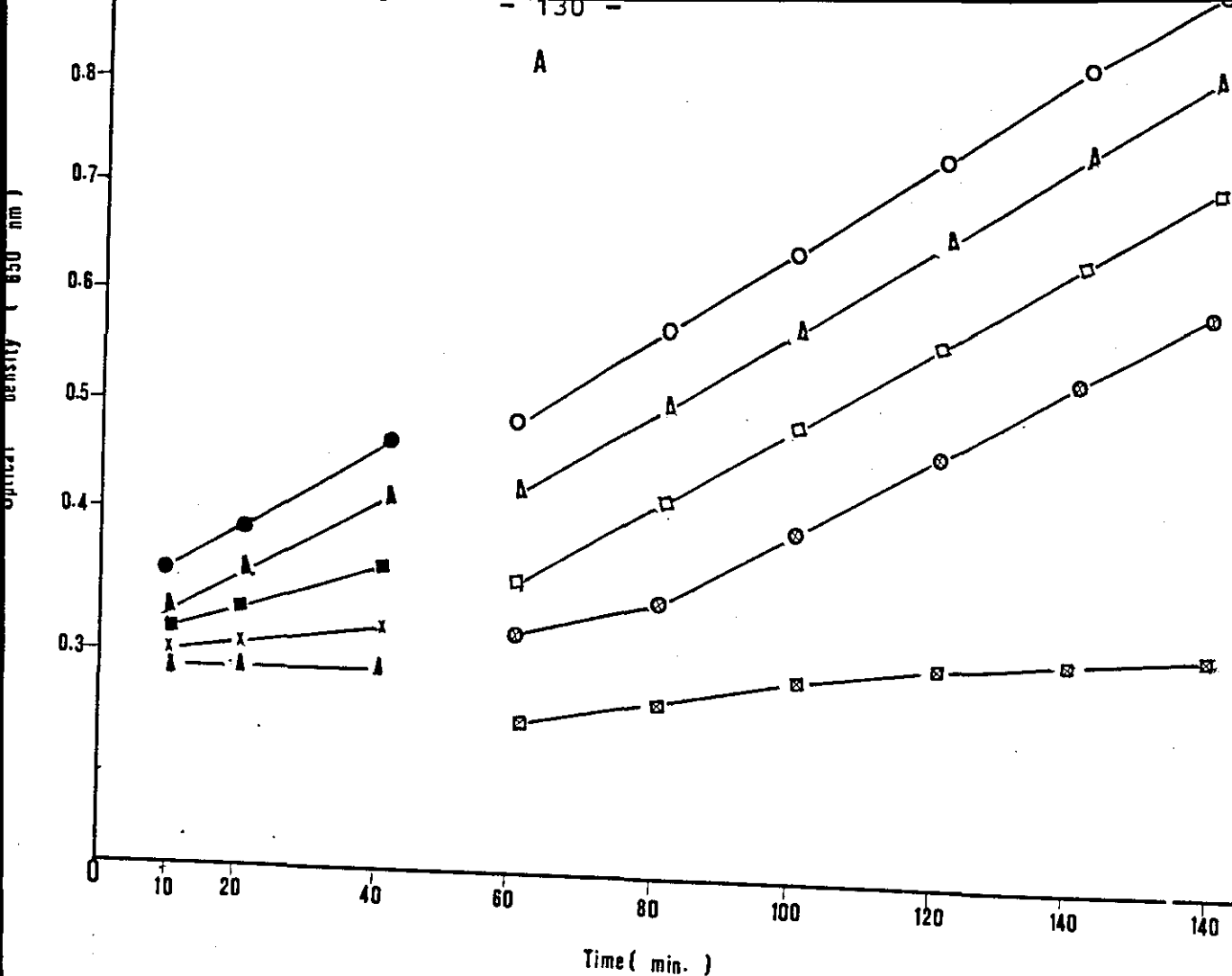
Oxprenolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 0.5;

○-○, 0.0; △-△, 0.5;

■-■, 1.0; x-x, 2.5; ∇-∇, 3.0;

□-□, 1.0; ⊗-⊗, 2.5; ⊠-⊠, 3.0.



A. Fig. 3.27: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing Atenolol, following addition of glucose and after resuspension in the same volume of growth medium at (40 min). Incubation temperature 37°C. Cell concentration 42×10^9 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 5.0;

○-○, 0.0; △-△, 5.0;

■-■, 10.0; x-x, 20.0; ∇-∇, 30.0

□-□, 10.0; ⊗-⊗, 20.0; ⊠-⊠, 30.0.

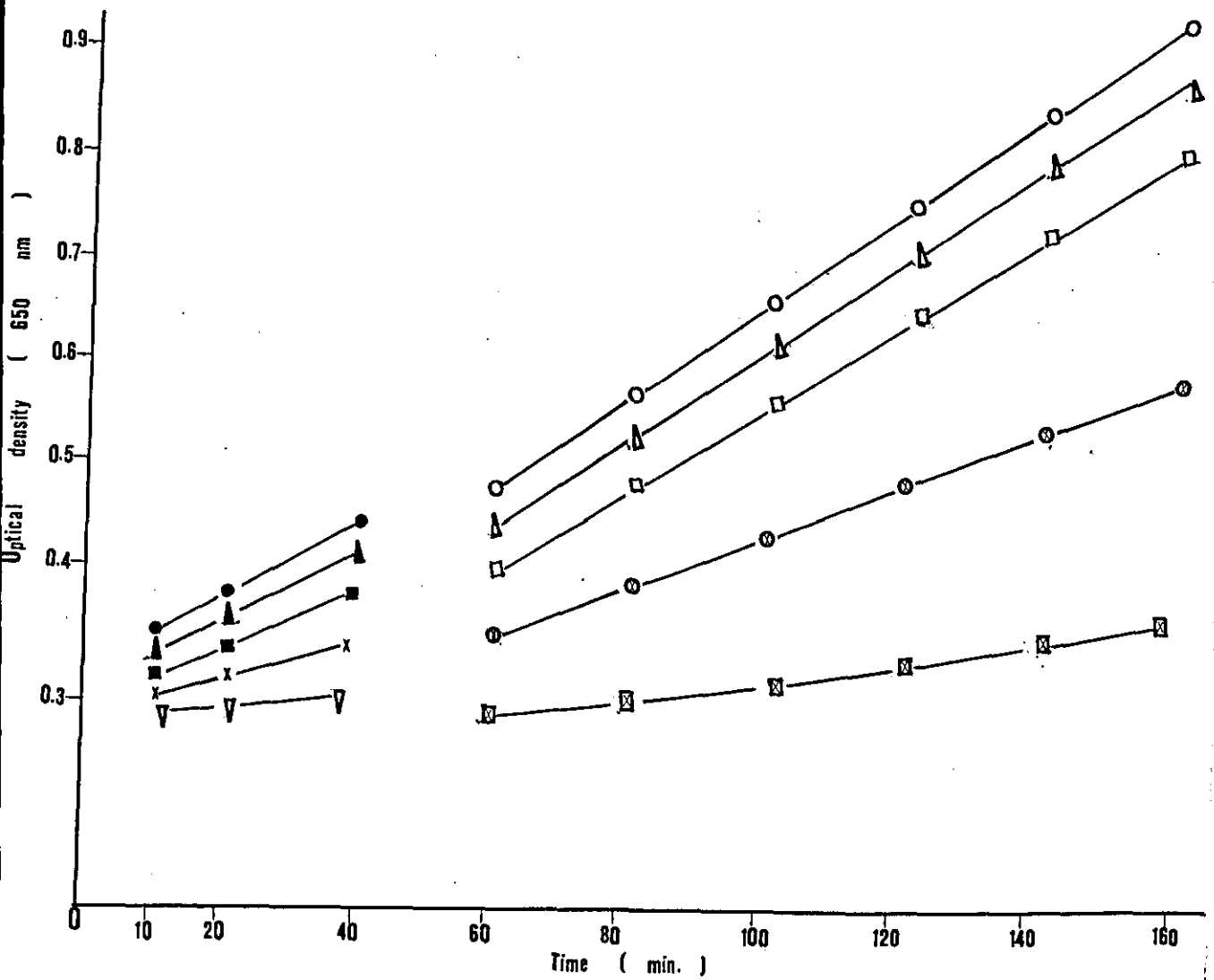
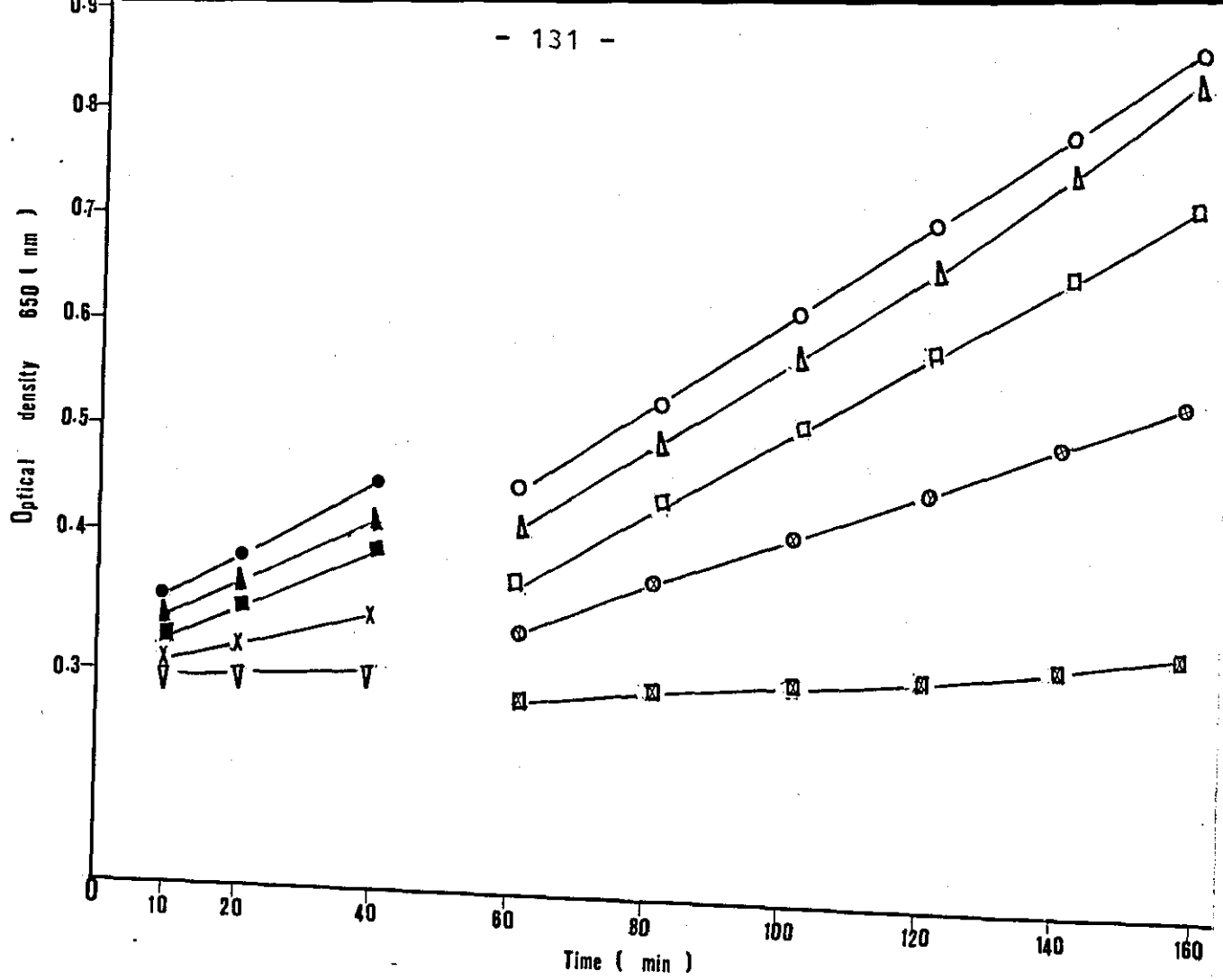
B. Fig. 3.28: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing Practolol, following addition of glucose and after resuspension in the same volume of growth medium at (40 min). Incubation temperature 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Practolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 5.0;

○-○, 0.0; △-△, 5.0;

■-■, 10.0; x-x, 20.0; ∇-∇, 30.0;

□-□, 10.0; ⊗-⊗, 20.0; ⊠-⊠, 30.0.



A. Fig. 3.29: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing Tetracaine, following addition of glucose and after resuspension in the same volume of growth medium at (40 min). Incubation temperature 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Tetracaine concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 0.25;

○-○, 0.0; △-△, 0.25;

■-■, 0.5; x-x, 1.0; ∇-∇, 1.5;

□-□, 0.5; ⊗-⊗, 1.0; ⊠-⊠, 1.5.

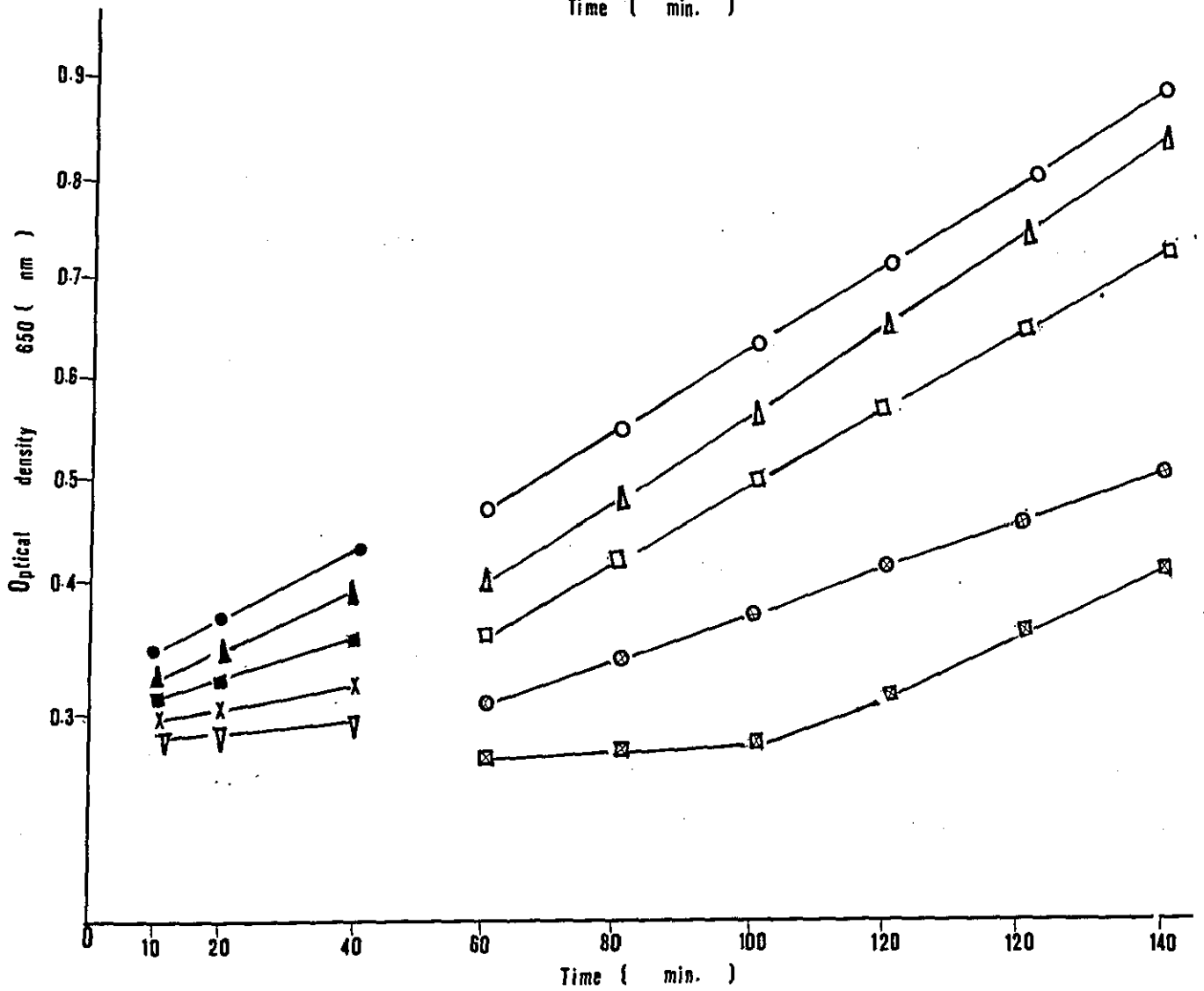
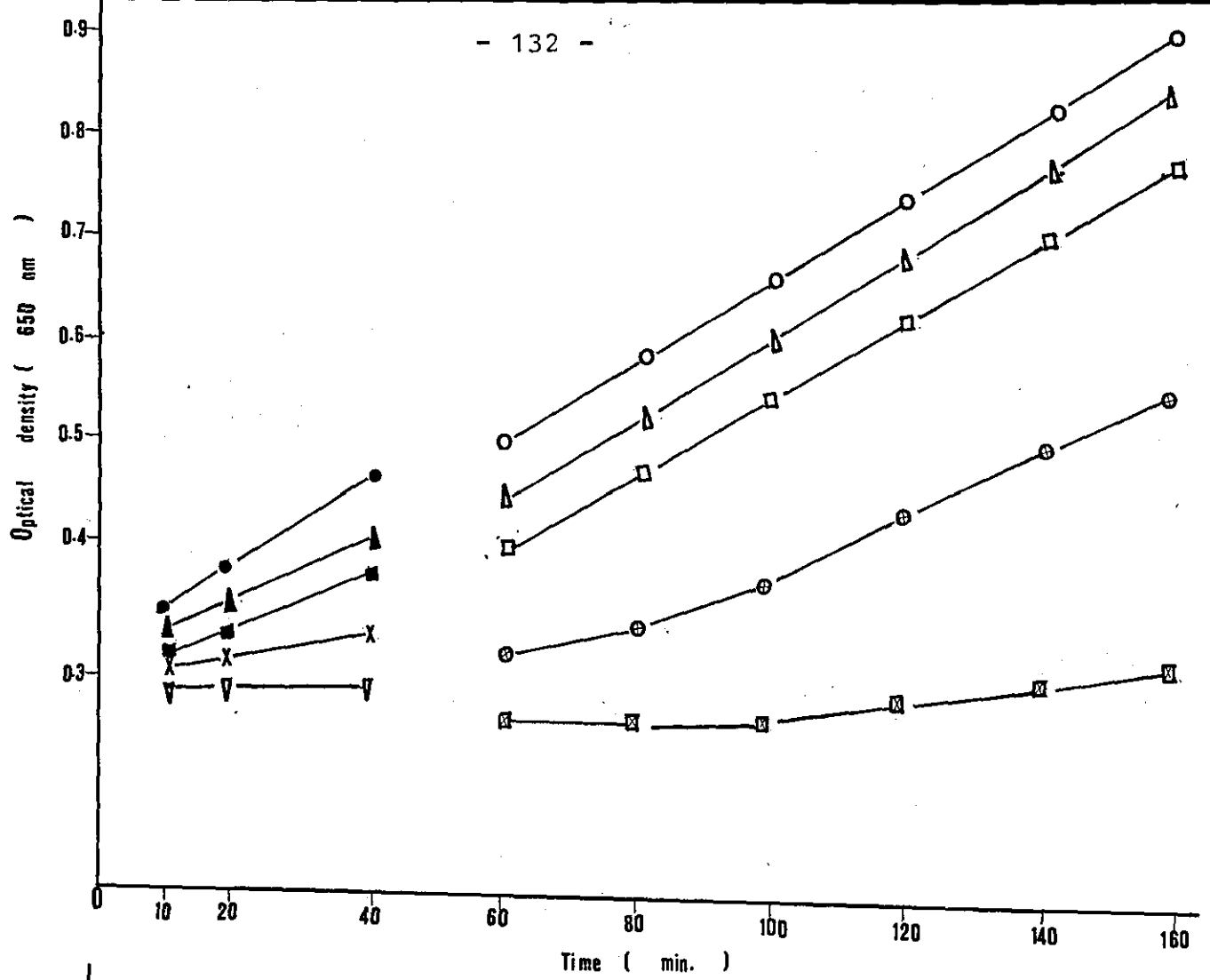
B. Fig. 3.30: Changes in optical density of cells of E.coli, initially suspended for 10 min in CFM containing Procaine, following addition of glucose and after resuspension in the same volume of growth medium at (40 min). Incubation temperature 37°C. Cell concentration (mg.ml⁻¹).

●-●, 0.0; ▲-▲, 5.0; ■-■, 10.0;

○-○, 0.0; △-△, 5.0; □-□, 10.0;

x-x, 20.0; ∇-∇, 30.0.

⊗-⊗, 20.0; ⊠-⊠, 30.0.



A. Fig. 3.31: Changes in the optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Propranolol, following addition of glucose and after resuspension in the same volume of growth medium at (100 min). Incubation temperature 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 0.3;

○-○, 0.0; △-△, 0.3;

■-■, 0.5; x-x, 0.75; ▽-▽, 1.0;

□-□, 0.5; ⊗-⊗, 0.75; ⊠-⊠, 1.0.

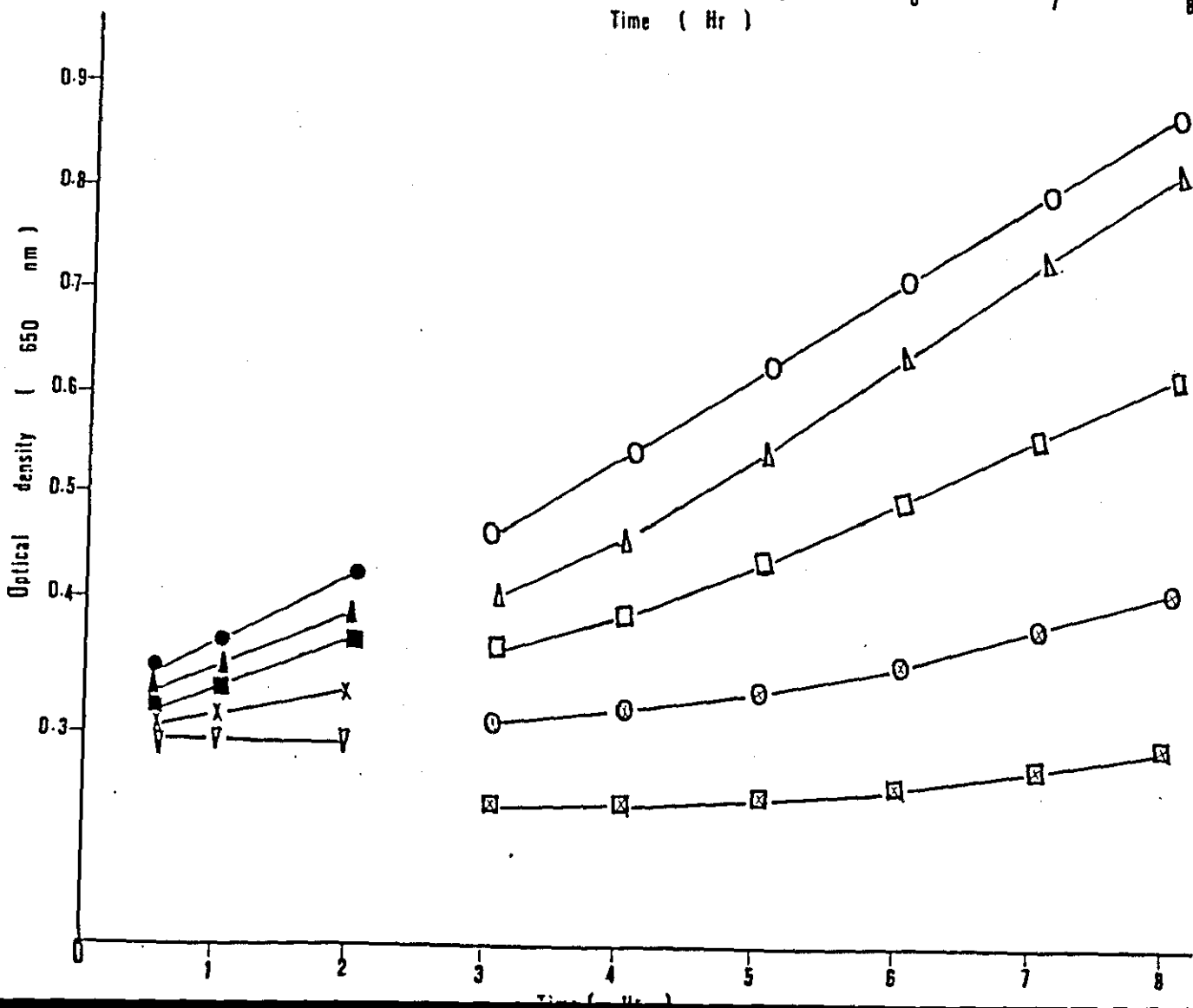
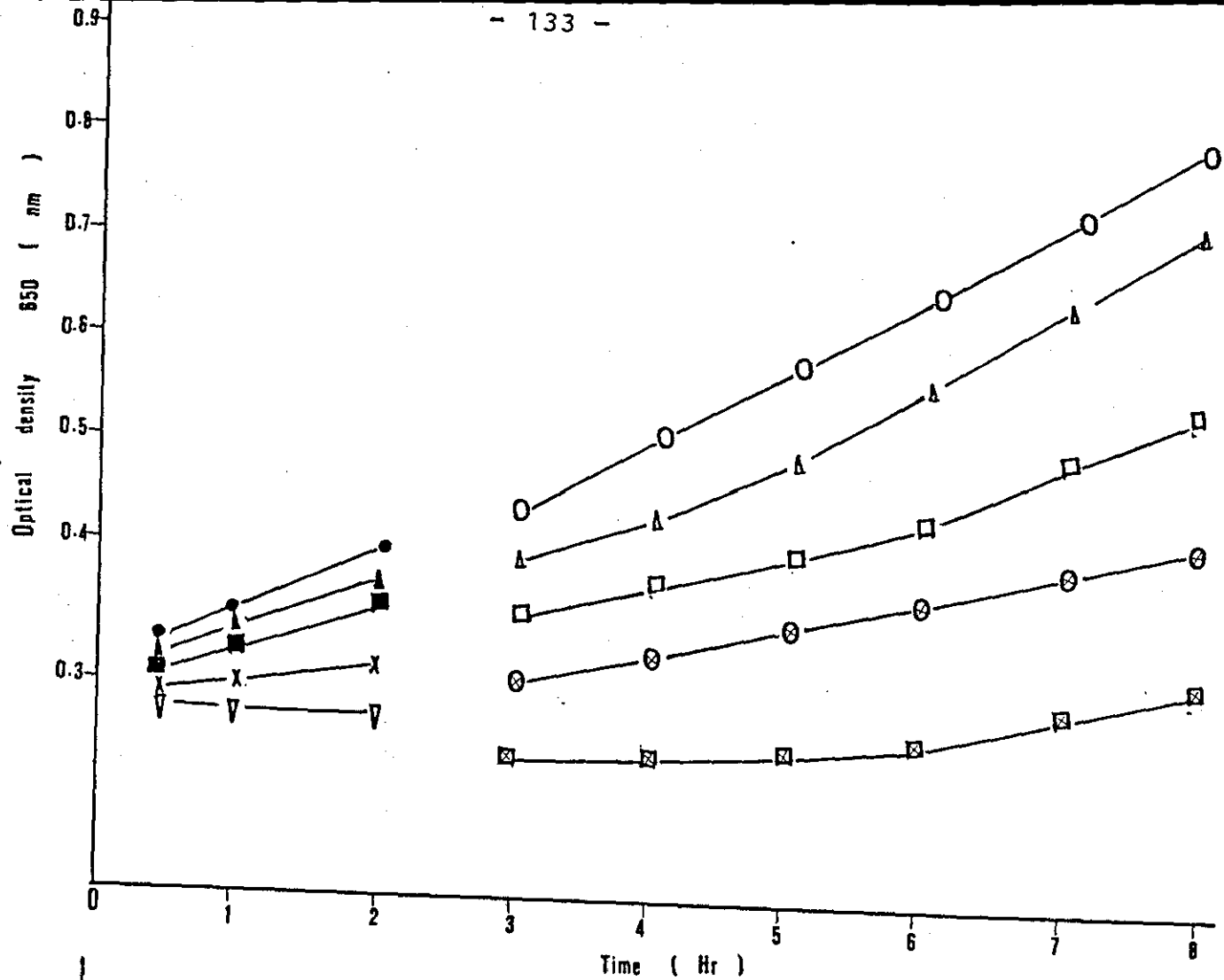
B. Fig. 3.32: Changes in the optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Oxprenolol, following addition of glucose and after resuspension in the same volume of growth medium at (100 min). Incubation temperature 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Oxprenolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 0.5;

○-○, 0.0; △-△, 0.5;

■-■, 1.0, x-x, 1.5; ▽-▽, 2.5;

□-□, 1.0; ⊗-⊗, 1.5; ⊠-⊠, 2.5.



A. Fig. 3.33: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Atenolol, following addition of glucose and after resuspension in the same volume of growth medium at (100 min). Incubation temperature 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 5.0;

○-○, 0.0; △-△, 5.0;

■-■, 10.0; x-x, 20.0; ▽-▽, 25.0;

□-□, 10.0; ⊗-⊗, 20.0; ⊠-⊠, 25.0.

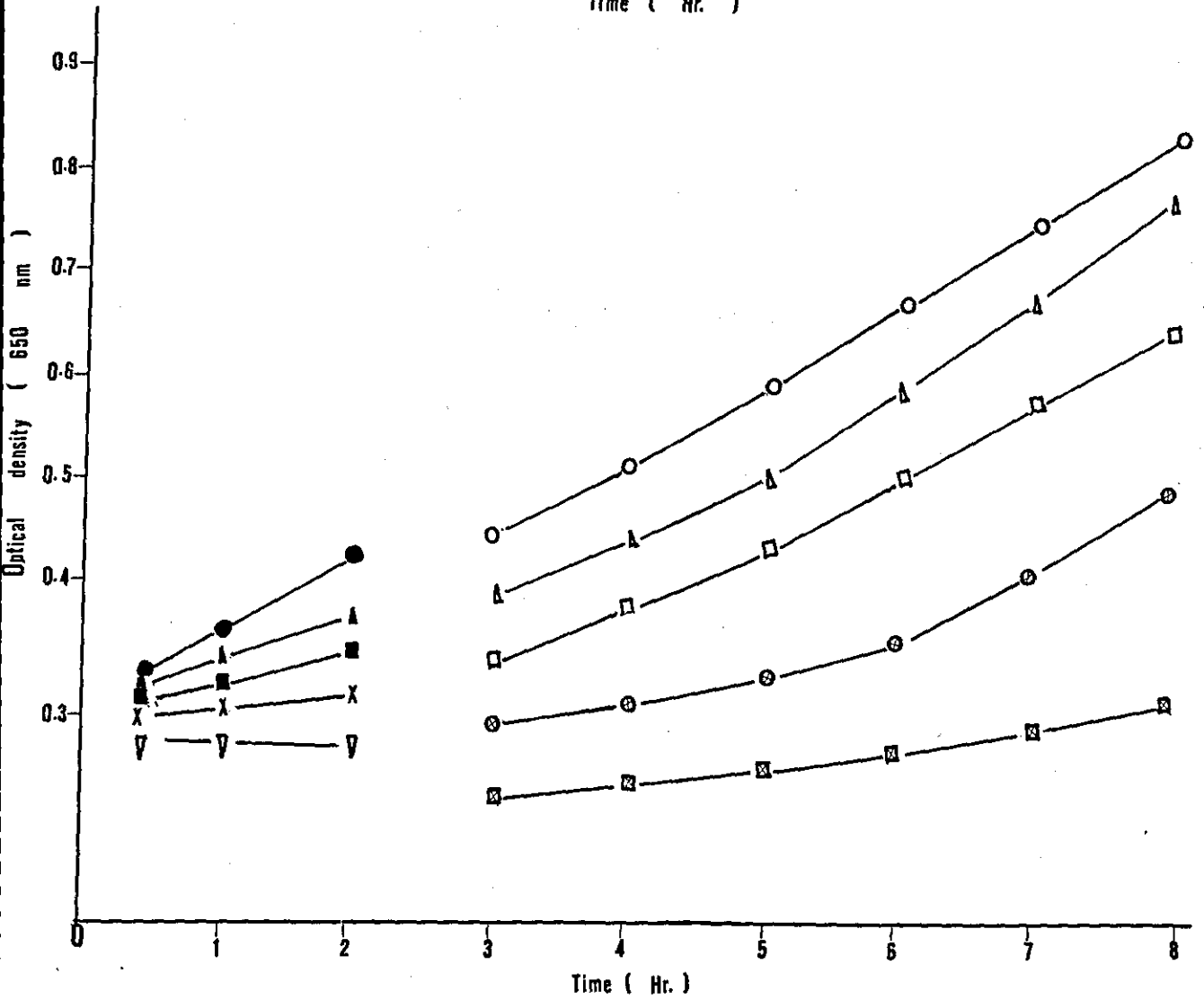
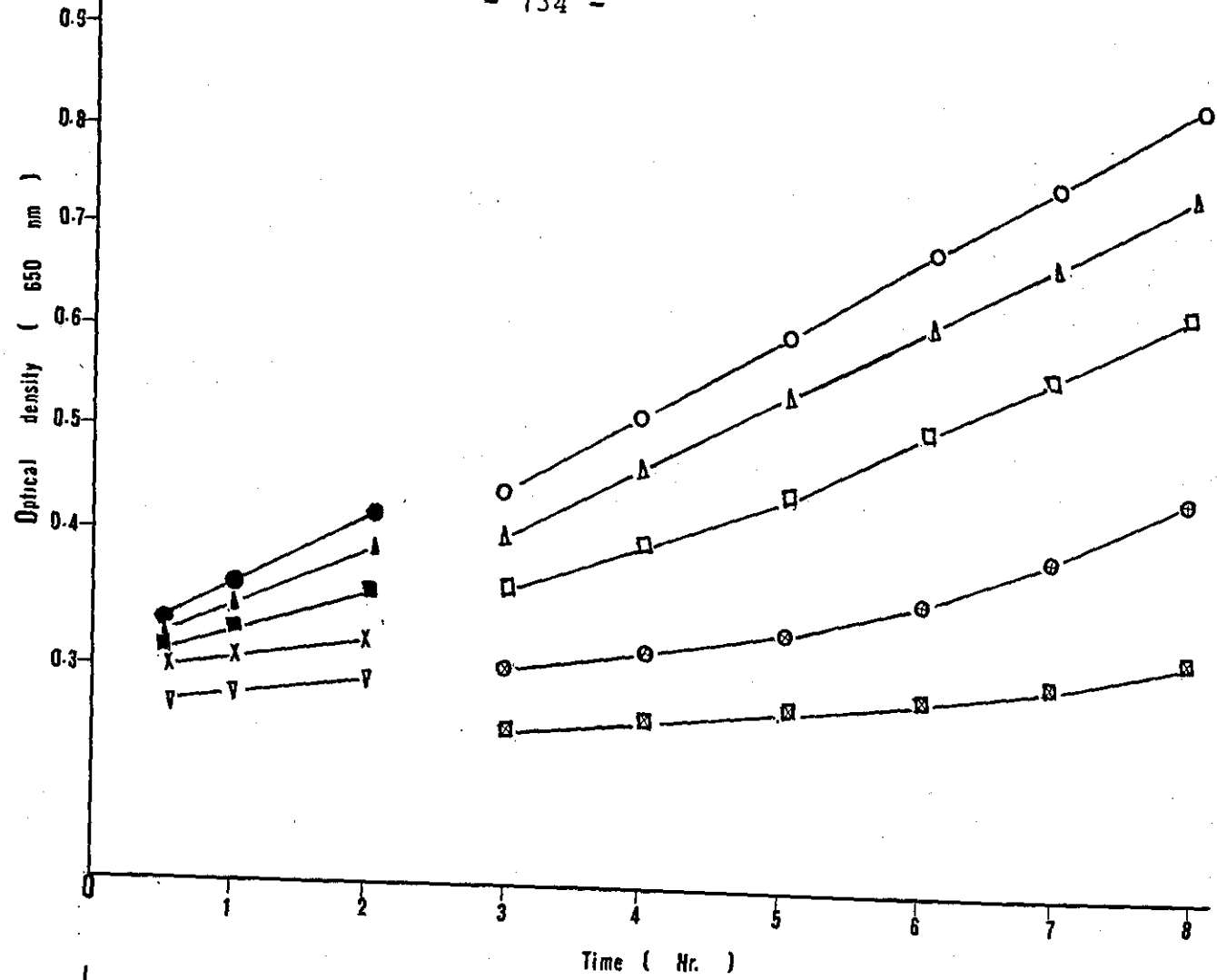
B. Fig. 3.34: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Practolol, following addition of glucose and after resuspension in the same volume of growth medium at (100 min). Incubation temperature 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Practolol concentration (mg.ml⁻¹)

●-●, 0.0; ▲-▲, 5.0;

○-○, 0.0; △-△, 5.0;

■-■, 10.0; x-x, 20.0; ▽-▽, 25.0;

□-□, 10.0; ⊗-⊗, 20.0; ⊠-⊠, 25.0.



A. Fig. 3.35: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Tetracaine, following addition of glucose and after resuspension in the same volume of growth medium at (100 min).

Incubation temperature 30°C . Cell concentration $3.2 \times 10^5 \text{ cells.ml}^{-1}$.

Tetracaine concentration (mg.ml^{-1})

●-●, 0.0; ▲-▲, 0.25;

○-○, 0.0; △-△, 0.25;

■-■, 0.5; x-x, 1.0; ∇-∇, 1.5.

□-□, 0.5; ⊗-⊗, 1.0; ⊠-⊠, 1.5.

B. Fig. 3.36: Changes in optical density of cells of C.albicans, initially suspended for 10 min in CFM containing Procaine, following addition of glucose and after resuspension in the same volume of growth medium at (100 min).

Incubation temperature 30°C . Cell concentration $3.2 \times 10^5 \text{ cells.ml}^{-1}$.

Procaine concentration (mg.ml^{-1})

●-●, 0.0; ▲-▲, 5.0.

○-○, 0.0; △-△, 5.0.

■-■, 10.0, x-x, 20.0; ∇-∇, 25.0;

□-□, 10.0; ⊗-⊗, 20.0; ⊠-⊠, 25.0.

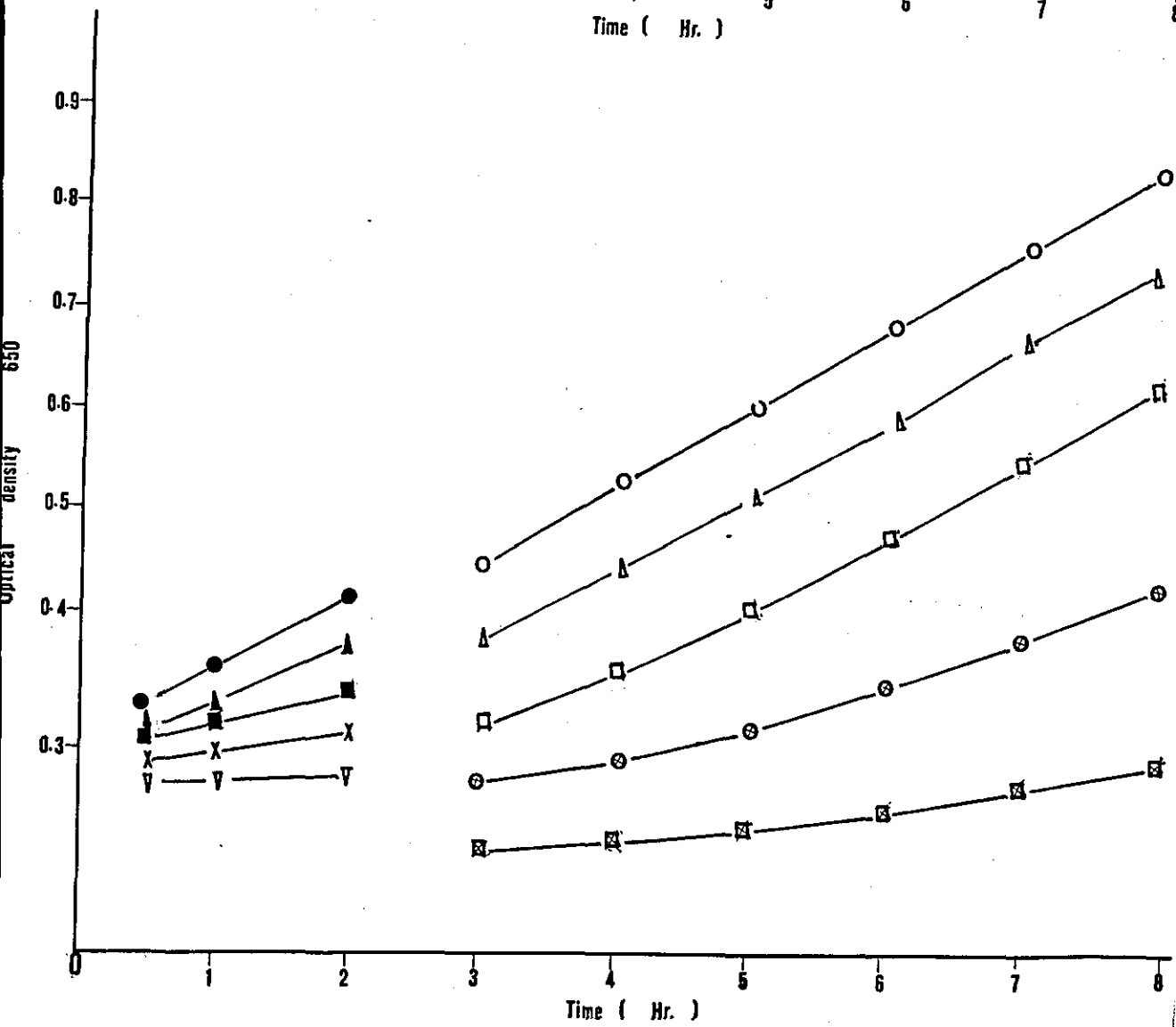
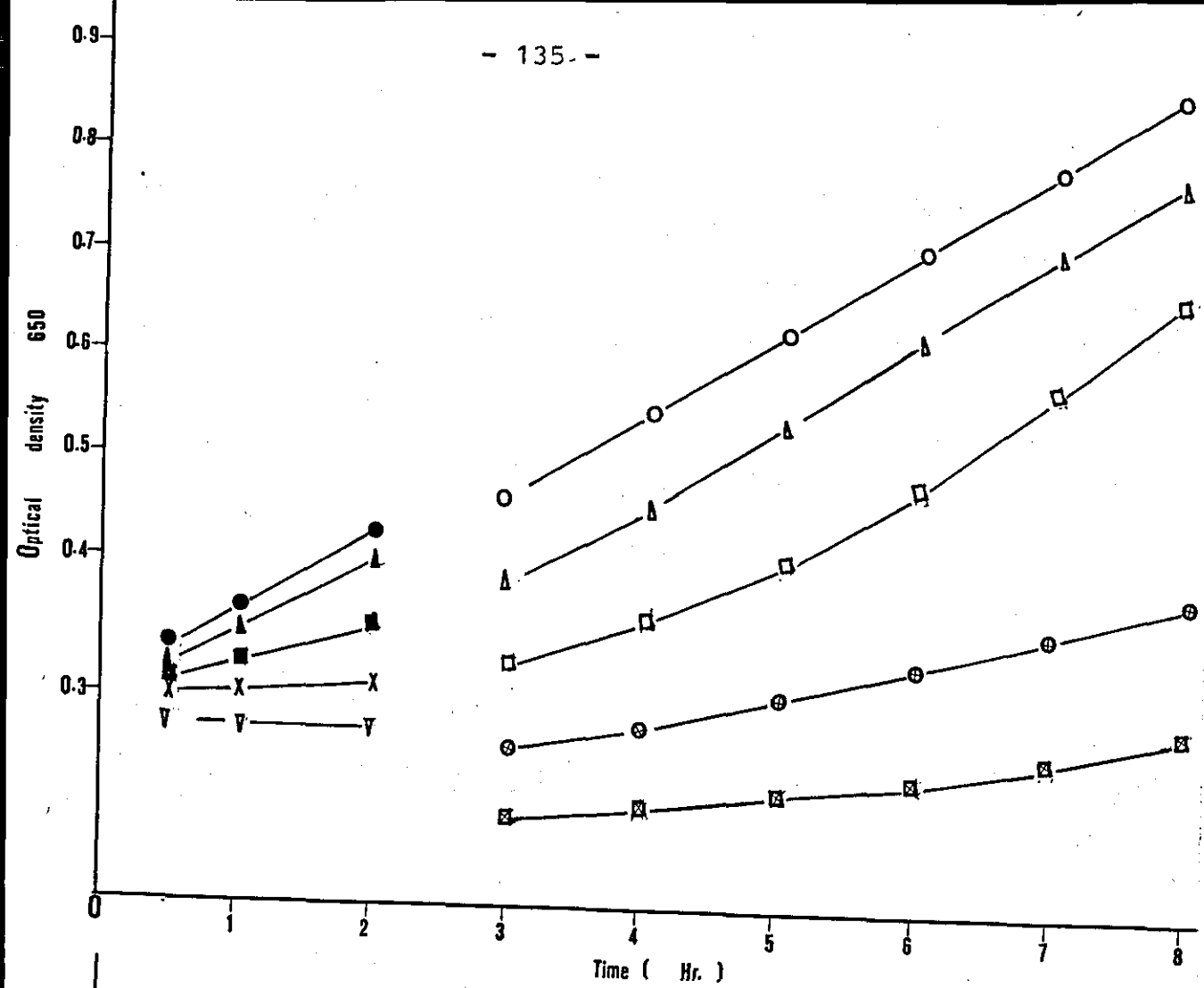


Fig. 3.37: The effect of propranolol O-O and
atenolol ●-● on the percentage viability
of cell suspension of E.coli in CFM and
maintained at 22°C. Contact time (15 min).
Cell concentration 4.2×10^9 cells.ml⁻¹.

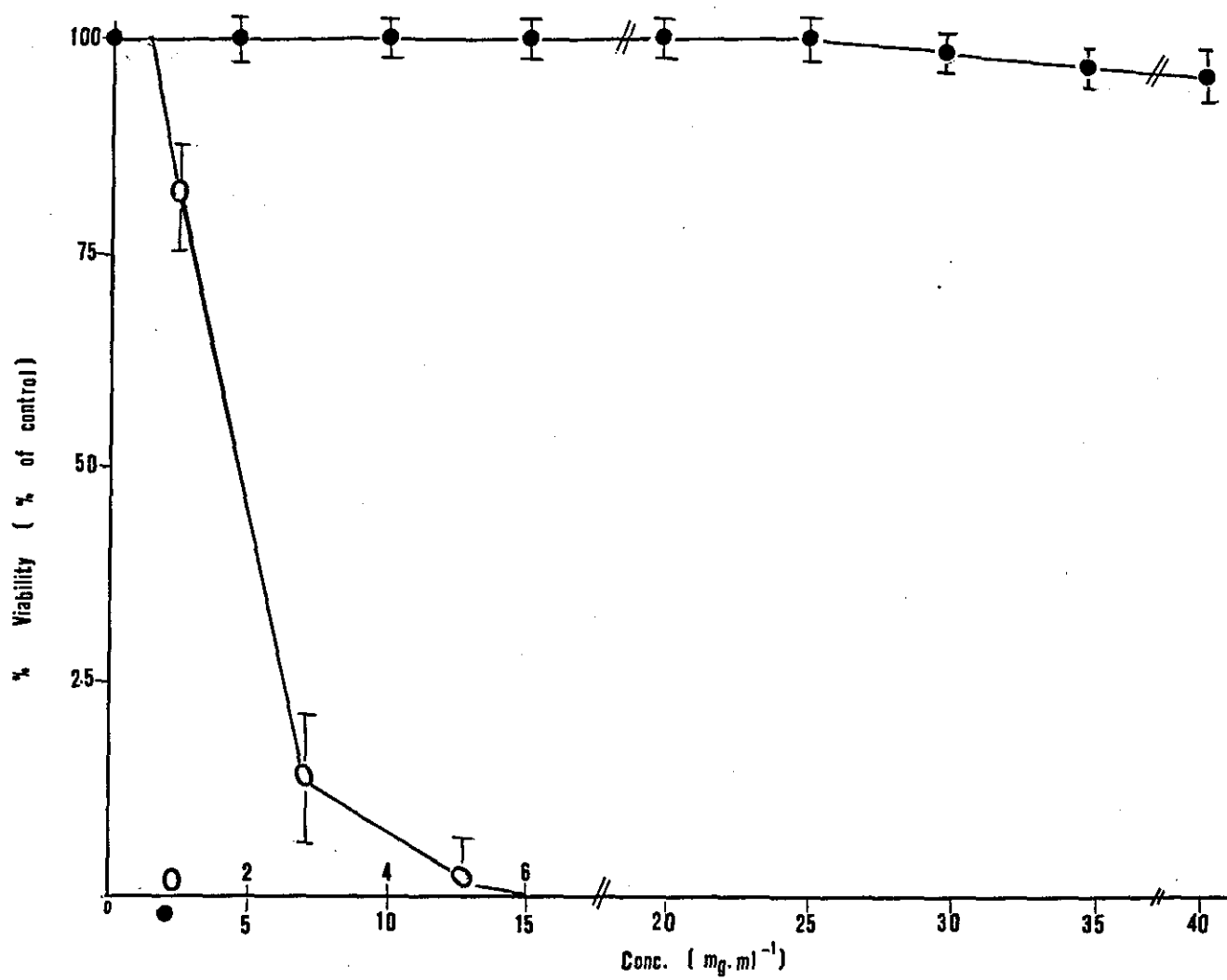
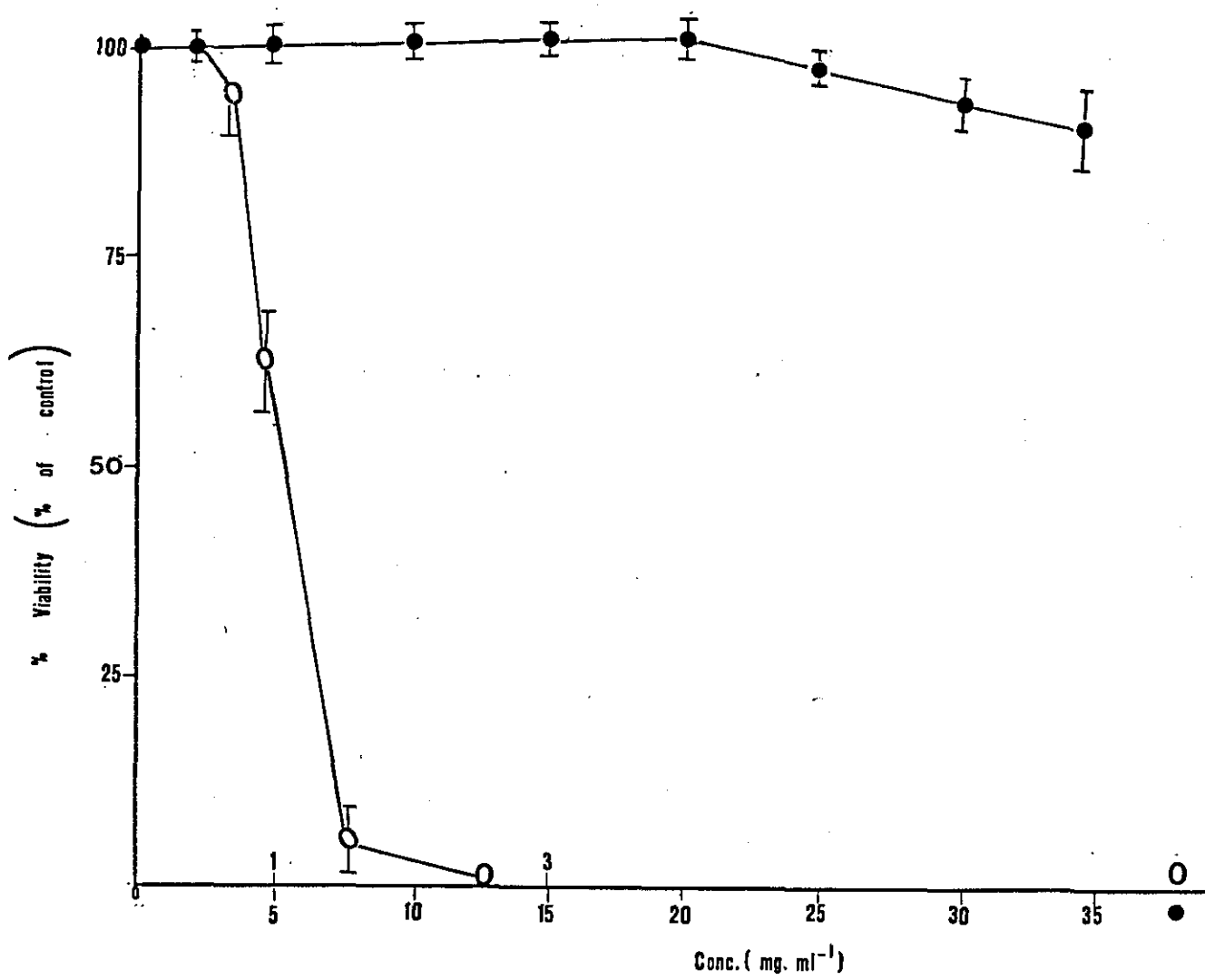
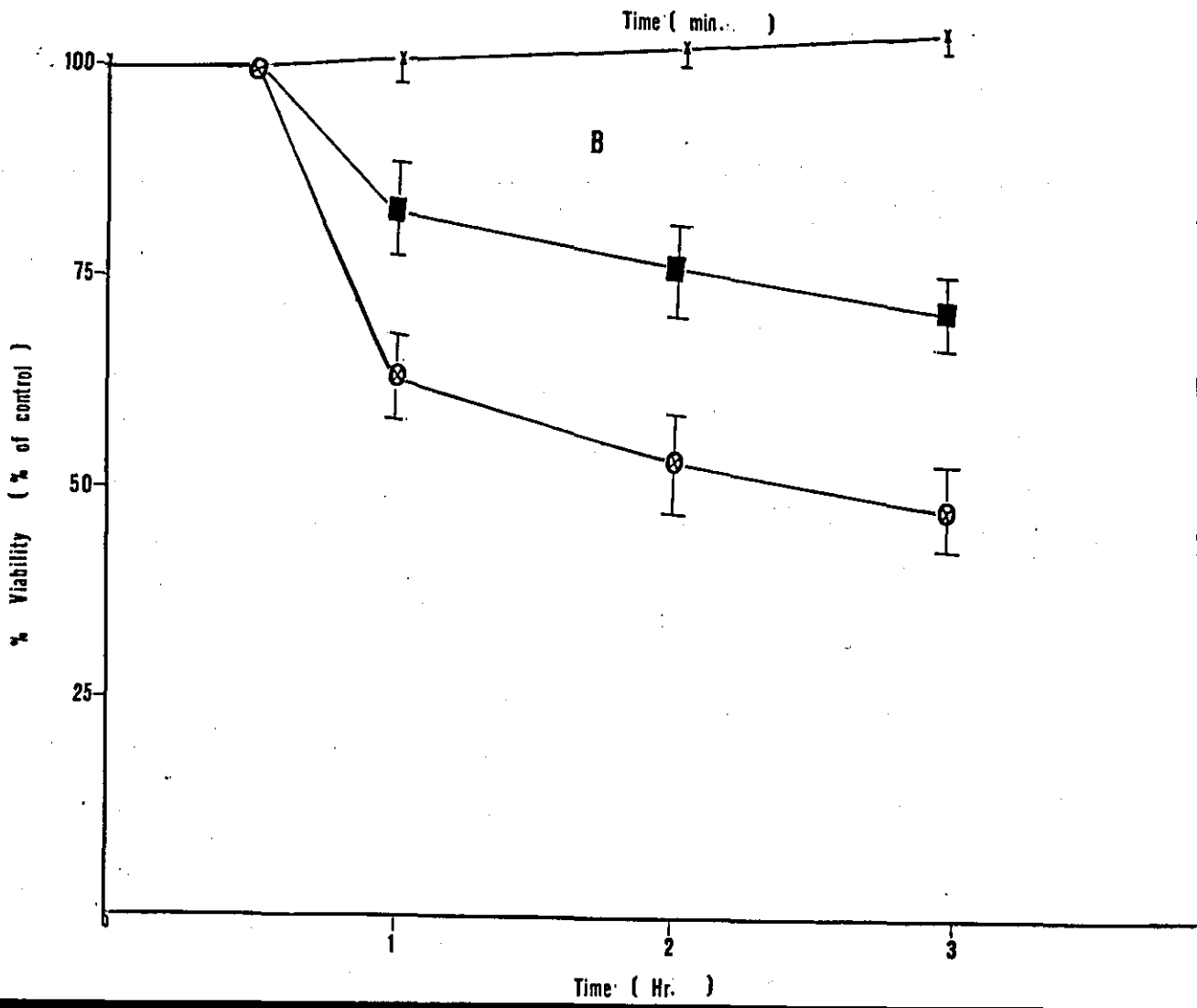
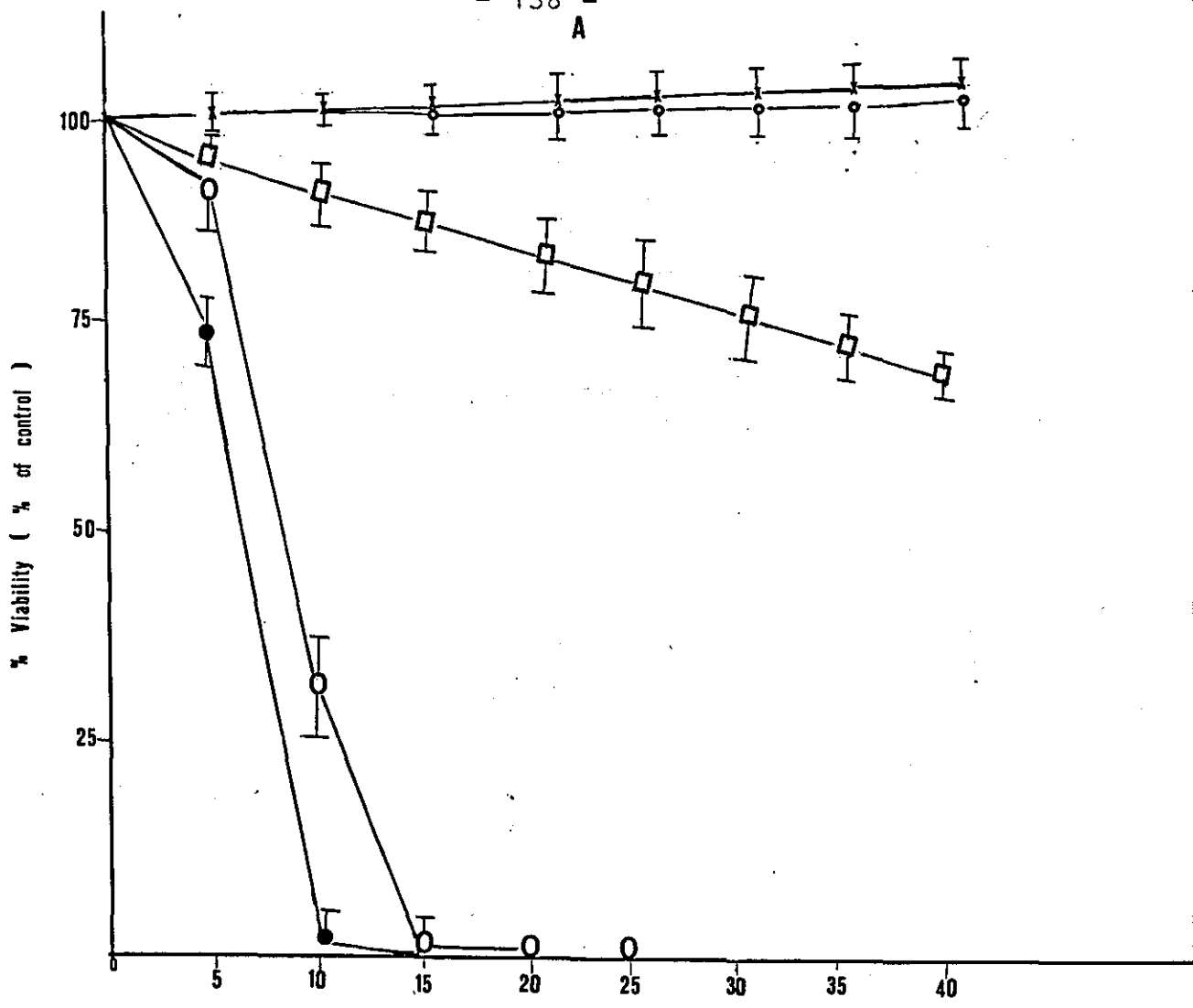


Fig. 3.38: The effect of propranolol O-O and
atenolol ●-● on the percentage viability
of cell suspension of C.albicans in CMF and
maintained at 22°C. Contact time (15 min).
Cell concentration 3.8×10^5 cells.ml⁻¹.



A. Fig. 3.39: The effect of contact time on the percentage viability of cell suspensions of E.coli in CFM containing different concentrations of propranol at 22°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹). Control, x-x, 0.0; O-O, 0.5; □□, 1.0; O-O, 2.5; ●-●, 5.0.

B. Fig. 3.40: The effect of contact time on the percentage viability of cell suspensions of E.coli in CFM containing different concentrations of atenolol at 22°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹), Control, x-x, 0.0; ■-■, 25.0; ⊗-⊗, 35.0.



A. Fig. 3.41: The effect of contact time on the percentage viability of cell suspensions of C.albicans in CFM containing different concentrations of propranolol at 22°C. Cell concentration 3.8×10^5 cells.ml⁻¹. Propranolol concentration (mg.ml⁻¹). Control, x-x, 0.0; O-O, 0.5; — —, 0.75; O-O, 1.0; O-O, 2.5.

B. Fig. 3.42: The effect of contact time on the percentage viability of cell suspensions of C.albicans in CFM containing different concentrations of atenolol at 22°C. Cell concentration 3.8×10^5 cells.ml⁻¹. Atenolol concentration (mg.ml⁻¹). Control, x-x, 0.0; ■-■, 25.0; O-O, 30.0.

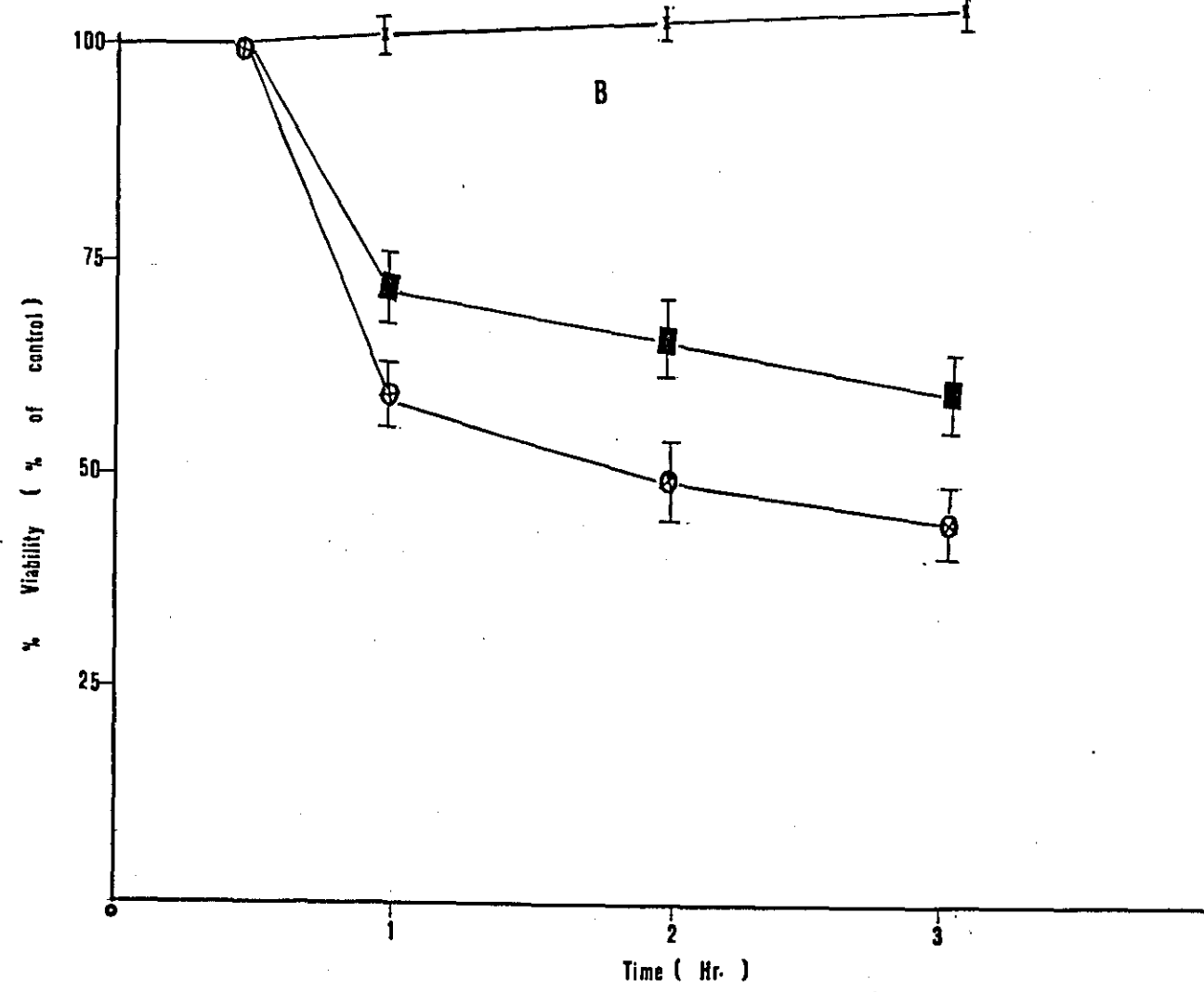
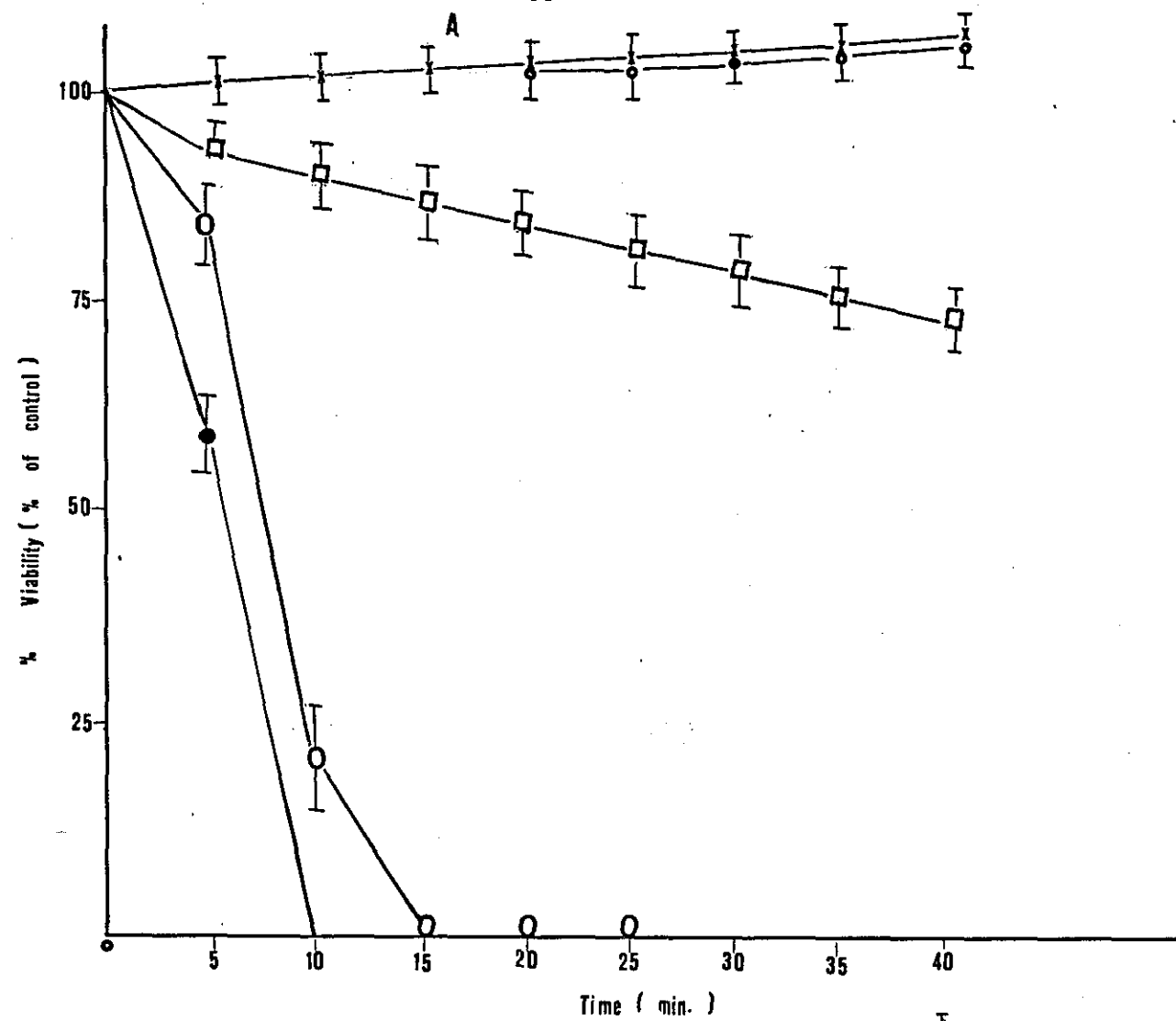


Fig. 3.43: TTC reduction by cells of E.coli suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol, and (D) Practolol at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Added substrate concentration 0.02M glucose.

(A) Propranolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.25
 x-x 0.50
 O-O 1.00
 ▲-▲ 7.5
 . . . Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.5
 x-x 2.0
 O-O 4.0
 ▲-▲ 12.0
 . . . Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 . . Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 . . Endogenous respiration

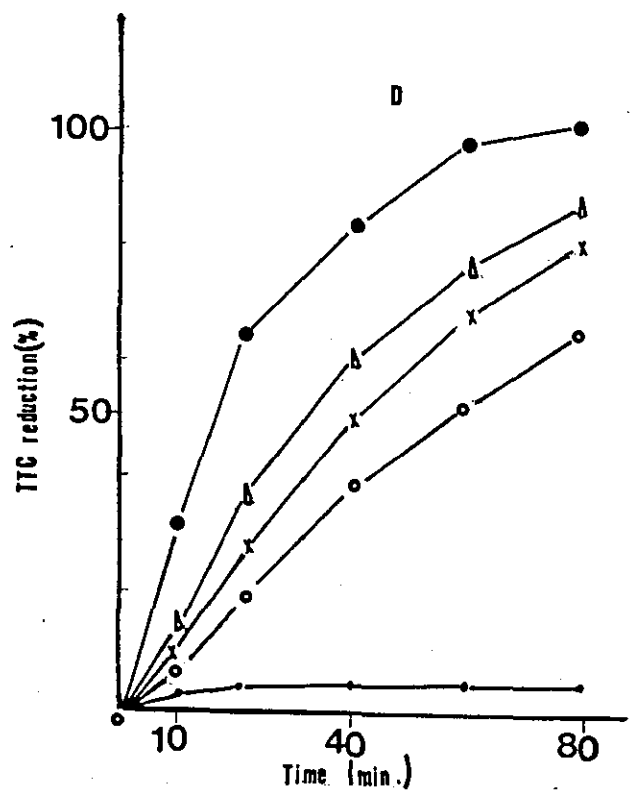
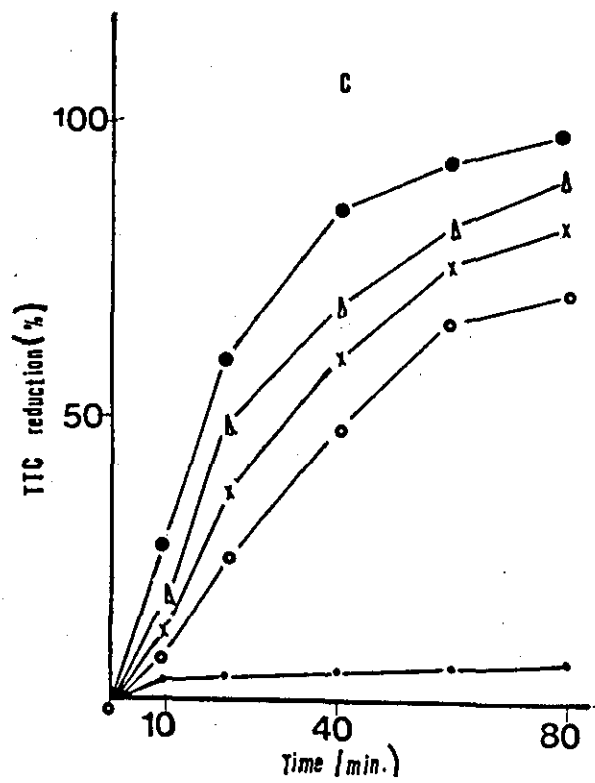
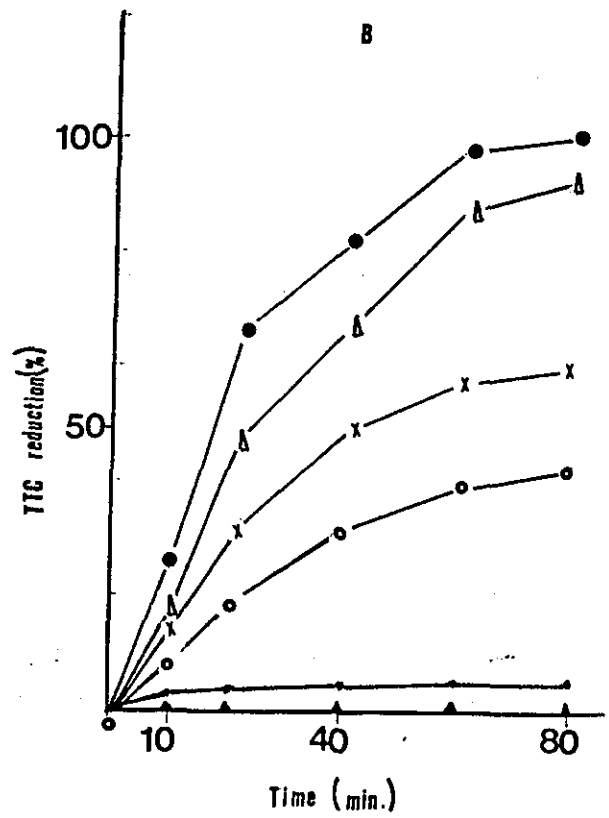
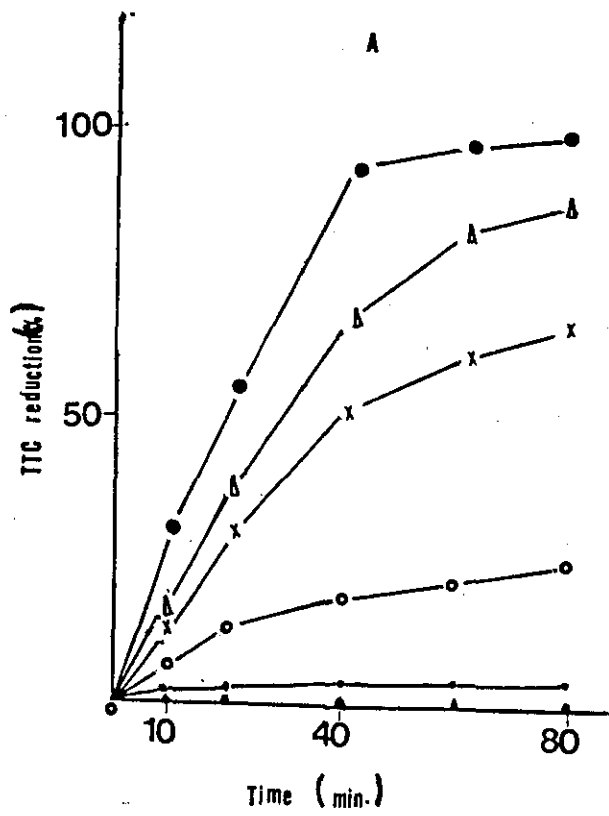


Fig. 3.44: TTC reduction by cells of E.coli suspended in CFM containing (A) Propranolol, (b) Oxprenolol, (C) Atenolol, and (D) Practolol at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Added substrate concentration 0.02M succinate.

(A) Propranolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.25
 x-x 0.50
 O-O 1.00
 ▲-▲ 7.5
 . . Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.5
 x-x 2.0
 O-O 4.0
 ▲-▲ 12.0
 . . Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 . . Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 . . Endogenous respiration

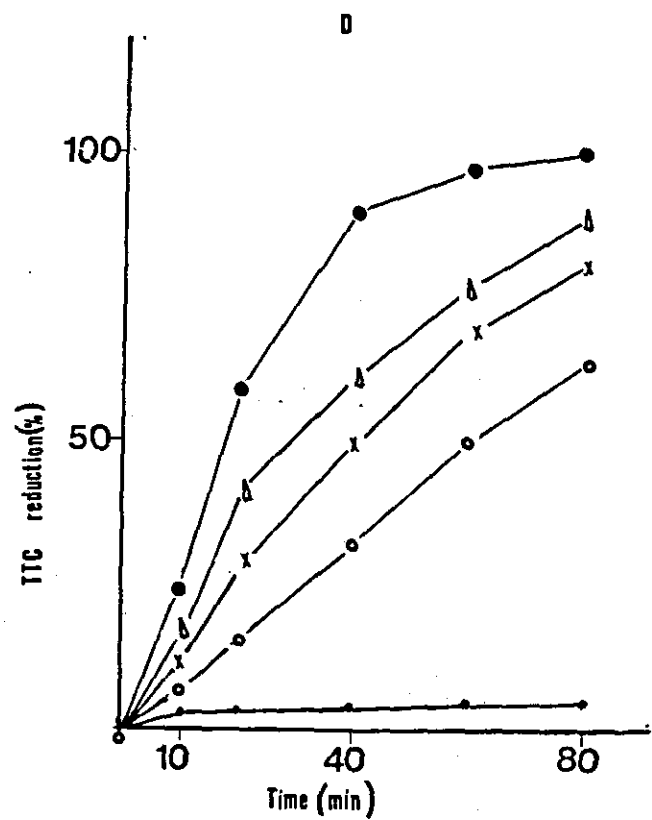
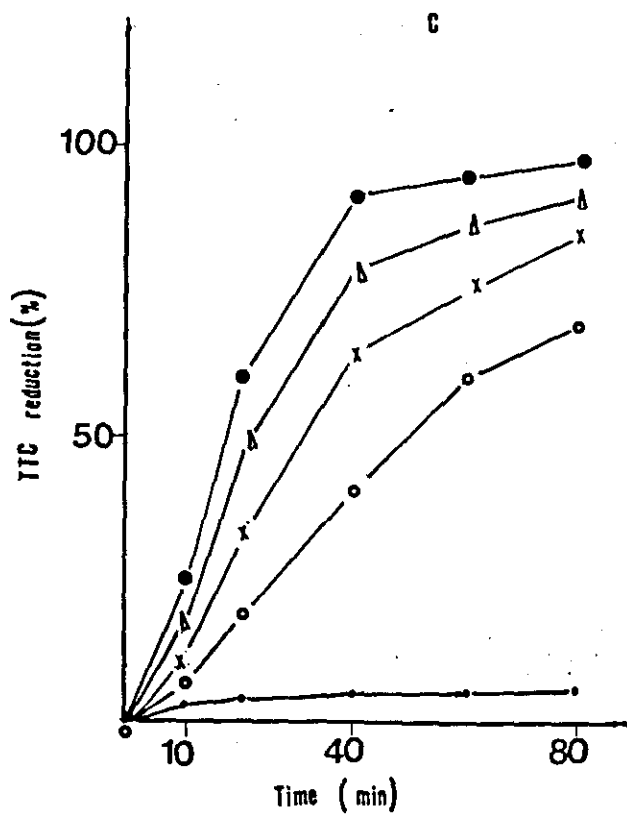
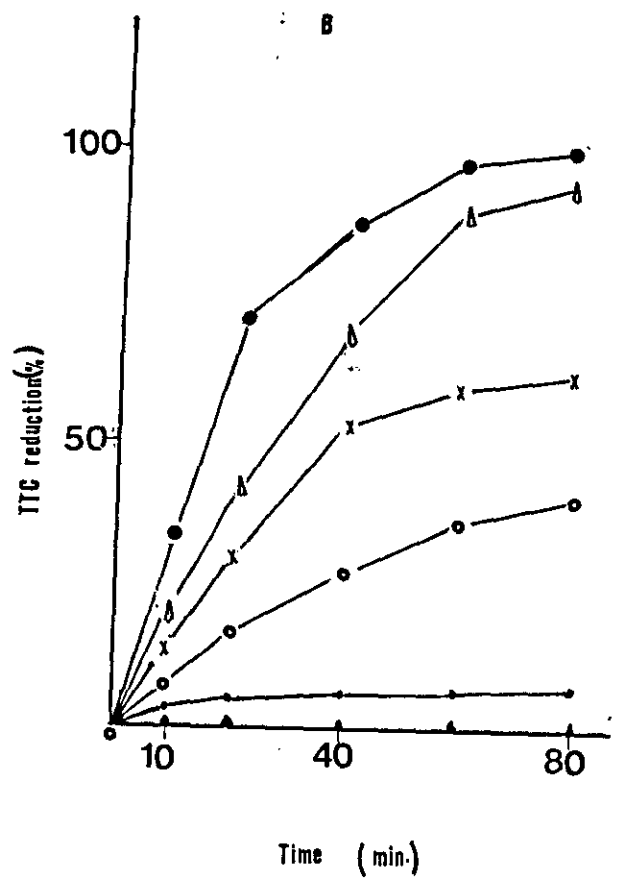
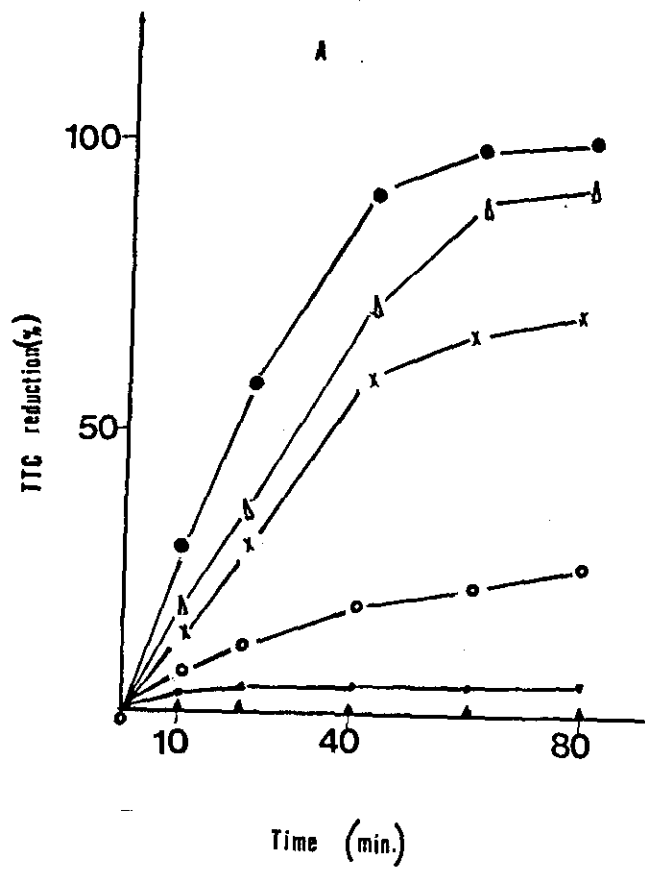


Fig. 3.45: The reduction by cells of E.coli suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol, and (D) Practolol 0.02 M malate.

(A) Propranolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.25
 x-x 0.50
 O-O 1.00
 ▲-▲ 7.5
 ... Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.5
 x-x 2.0
 O-O 4.0
 ▲-▲ 12.0
 ... Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

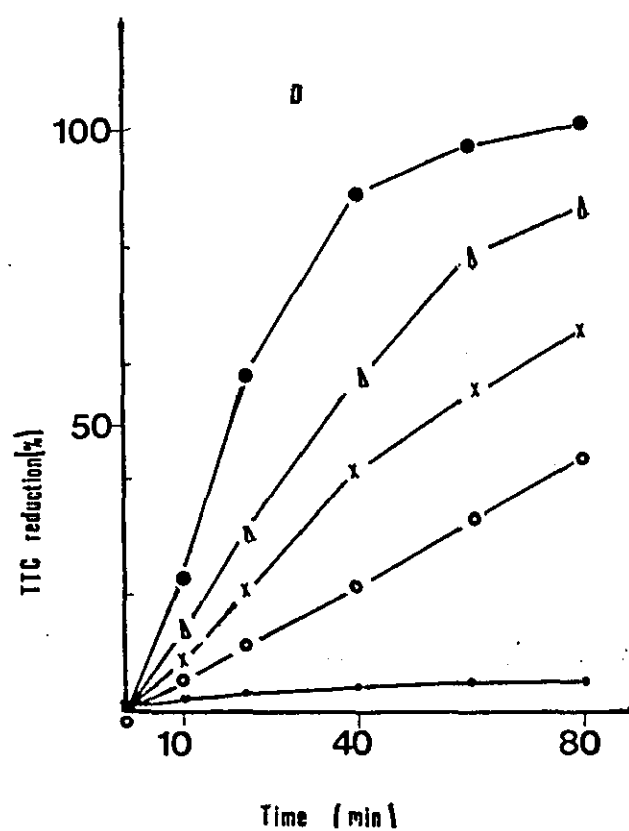
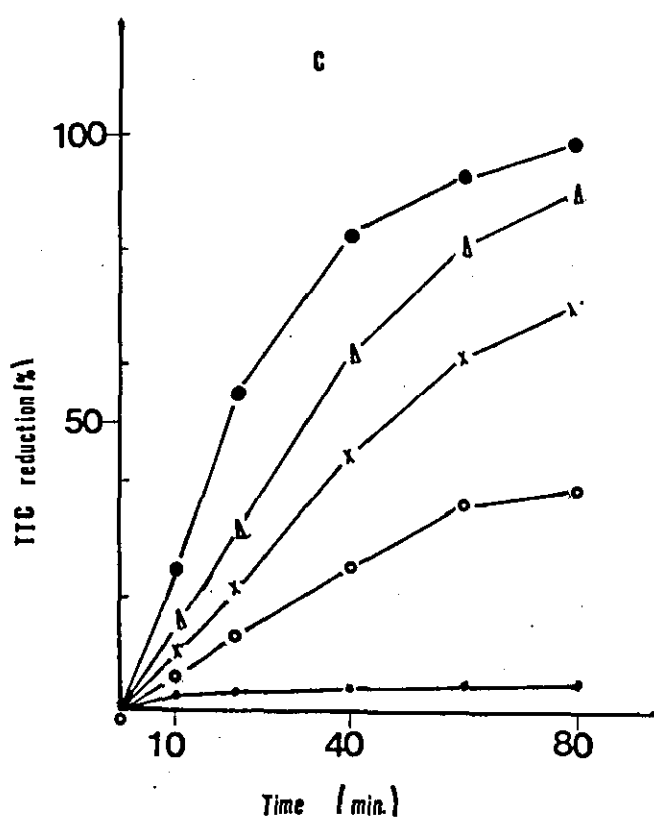
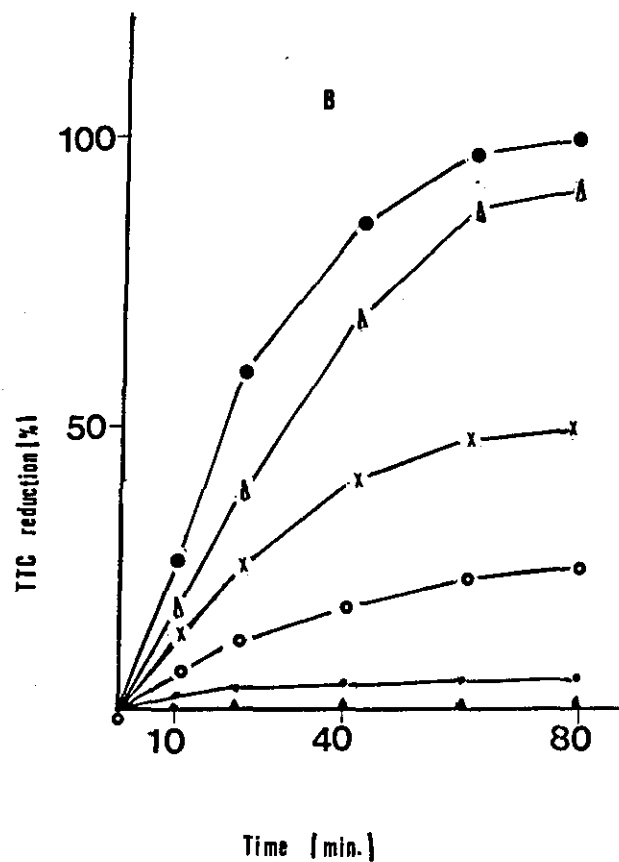
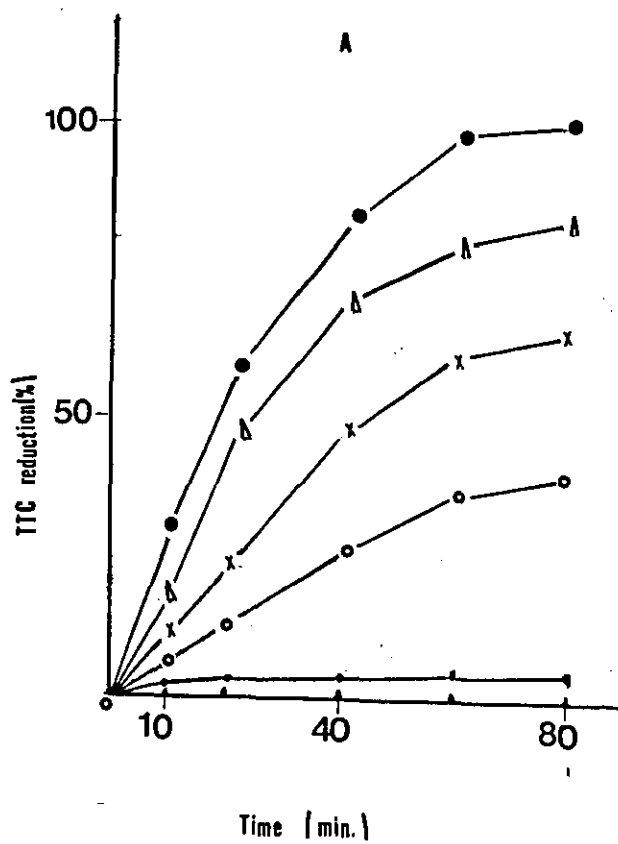


Fig. 3.46: TTC reduction by cells of E.coli suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol and (D) Practolol at 37°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Added substrate concentration 0.02M Lactate.

(A) Propranolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.25
 x-x 0.50
 O-O 1.00
 ▲-▲ 7.5
 ... Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ Δ 0.5
 x-x 2.0
 O-O 4.0
 ▲-▲ 12.0
 ... Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

(C) Practolol (mg.ml⁻¹)

●-○ 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

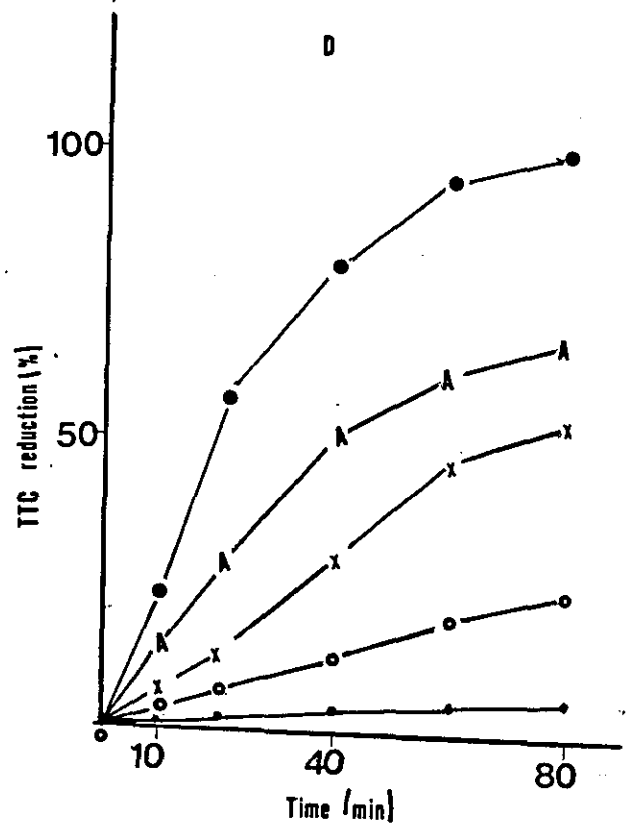
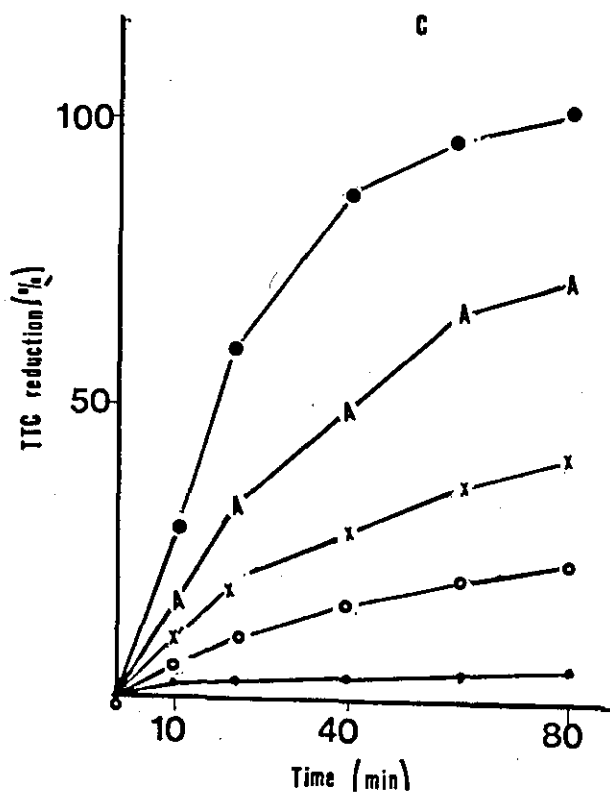
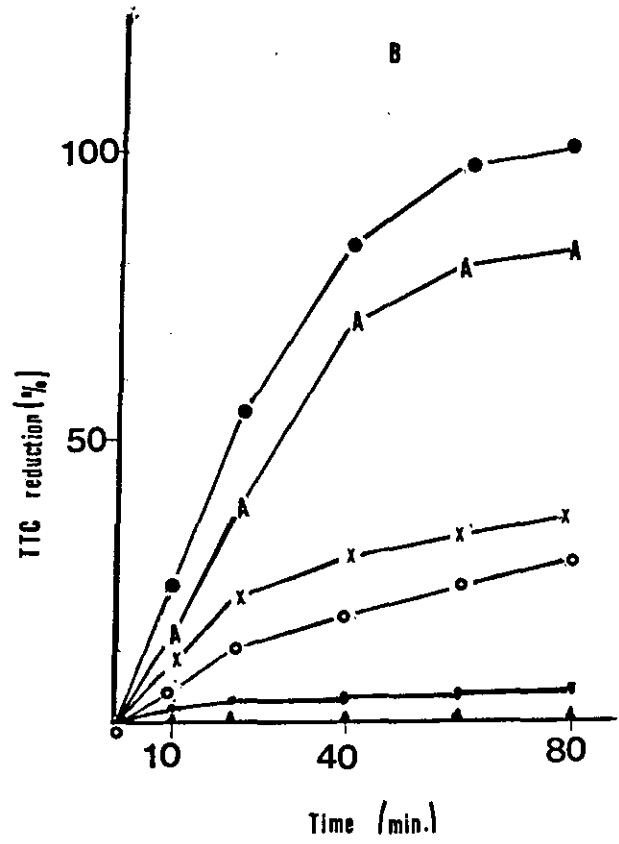
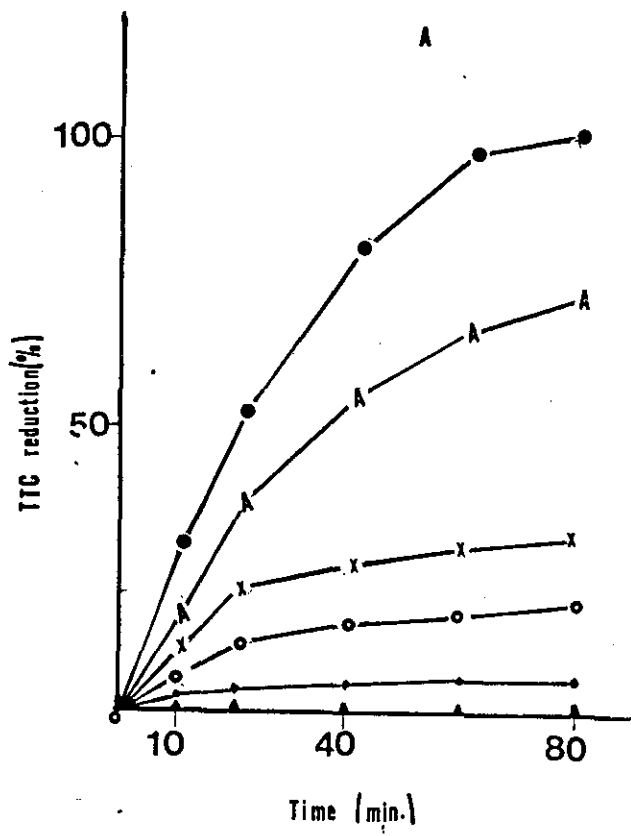


Fig. 3.47: TTC reduction by cells of C.albicans suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol and (D) Practolol at 30°C. Cell concentration 3.8×10^5 cells.ml⁻¹. Added substrate concentration 0.02 M d-glucose.

(A) Propranolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.15
 x-x 0.35
 O-O 0.75
 Δ-Δ 5.0
 --- Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.85
 x-x 1.00
 O-O 2.0
 Δ-Δ 7.5
 --- Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 --- Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 --- Endogenous respiration

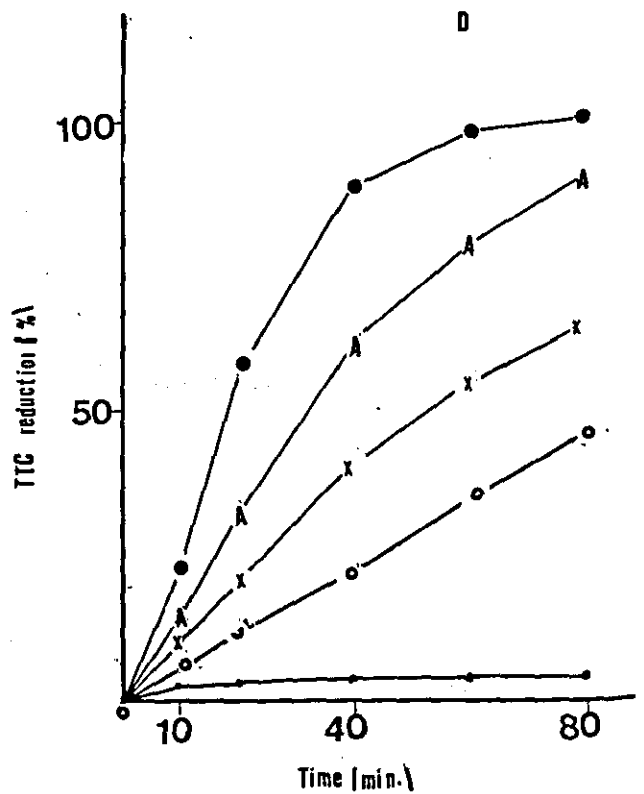
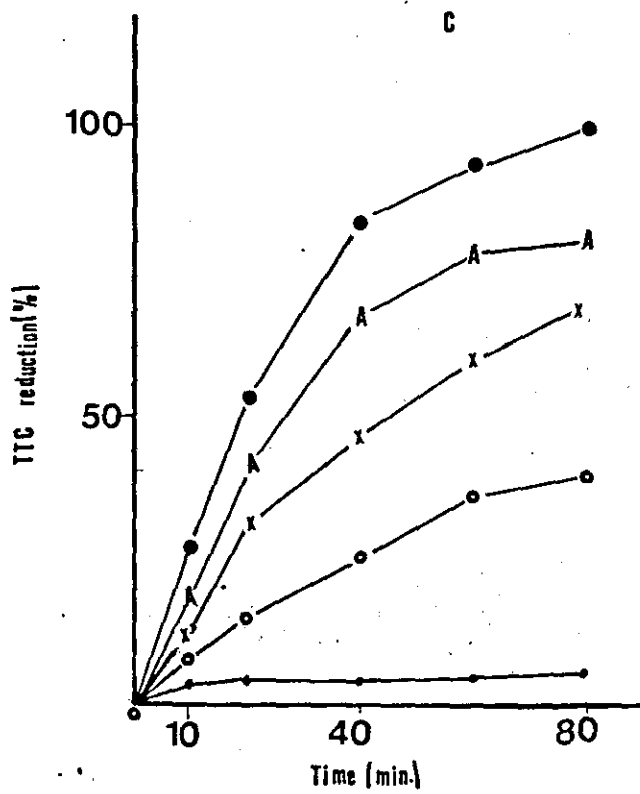
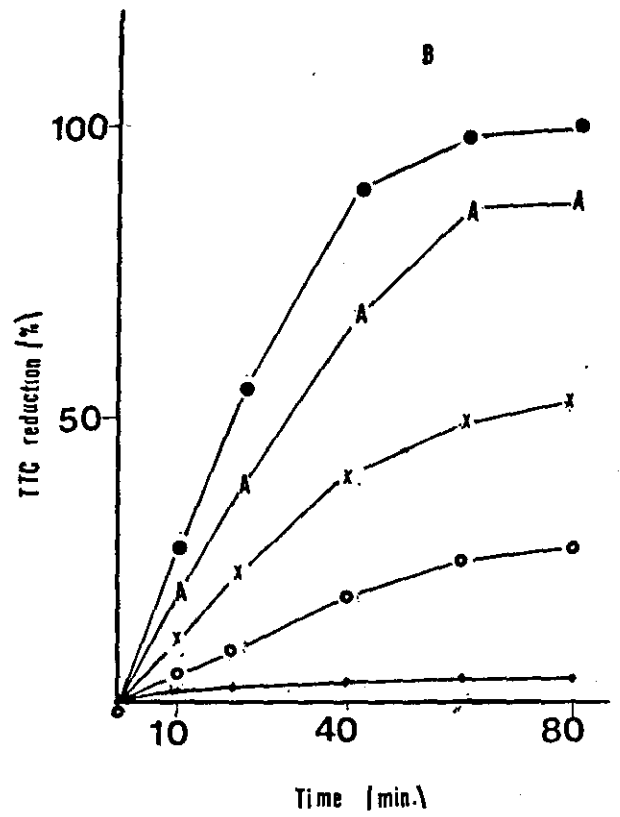
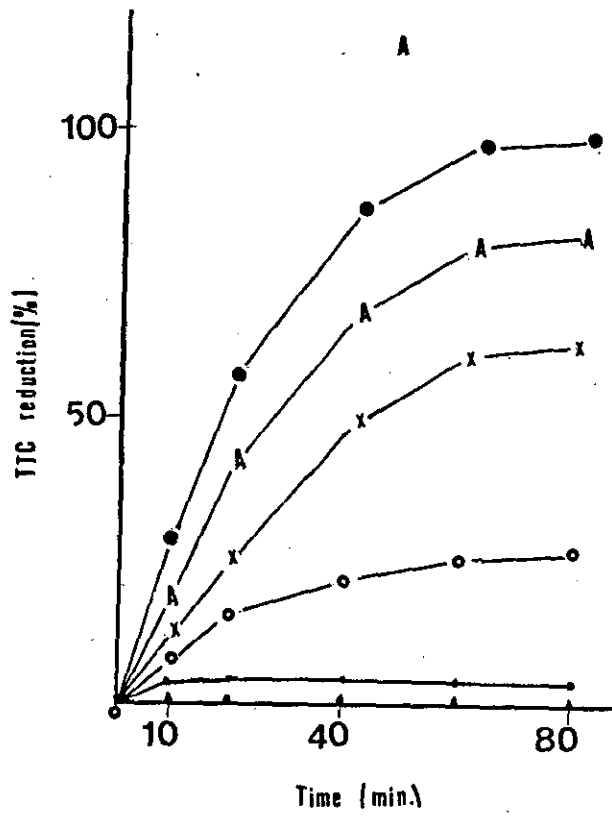


Fig. 3.48: TTC reduction by cells of C.albicans suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol and (D) Practolol at 30°C. Cell concentration 3.8×10^5 cells.ml⁻¹. Added substrate concentration 0.02M succinate.

(A) Propranolol (mg. ml⁻¹)

●-● 0.0
 Δ-Δ 0.15
 x-x 0.35
 O-O 0.75
 ▲-▲ 5.0
 ... Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.35
 x-x 1.00
 O-O 2.0
 ▲-▲ 7.5
 ... Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 4.0
 O-O 30.0
 ... Endogenous respiration

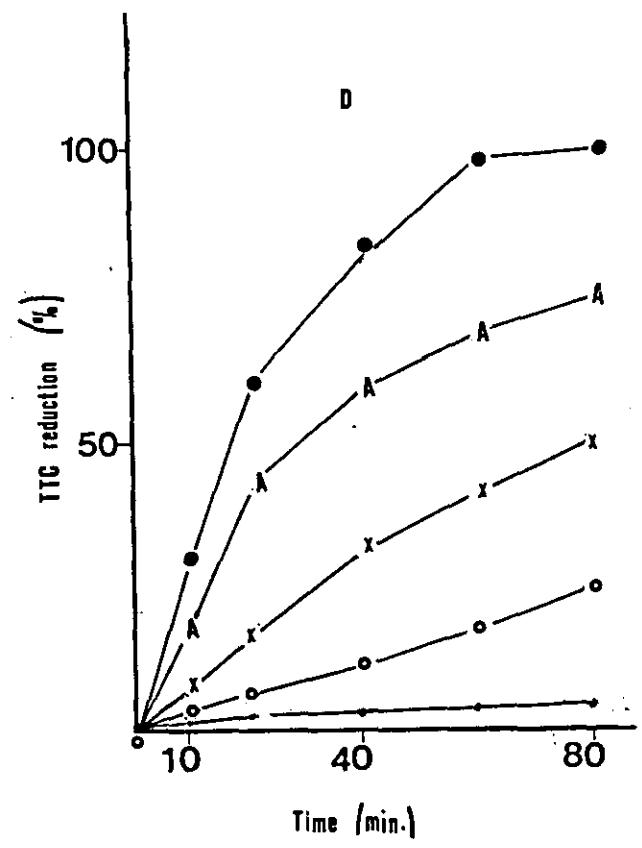
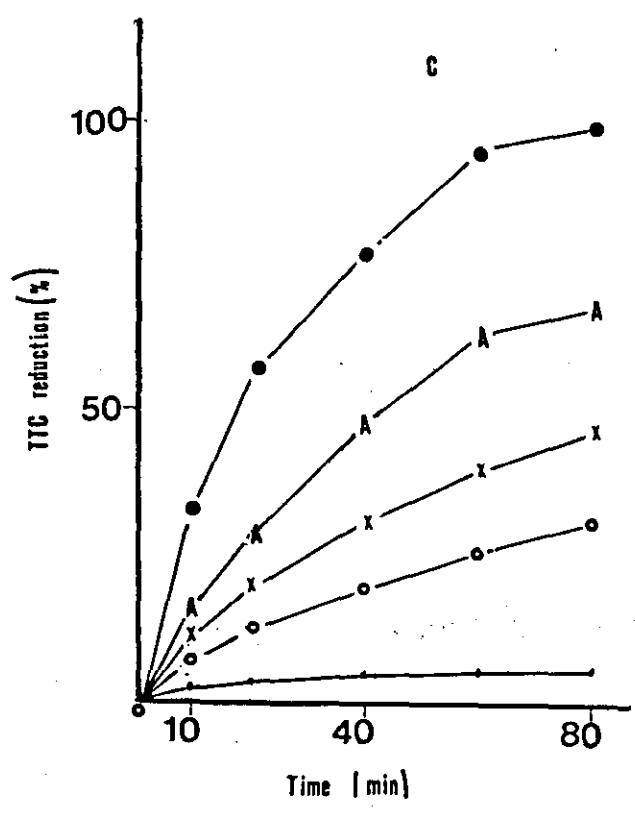
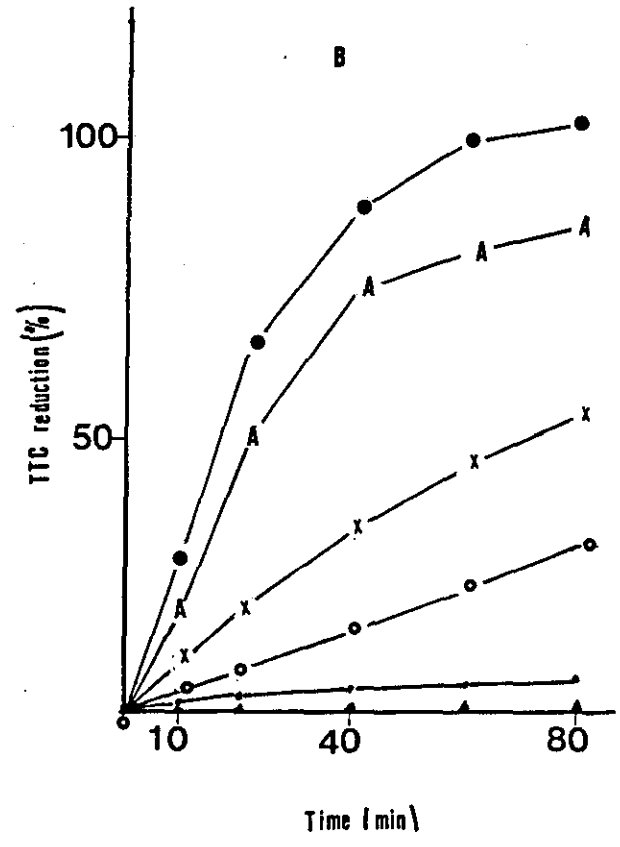
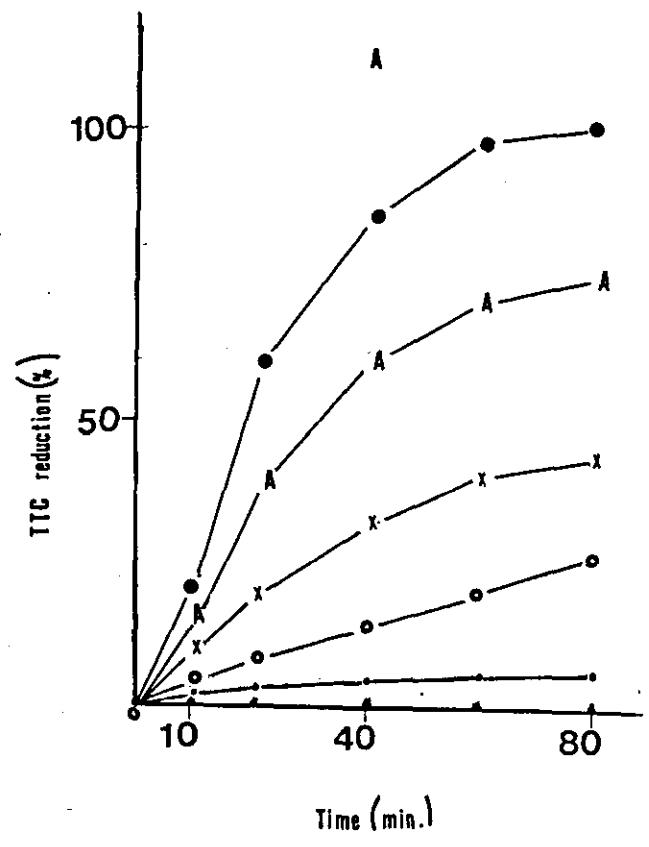


Fig. 3.49: TTC reduction by cells of C.albicans suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol, and (D) Practolol at 30°C. Cell concentration 3.8×10^5 cells.ml⁻¹. Added substrate concentration 0.02M Malate.

(A) Propranolol (mg.ml⁻¹)

●-●	0.0
Δ-Δ	0.15
x-x	0.35
O-O	0.75
▲-▲	5.0
.-.	Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-●	0.0
Δ-Δ	0.35
x-x	1.00
O-X	2.00
▲-▲	7.5
.-.	Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-●	0.0
Δ-Δ	4.0
x-x	12.0
O-O	30.0
. .	Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-●	0.1
Δ-Δ	4.0
x-x	12.0
O-O	30.0
. .	Endogenous respiration

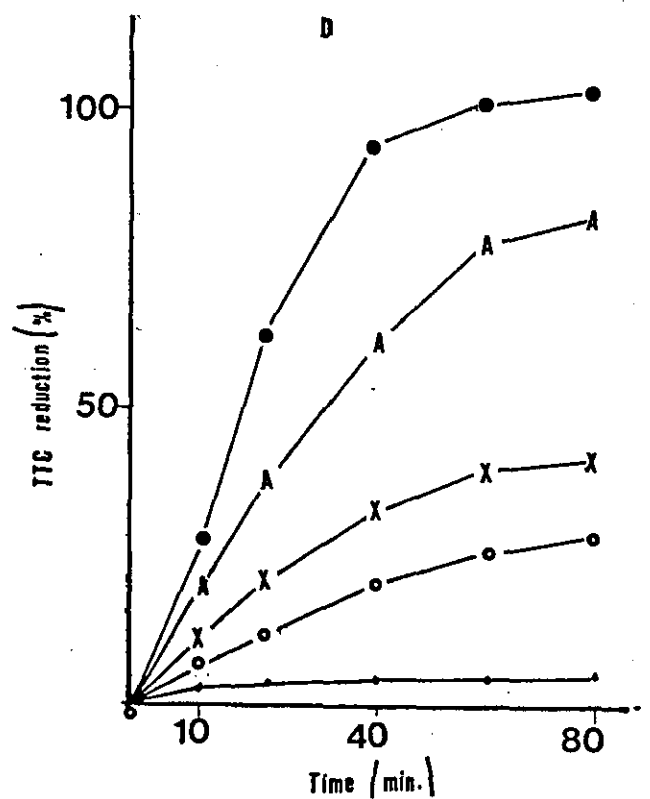
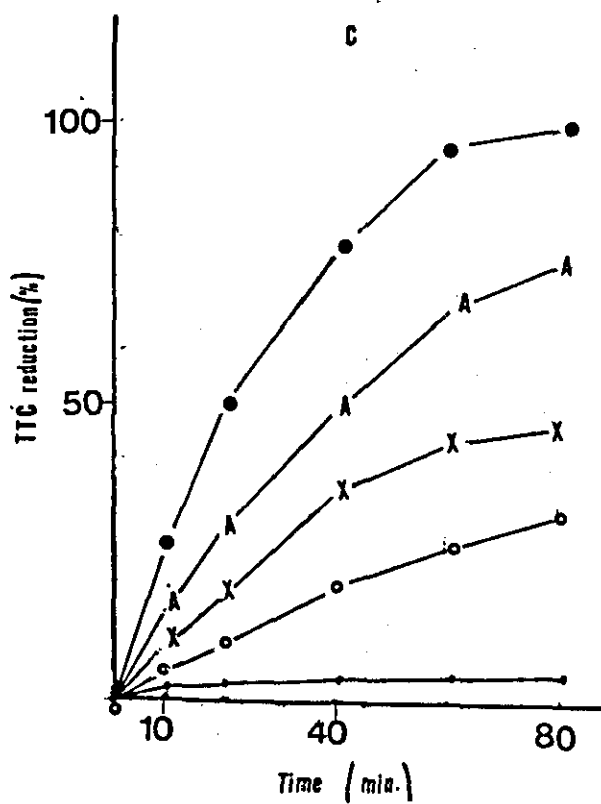
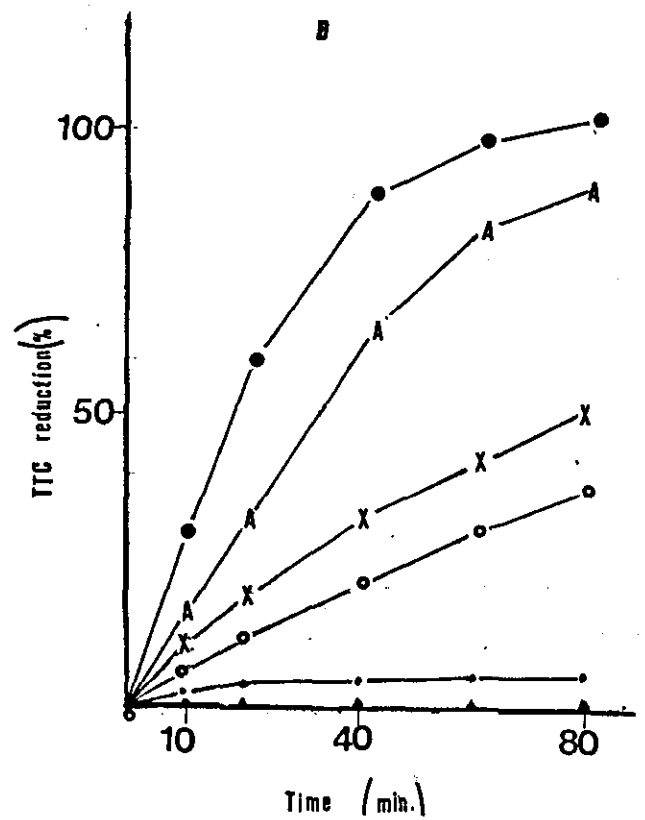
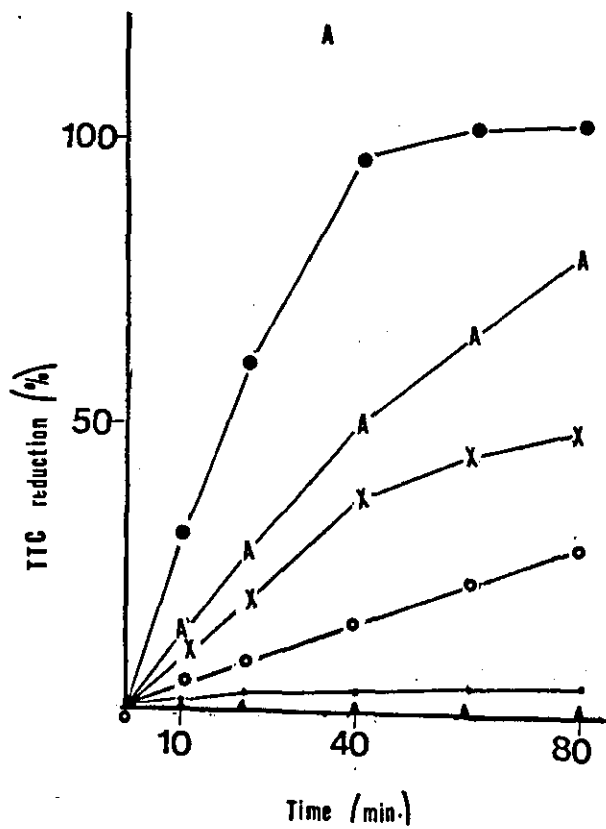


Fig. 3.50: TTC reduction by cells of C.albicans suspended in CFM containing (A) Propranolol, (B) Oxprenolol, (C) Atenolol and (D) Practolol at 30°C. Cell concentration 3.8×10^5 cells.ml⁻¹. Added substrate concentration 0.02m lactate.

(A) Propranolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.15
 x-x 0.35
 O-O 0.75
 ▲-▲ 5.0
 ... Endogenous respiration

(B) Oxprenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 0.35
 x-x 1.00
 O-O 2.00
 ▲-▲ 7.5
 ... Endogenous respiration

(C) Atenolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

(D) Practolol (mg.ml⁻¹)

●-● 0.0
 Δ-Δ 4.0
 x-x 12.0
 O-O 30.0
 ... Endogenous respiration

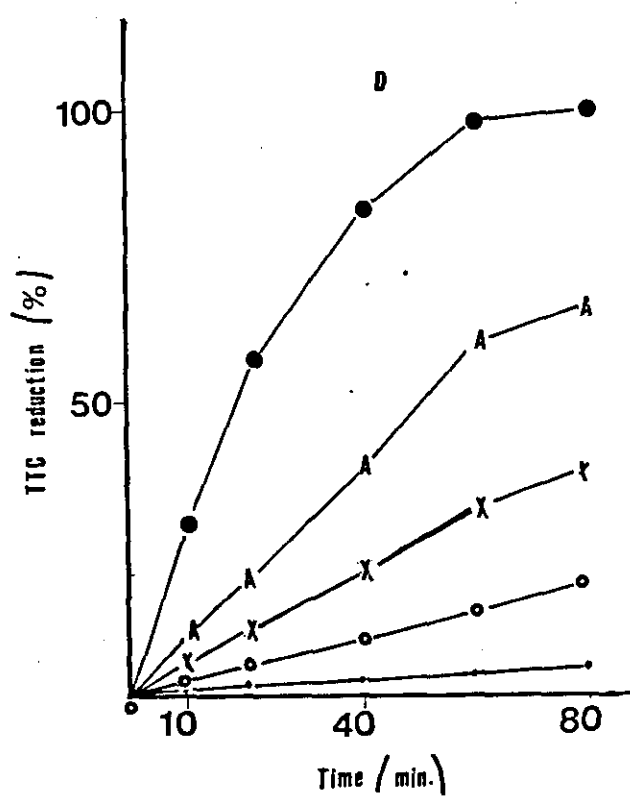
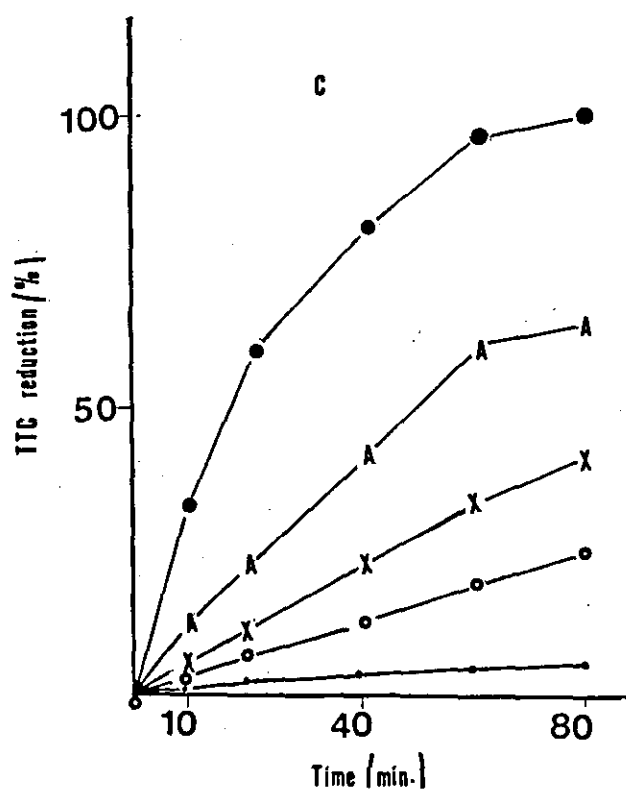
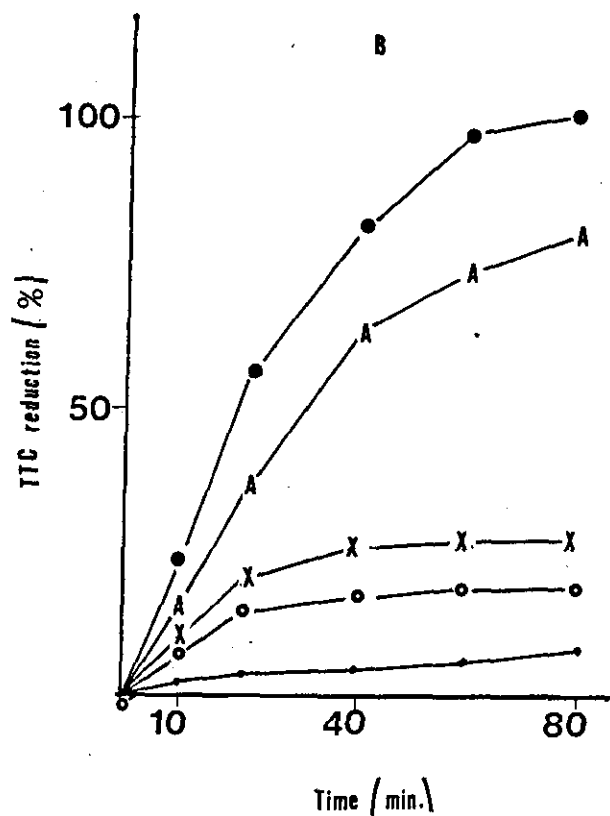
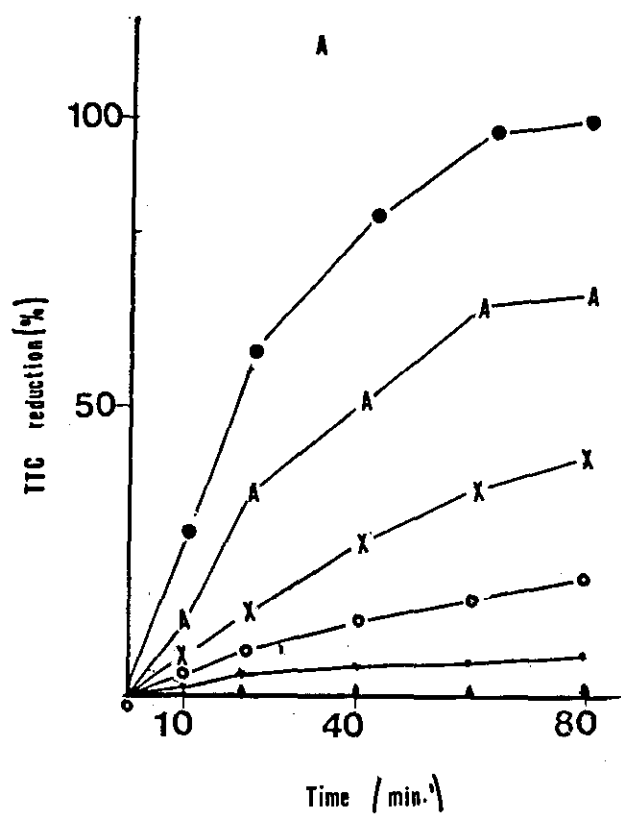
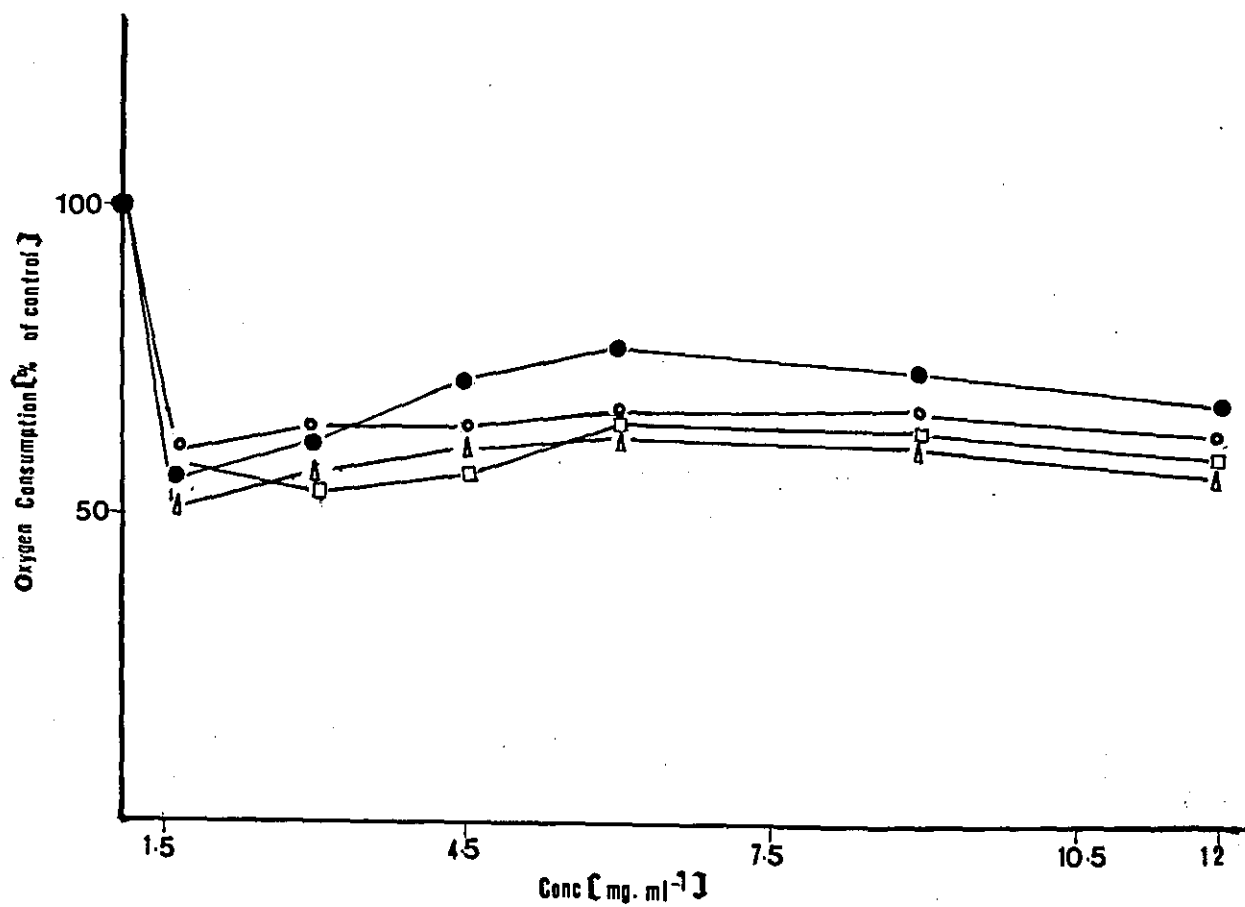
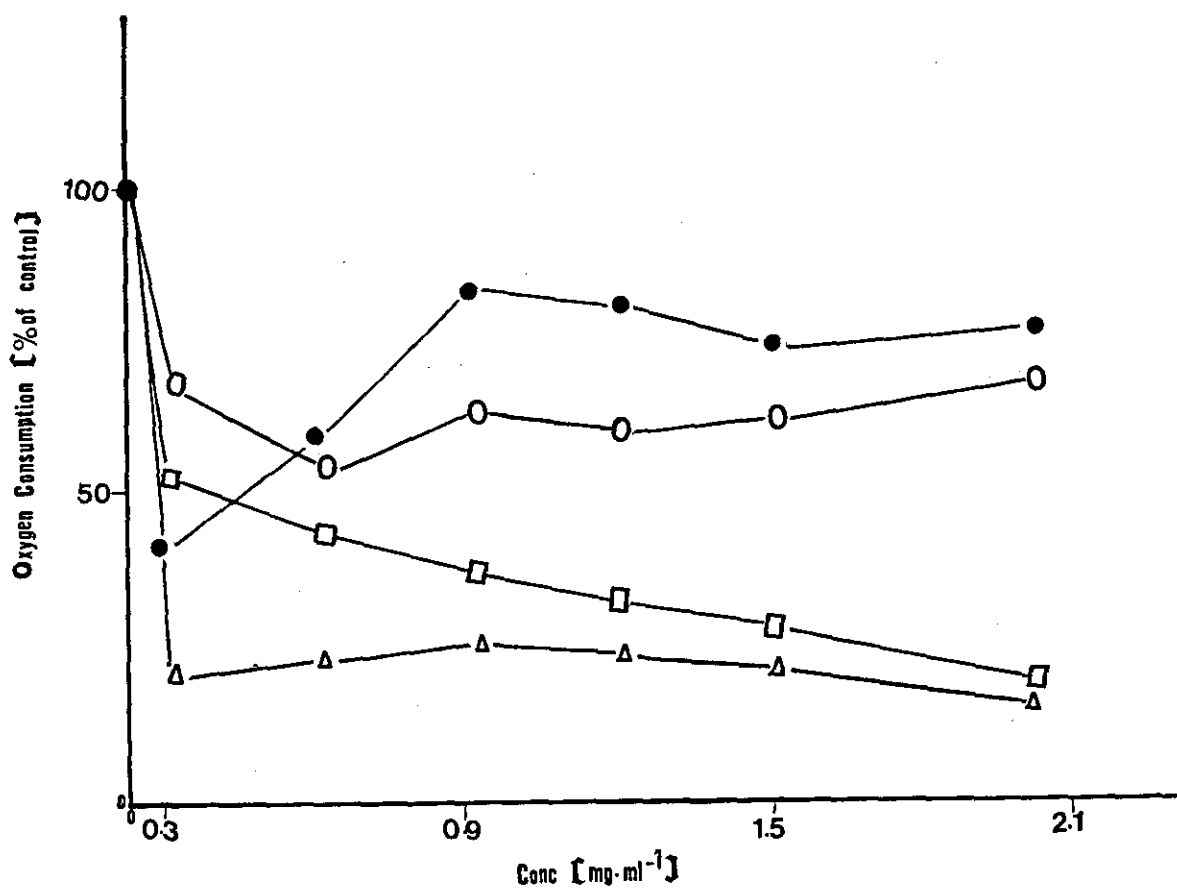


Fig. 3.51: The effect of Propranolol on the oxygen consumption of E.coli suspended in CFM at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □-□; succinate, Δ-Δ; Malate, ●-●; Lactate O-O.

Fig. 3.52: The effect of Metoprolol on the oxygen consumption of E.coli suspended in CFM at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □ □; succinate, Δ-Δ; Malate, ●-●; Lactate, O-O.



A. Fig. 3.53: The effect of Atenolol on the oxygen consumption of E.coli suspended in CFM at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □ □; Succinate, Δ-Δ; Malate, ●-●; Lactate, O-O.

B. Fig. 3.54: The effect of Dinitrophenol on the oxygen consumption of E.coli suspended in CFM at 37°C. Cell concentration 4.0×10^9 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □ □; Succinate, Δ Δ; Malate ●-●; Lactate O-O.

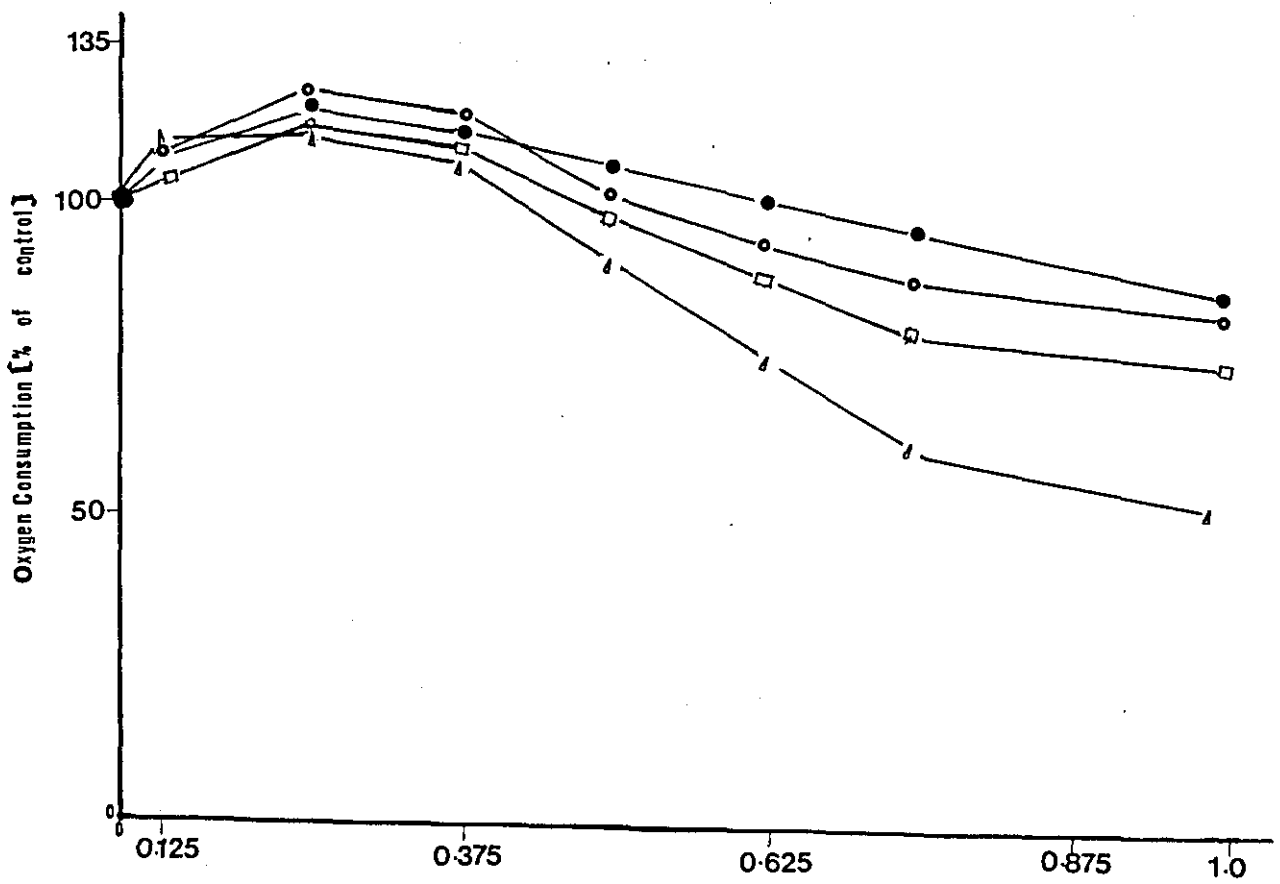
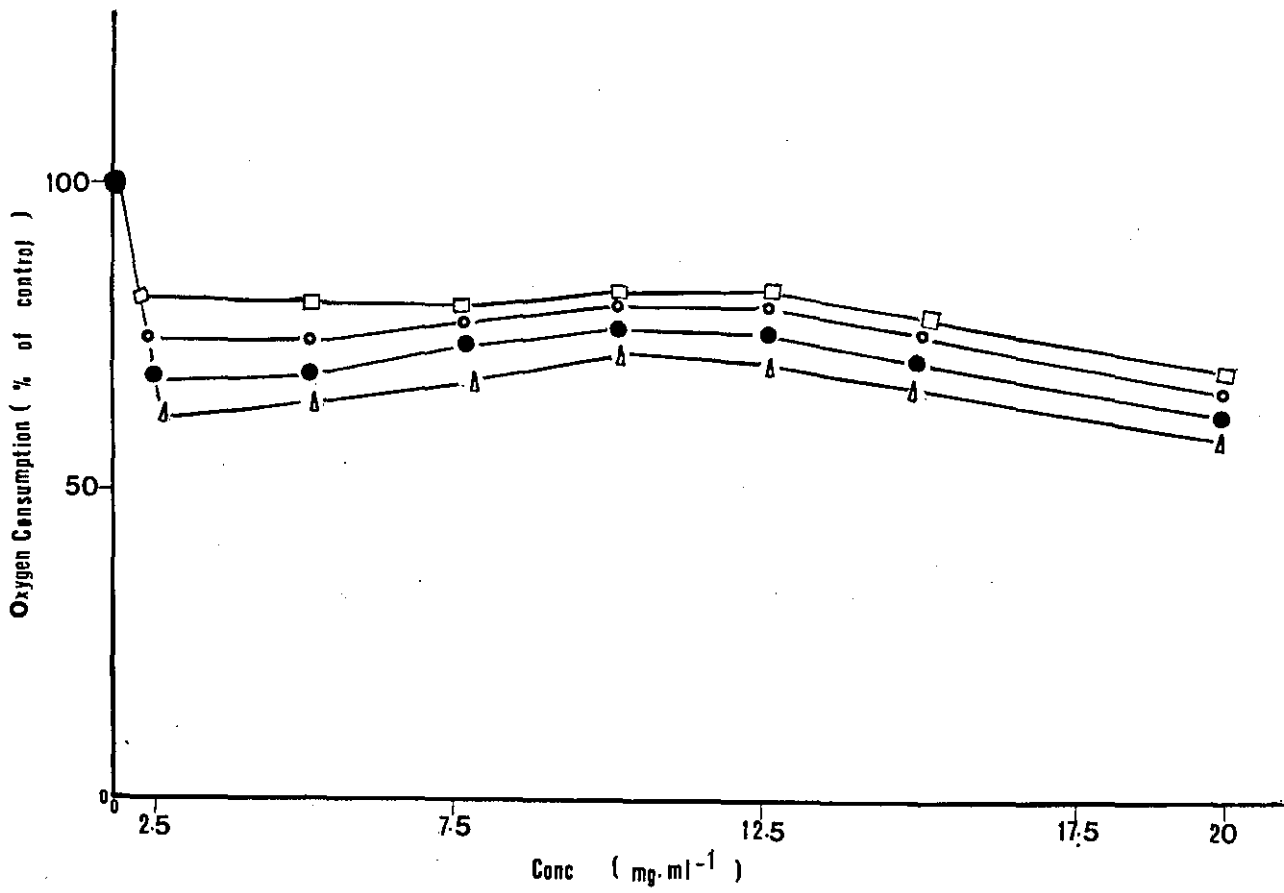
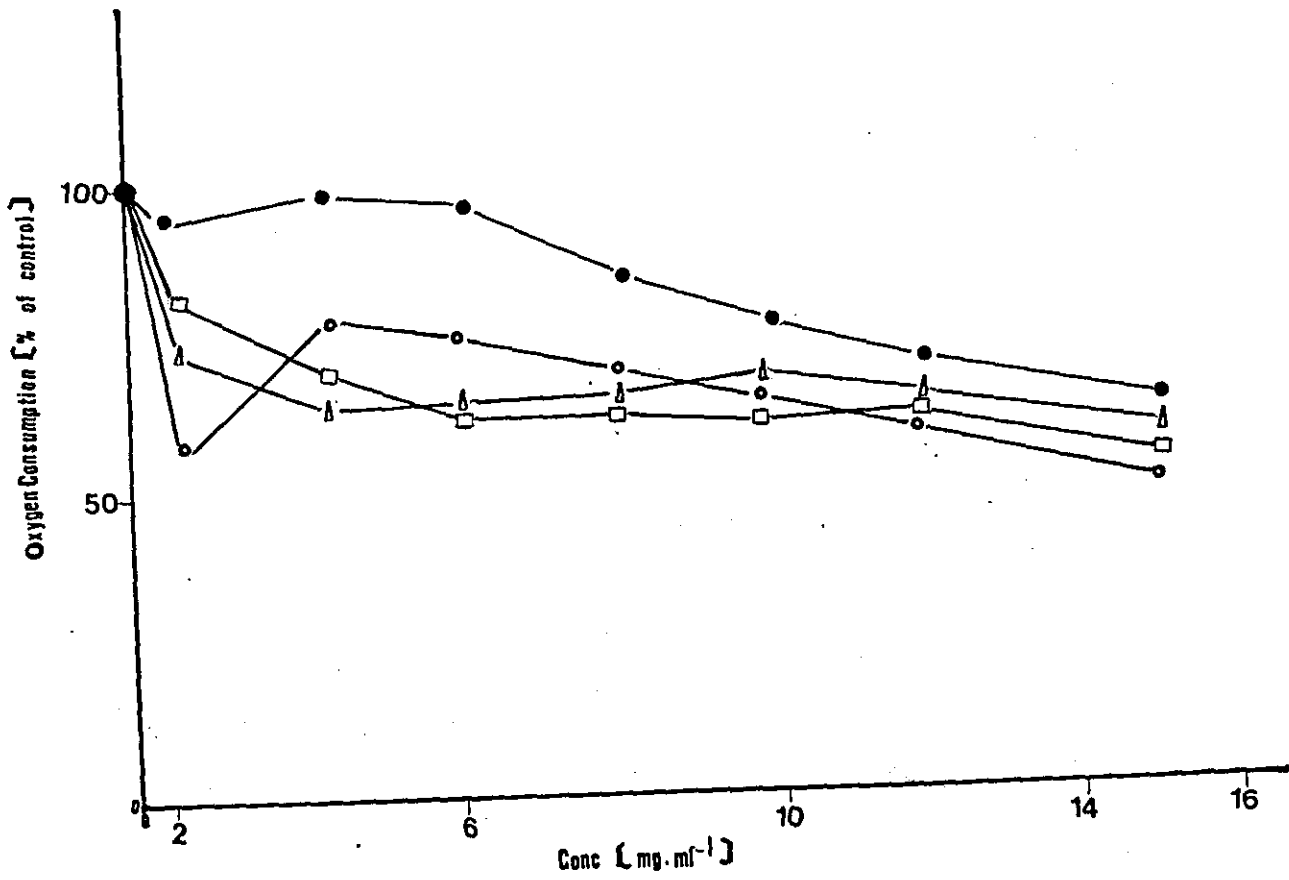
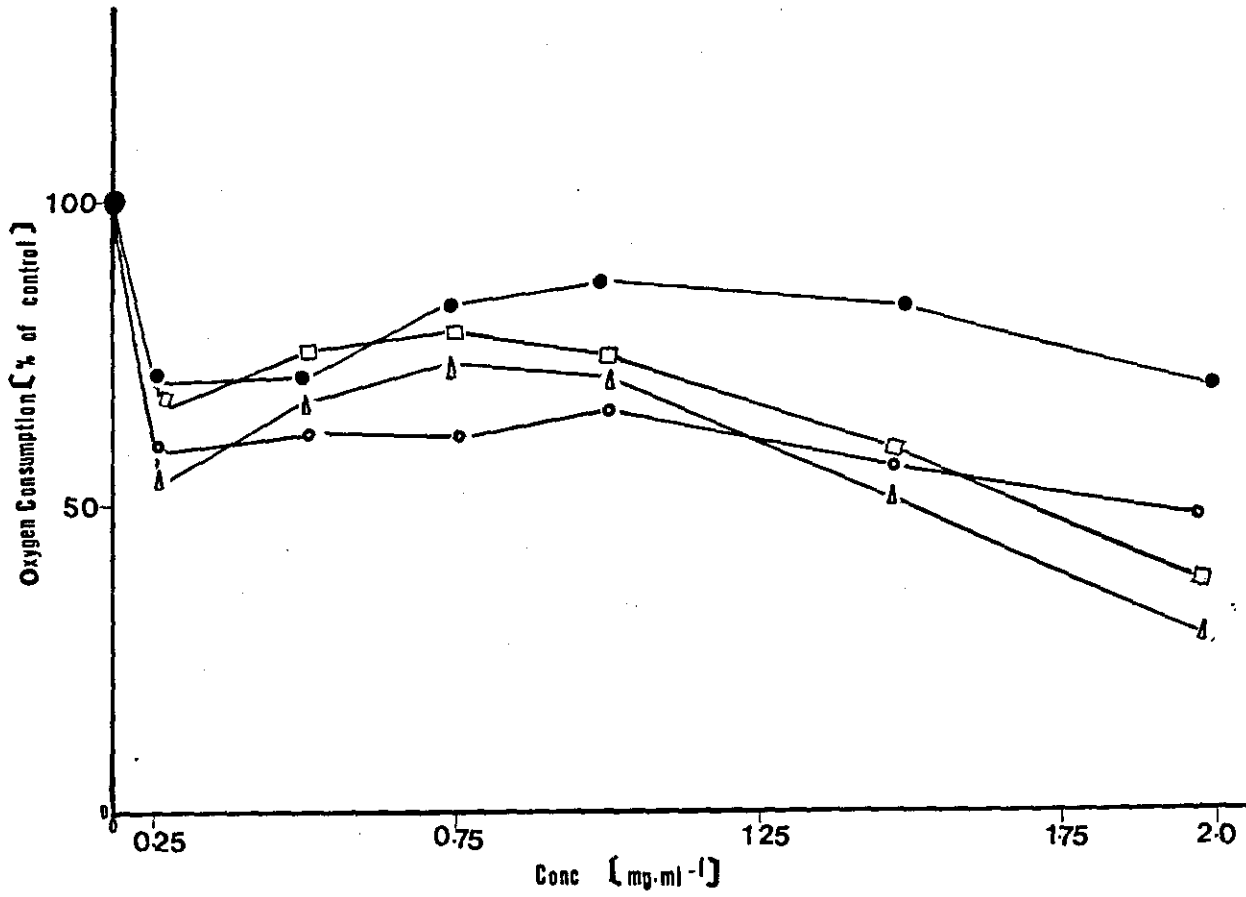


Fig. 3.55: The effect of Propranolol on the oxygen consumption of C.albicans suspended in CFM at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □ □; Succinate, Δ Δ; Malate, ●-●; Lactate, O-O.

Fig. 3.56: The effect of Metoprolol on the oxygen consumption of C.albicans suspended in CFM at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □ □; Succinate, Δ-Δ; Malate, ●-●; Lactate, O-O.



A. Fig. 3.57: The effect of Atenolol on the oxygen consumption of C.albicans suspended in CFM at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2m; Glucose, □ □; Succinate, Δ-Δ; Malate, ●-●; Lactate, O-O.

B. Fig. 3.58: The effect of DNP on the oxygen consumption of C.albicans suspended in CFM at 30°C. Cell concentration 3.2×10^5 cells.ml⁻¹. Contact time 10 min.

Substrate 0.2M; Glucose, □ □; Succinate, Δ Δ; Malate, ●-●; Lactate, O-O.

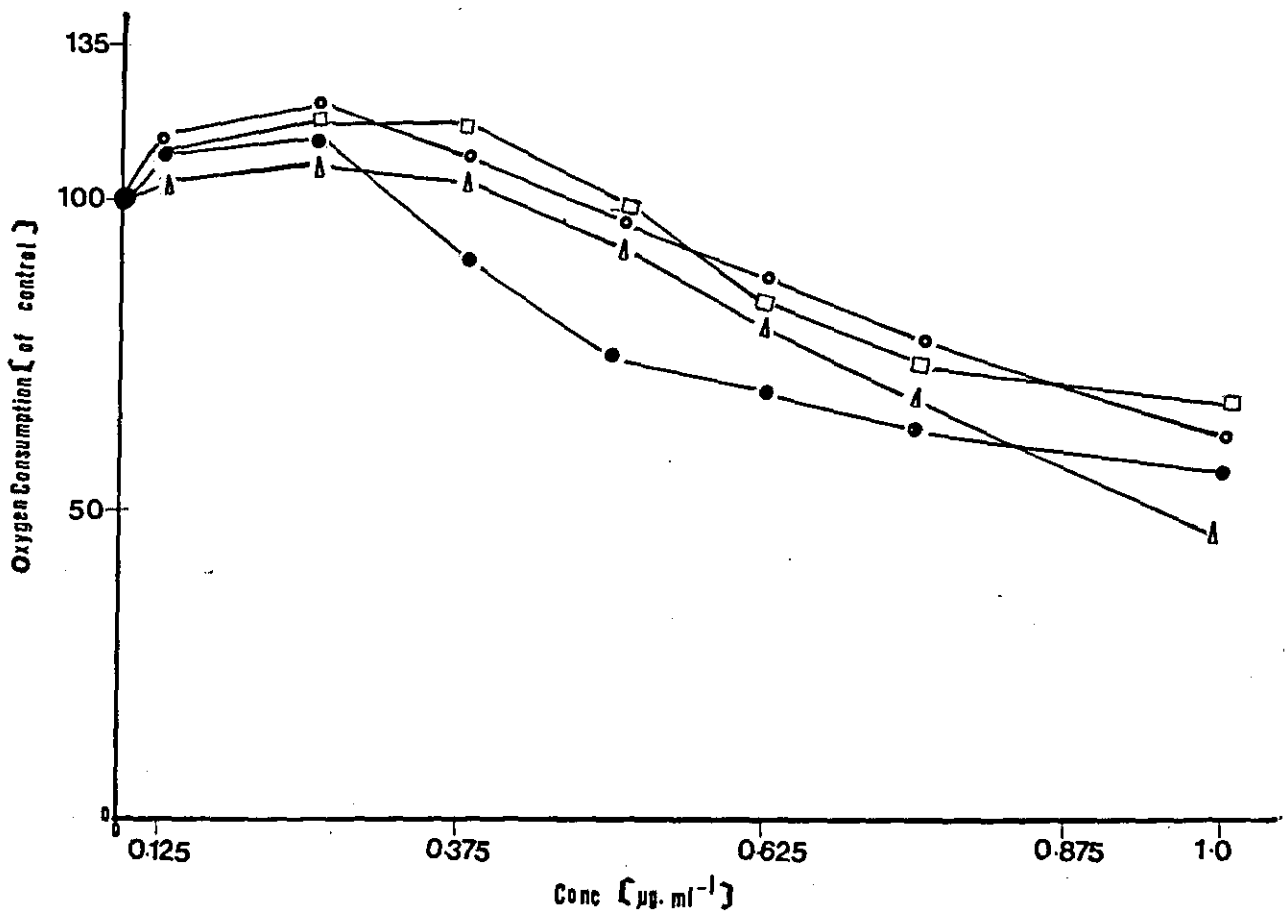
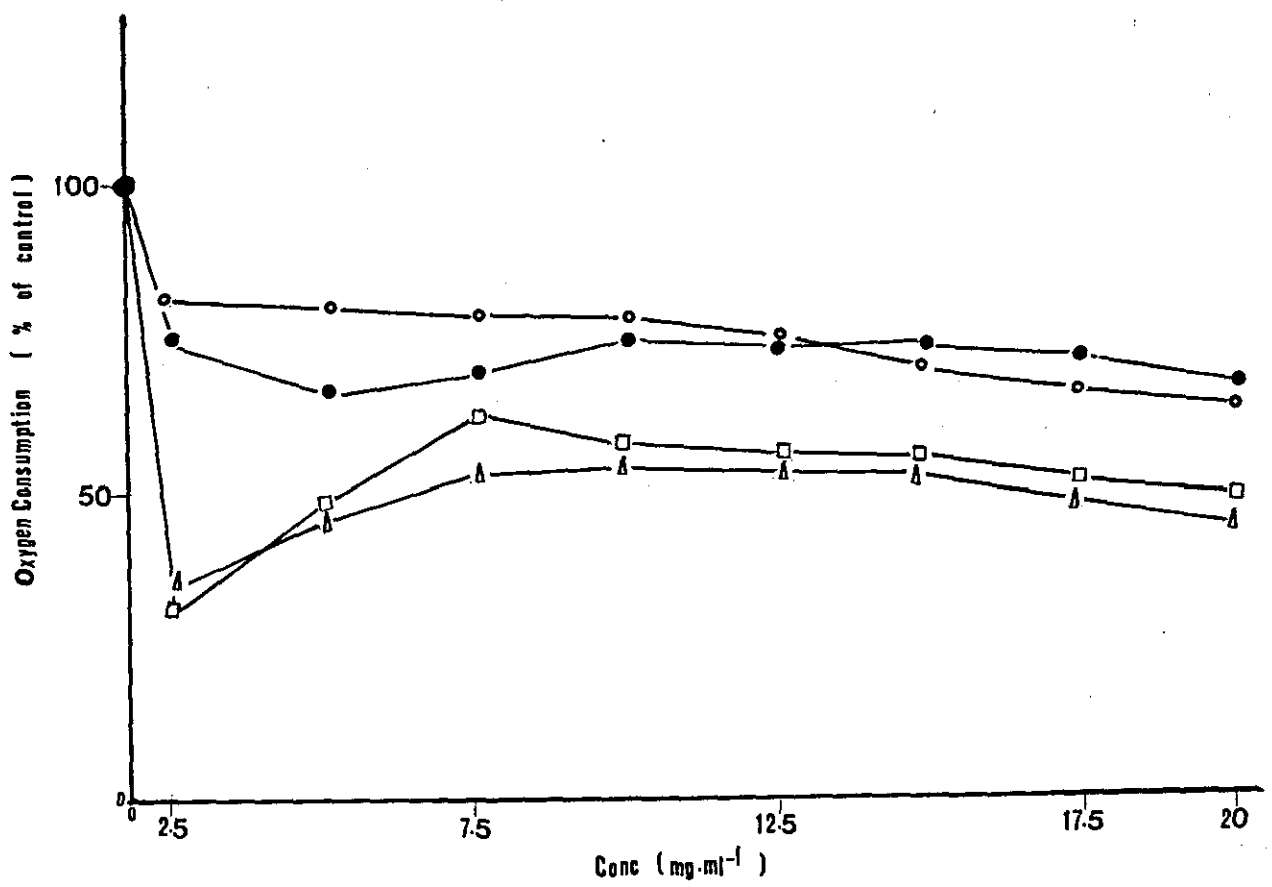


Fig. 3.59: The effect of time on the influence of β -blockers and DNP on the oxygen consumption of cells of E.coli and C.albicans suspended in CFM, with 0.2M glucose as added substrate.

(A) E.Coli

Cell concentration - 4.2×10^9 cells.ml⁻¹

Temperature 37°C

Drug Concentration
(mg.ml⁻¹)

□-□ Atenolol	15.0
O-O Metoprolol	10.0
▲-▲ Propranolol	2.0
⊗-⊗ DNP	0.50

(B) C.albicans

Cells concentration - 3.8×10^9 cells.ml⁻¹

Temperature 30°C

Drug Concentration
(mg.ml⁻¹)

■-■ Atenolol	10.0
x-x Metoprolol	8.0
Δ-Δ Propranolol	1.0
●-● DNP	0.5

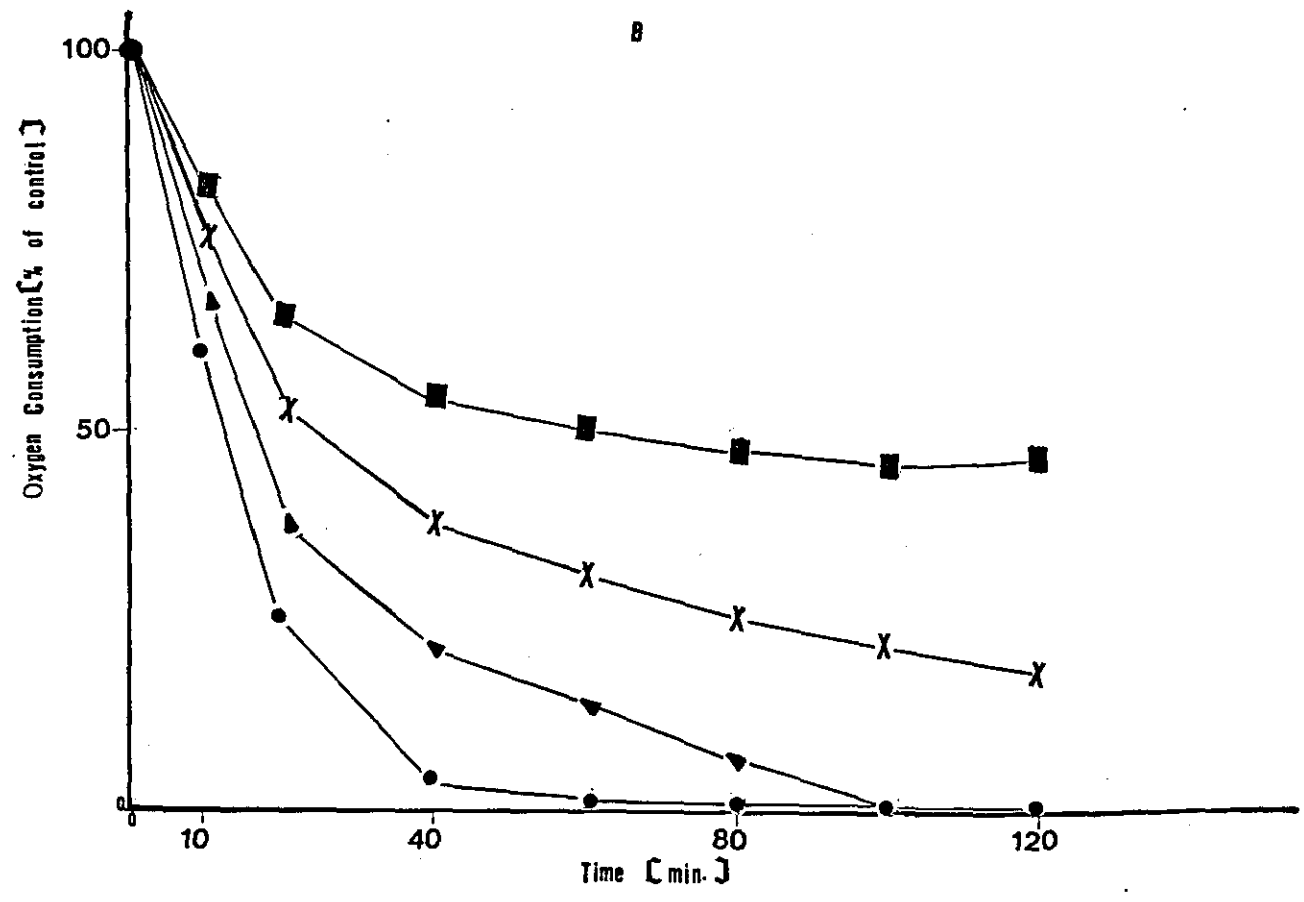
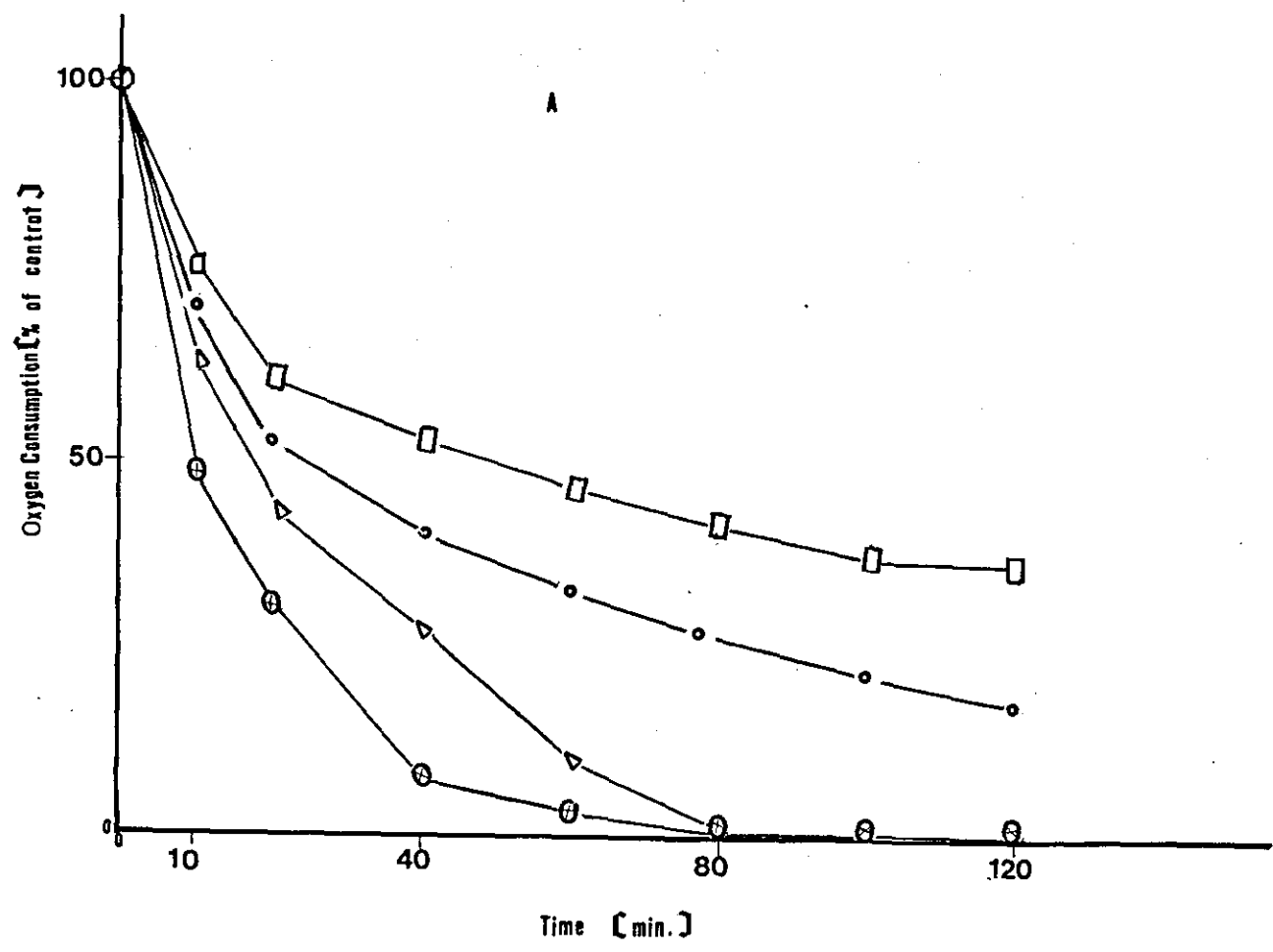


Fig. 3.60: The effect of β -blockers on the uptake of ^{14}C -glucose by cells of (A) E.coli and (B) C.albicans suspended in CFM.

T_1 = Time for control to reach pre-fixed CPM

T_2 = Time for treated cultures (Practolol or Propranolol) to reach same CPM.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

Temperature 37°C

Drug concentration (mg.ml⁻¹)

⊗-⊗ 0.0 control
o-o 3.5 Practolol
●-● 0.25 Propranolol

(B)

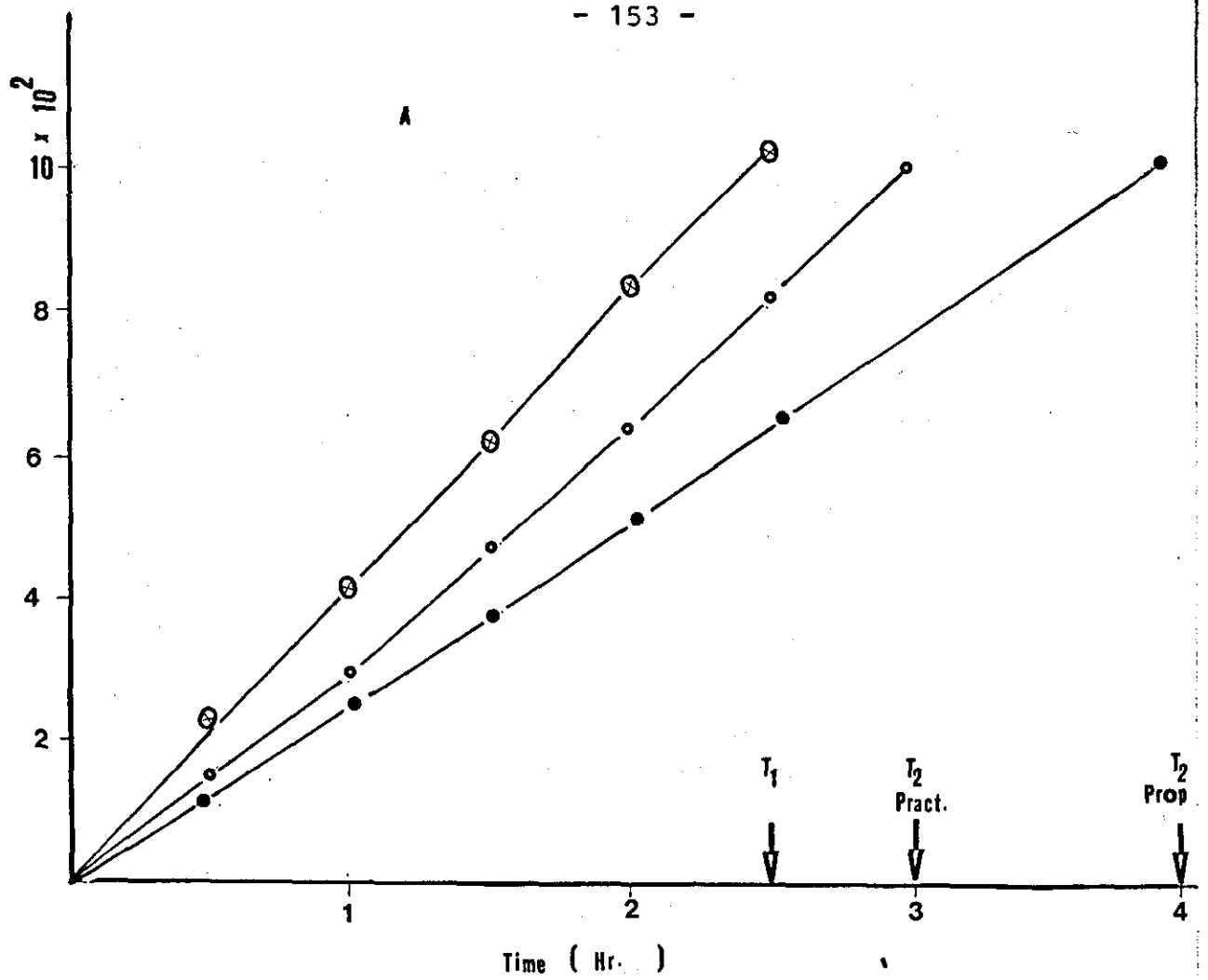
Cell concentration 3.8×10^5 cells.ml⁻¹

Temperature 30°C

Drug concentration (mg.ml⁻¹)

□-□ 0.0 control
x-x 2.0 Practolol
Δ-Δ 0.15 Propranolol

CPM / ml of cells



CPM / ml of cells

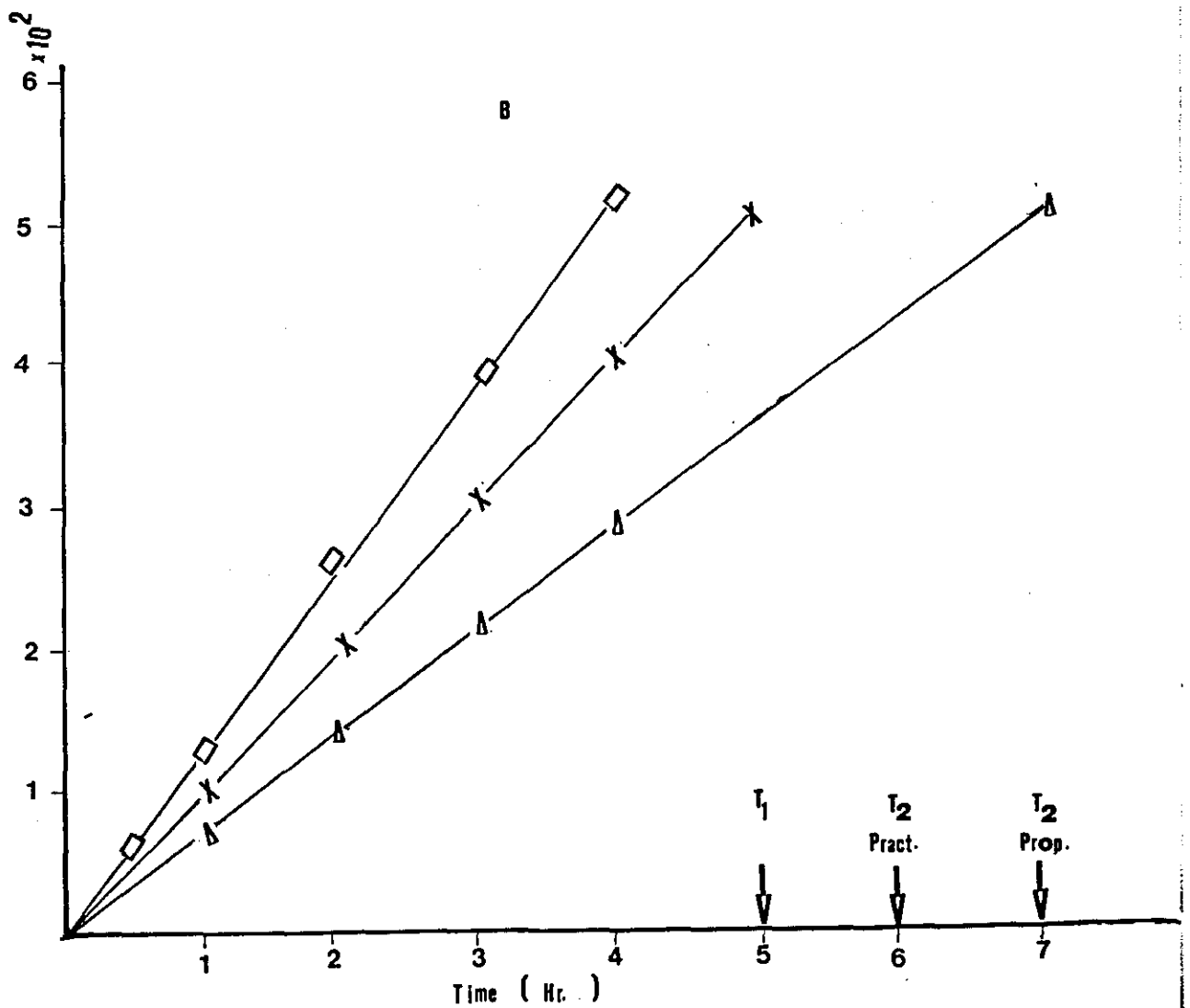


Fig. 3.61: The effect of β -blockers on the uptake of ^{14}C -mannose by cells of (A) E.coli and (B) C.albicans suspended in growth medium.

T_1 = Time for control to reach pre-fixed CPM

T_2 = Time for treated cultures (Practolol or Propranolol) to reach same CPM.

(A)

Cell concentration 4.2×10^9 cells.ml $^{-1}$

Temperature 37°C

Drug concentration (mg.ml $^{-1}$)

⊗-⊗ 0.0 (control)

O-O 3.5 (Practolol)

●-● 0.25 (Propranolol)

(B)

Cell concentration 3.8×10^5 cells.ml $^{-1}$

Temperature 30°C

Drug concentration (mg.ml $^{-1}$)

□ □ 0.0 (control)

x-x 2.0 (Practolol)

Δ-Δ 0.15 (Propranolol)

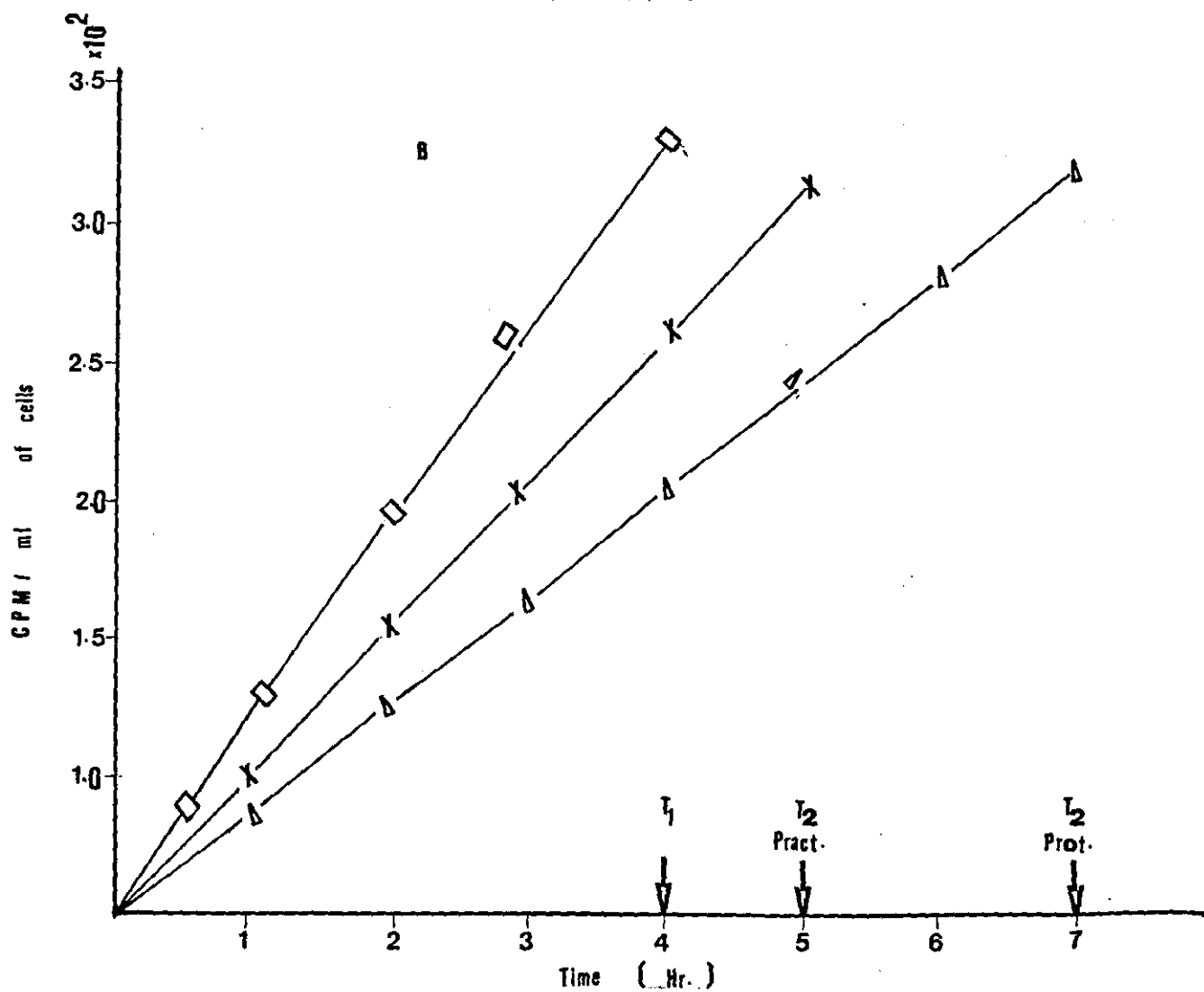
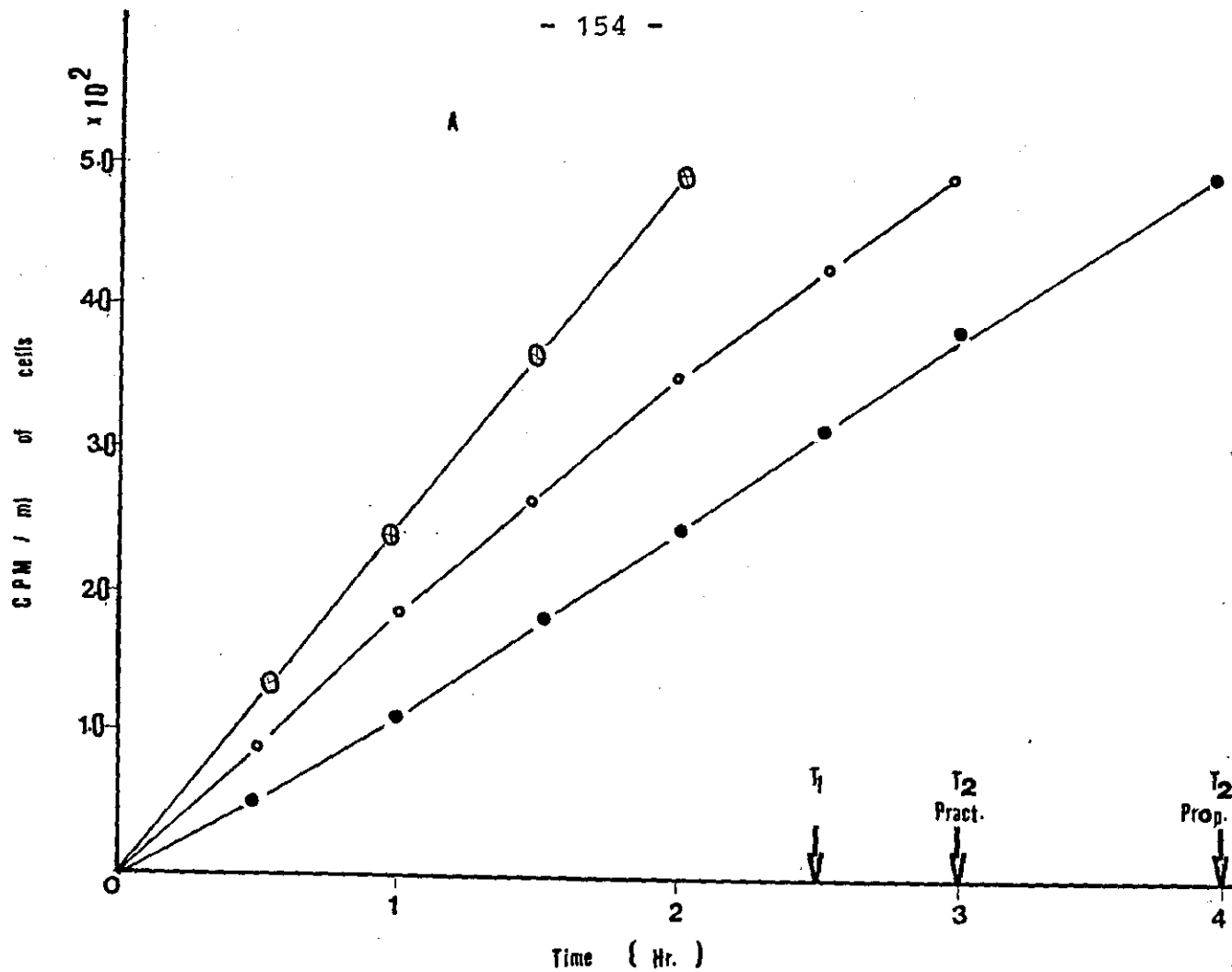


Fig. 3.62: Pentose release from cells of E.coli suspended in CFM at 22°C after treatment with different concentrations of β -blockers. Cell concentrations 4.2×10^9 cells.ml⁻¹.

(A)

o-o Oxprenolol

▲-▲ Atenolol

--- amount of pentose released from cells after boiling for 5 min.

(B)

●-● Propranolol

Δ-Δ Practolol

--- amount of pentose released from cells after boiling for 5 min.

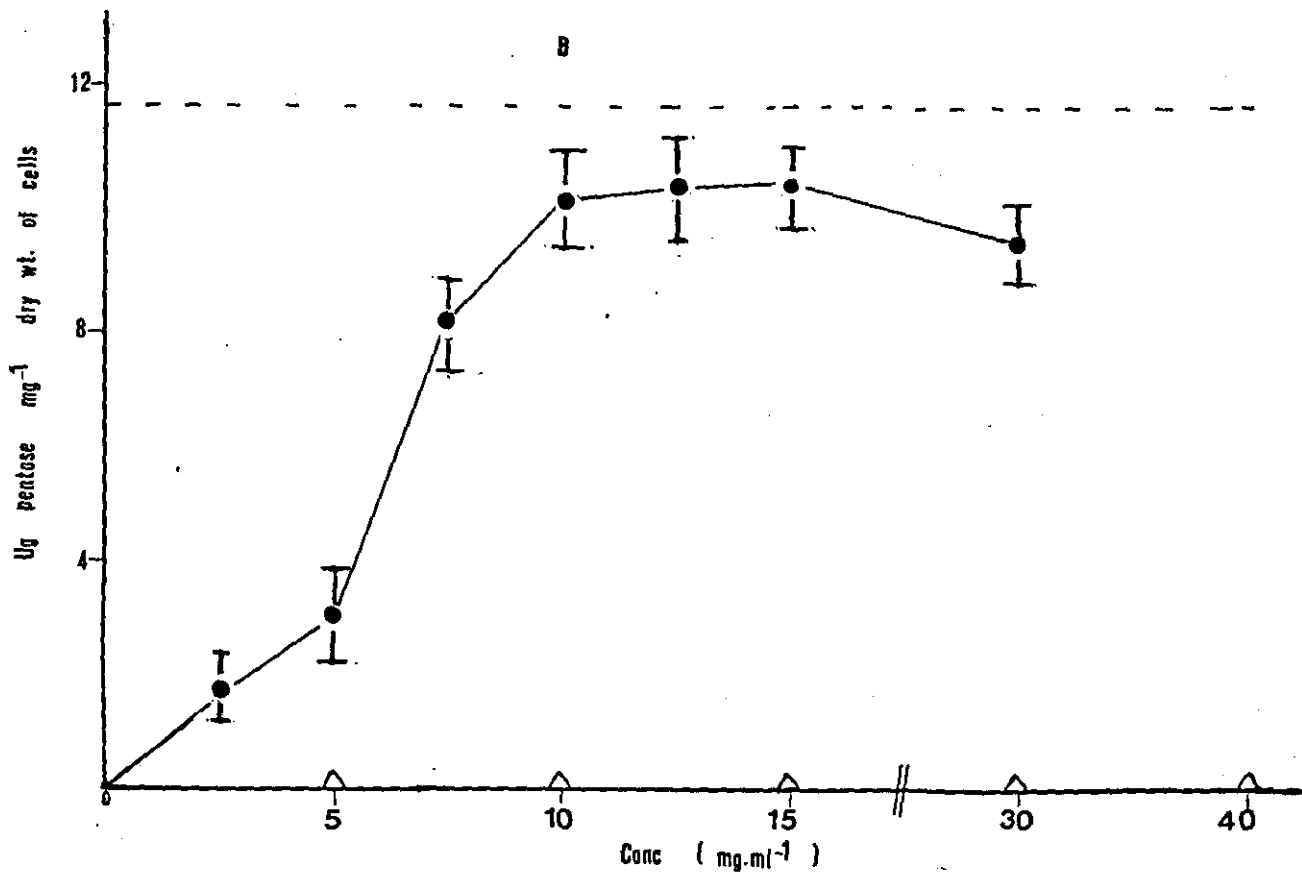
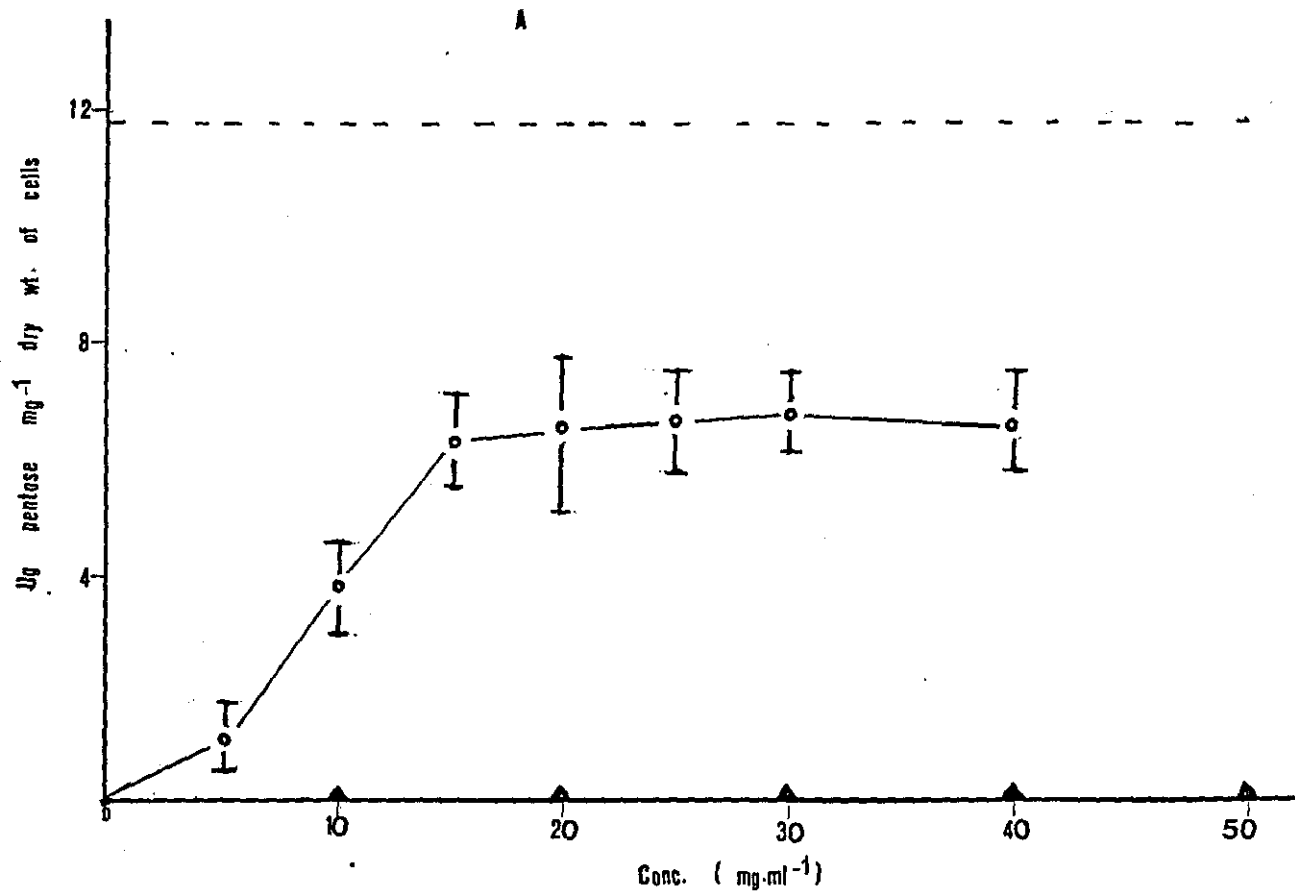


Fig. 3.63: Pentose release from cells of C.albicans suspended in CFM at 22°C after treatment with different concentrations of β -blockers. Cell concentration 3.8×10^5 cells.ml⁻¹. Contact time 15 min.

(A)

O-O Oxprenolol

▲-▲ Atenolol

--- amount of pentose released from cells after boiling for 5 min.

(B)

●-● Propranolol

Δ-Δ Proctolol

--- amount of pentose released from cells after boiling for 5 min.

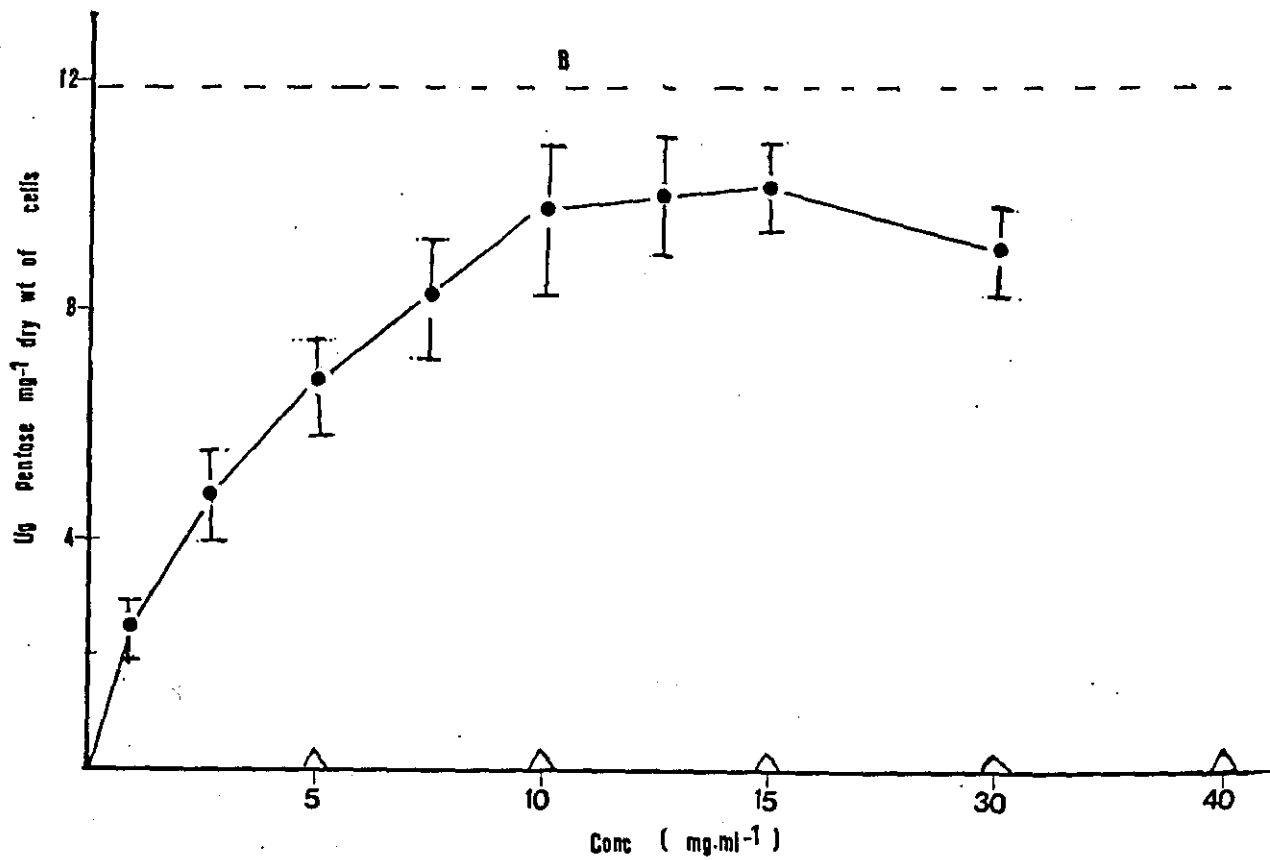
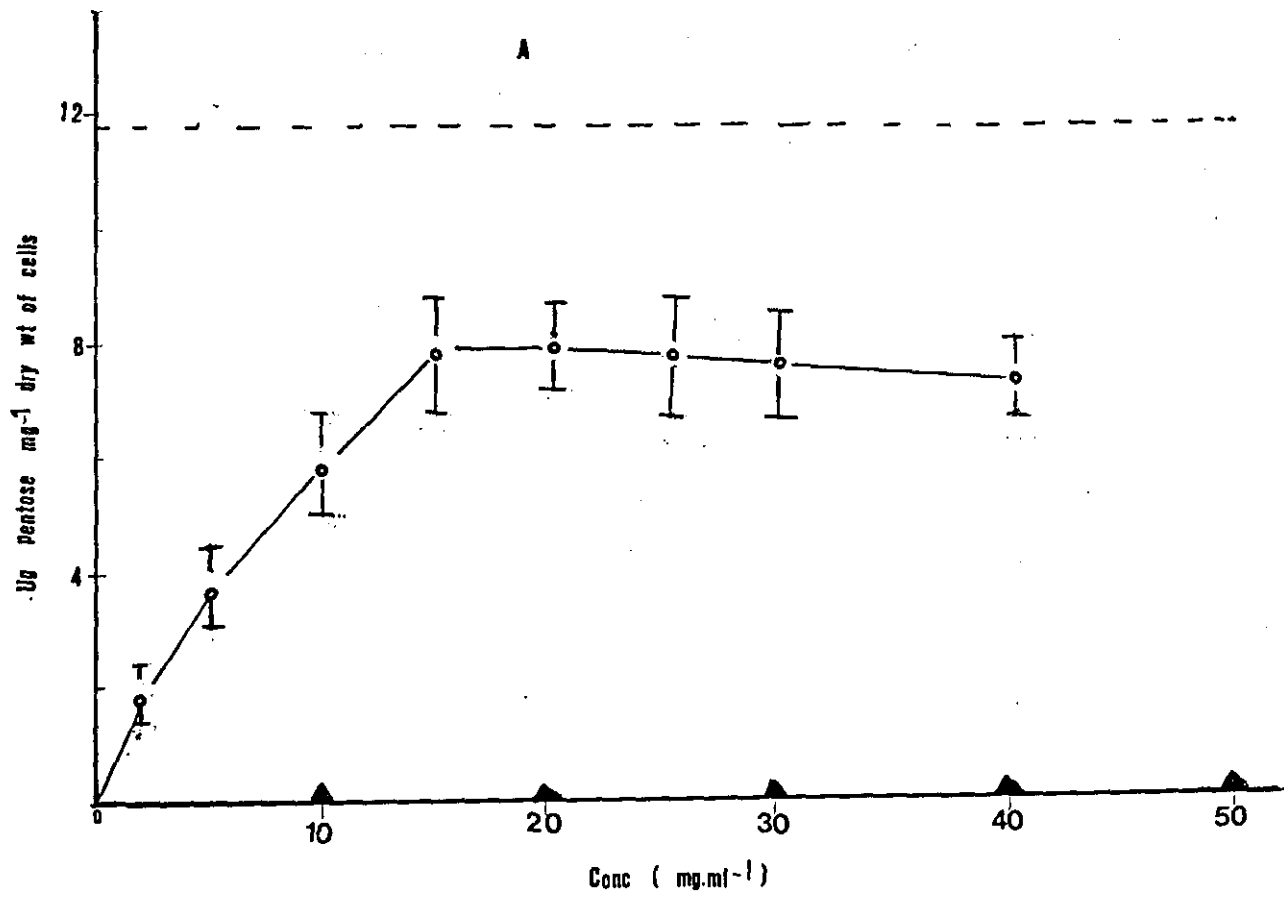


Fig. 3.64: Pentose release from cells of E.coli suspended in CFM at 22°C after treatment with fixed concentrations of β -blockers. Cell concentration 4.2×10^9 cells.ml⁻¹.

(A)

▲-▲	5 mg.ml ⁻¹	Propranolol
○-○	10 mg.ml ⁻¹	Propranolol
⊗-⊗	20 mg.ml ⁻¹	Propranolol
□-□	35 mg.ml ⁻¹	Atenolol

(B)

Δ-Δ	10 mg.ml ⁻¹	Oxprenolol
x-x	20 mg.ml ⁻¹	Oxprenolol
●-●	25 mg.ml ⁻¹	Oxprenolol
□-□	35 mg.ml ⁻¹	Practolol

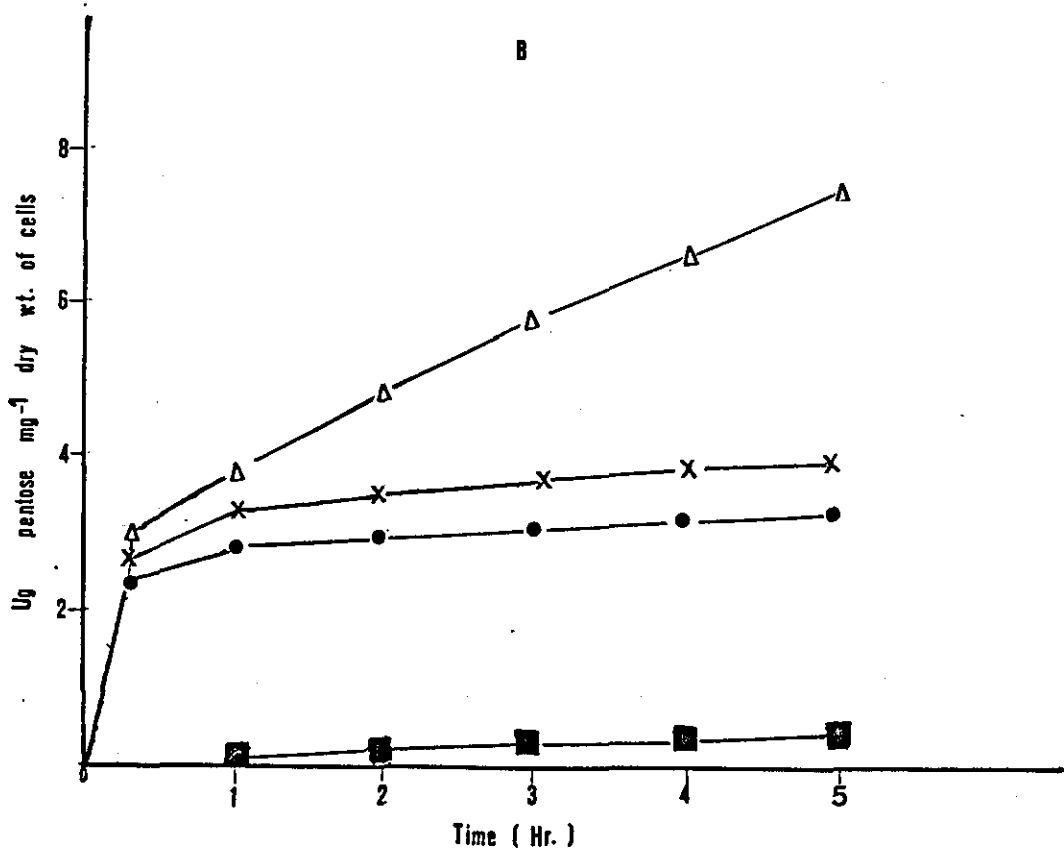
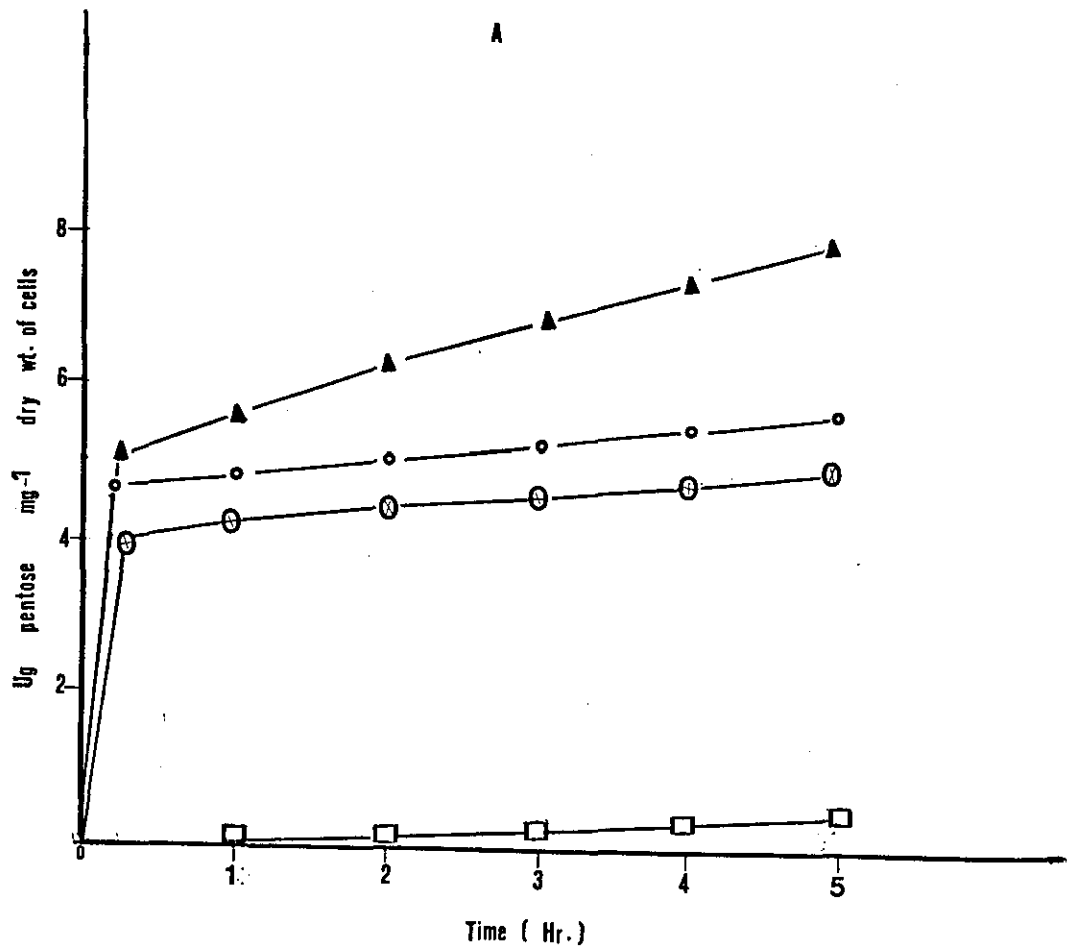


Fig. 3.65: Pentose release from cells of C.albicans suspended in CFM at 22°C after treatment with fixed concentrations of β -blockers. Cell concentration 3.8×10^5 cells.ml⁻¹.

(A)

▲-▲	5 mg.ml ⁻¹	Propranolol
O-O	10 mg.ml ⁻¹	Propranolol
⊗-⊗	20 mg.ml ⁻¹	Propranolol
□-□	35 mg.ml ⁻¹	Atenolol

(B)

Δ Δ	10 mg.ml ⁻¹	Oxprenolol
x-x	20 mg.ml ⁻¹	Oxprenolol
O-O	25 mg.ml ⁻¹	Oxprenolol
■-■	35 mg.ml ⁻¹	Practolol

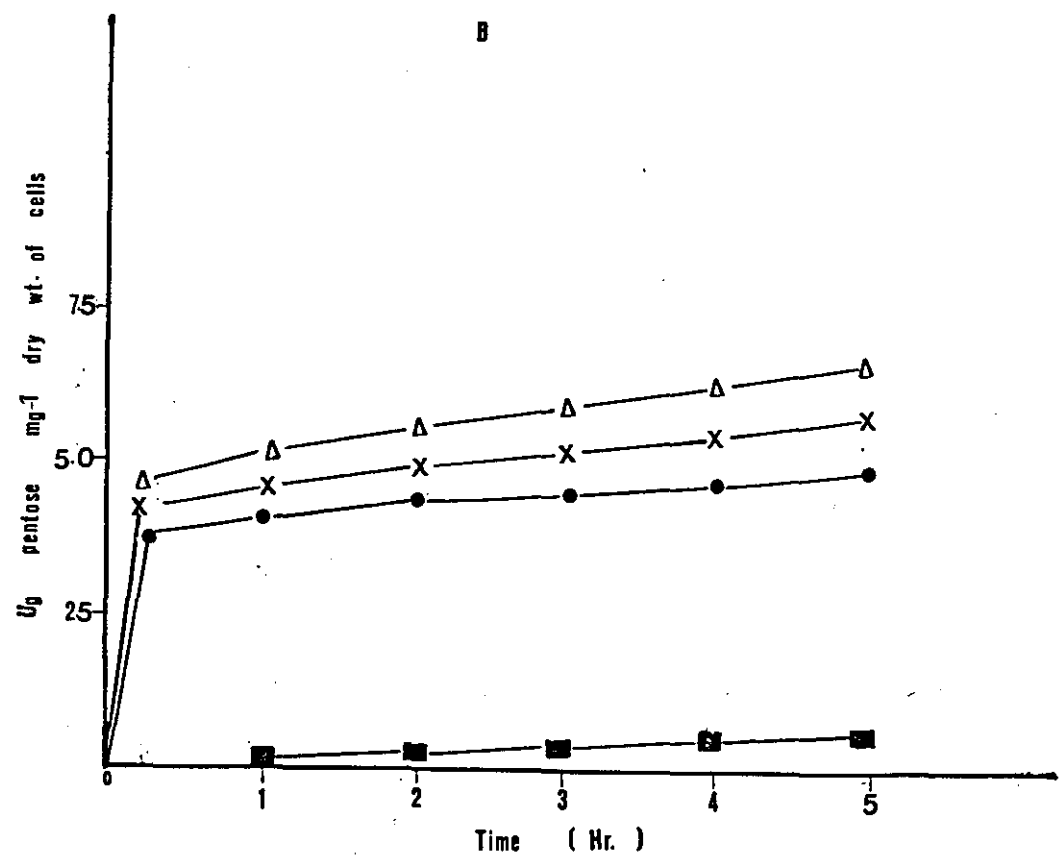
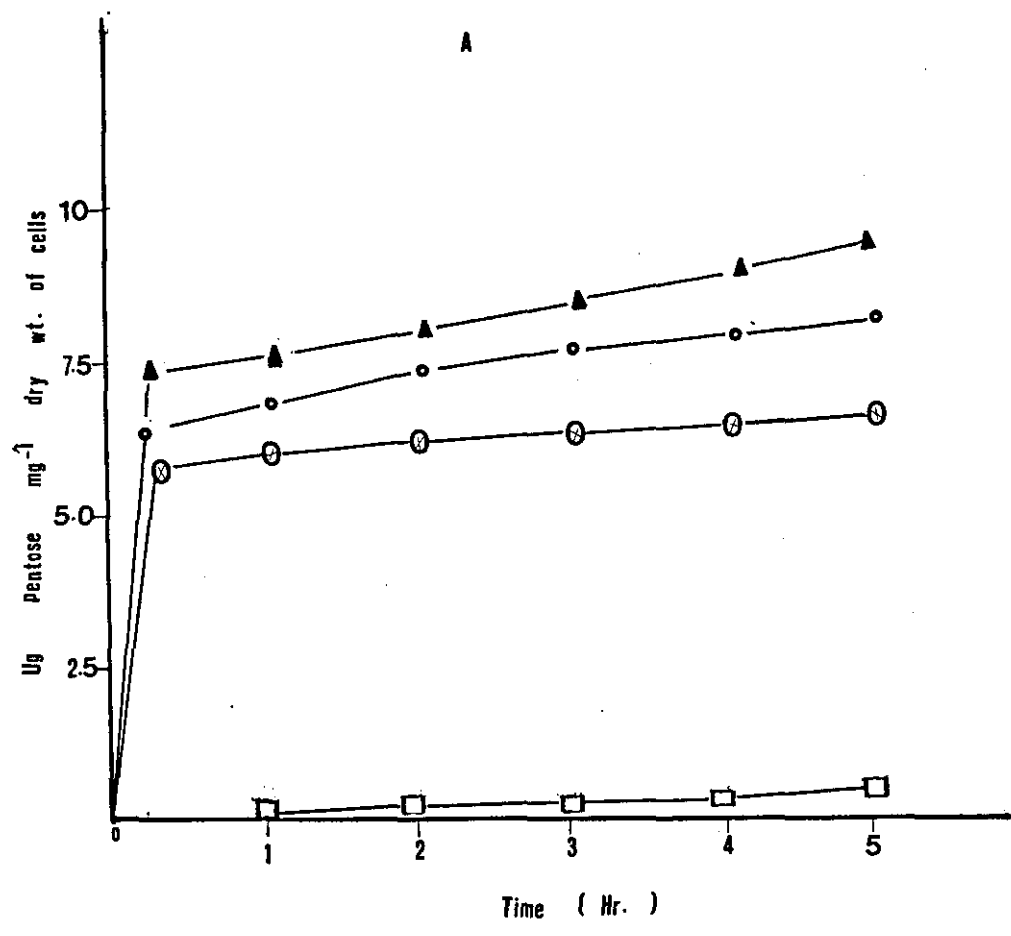


Fig. 3.66: Pentose release from cells of E.coli suspended in CFM at 0°C after treatment with fixed concentrations of β -blockers. Cell concentration 4.2×10^9 cells.ml⁻¹.

(A)

▲-▲ 5 mg.ml⁻¹ Propranolol
O-O 10 mg.ml⁻¹ Propranolol
⊗-⊗ 20 mg.ml⁻¹ Propranolol

(B)

Δ-Δ 1.0 mg.ml⁻¹ Oxprenolol
x-x 20 mg.ml⁻¹ Oxprenolol
●-● 25 mg.ml⁻¹ Oxprenolol

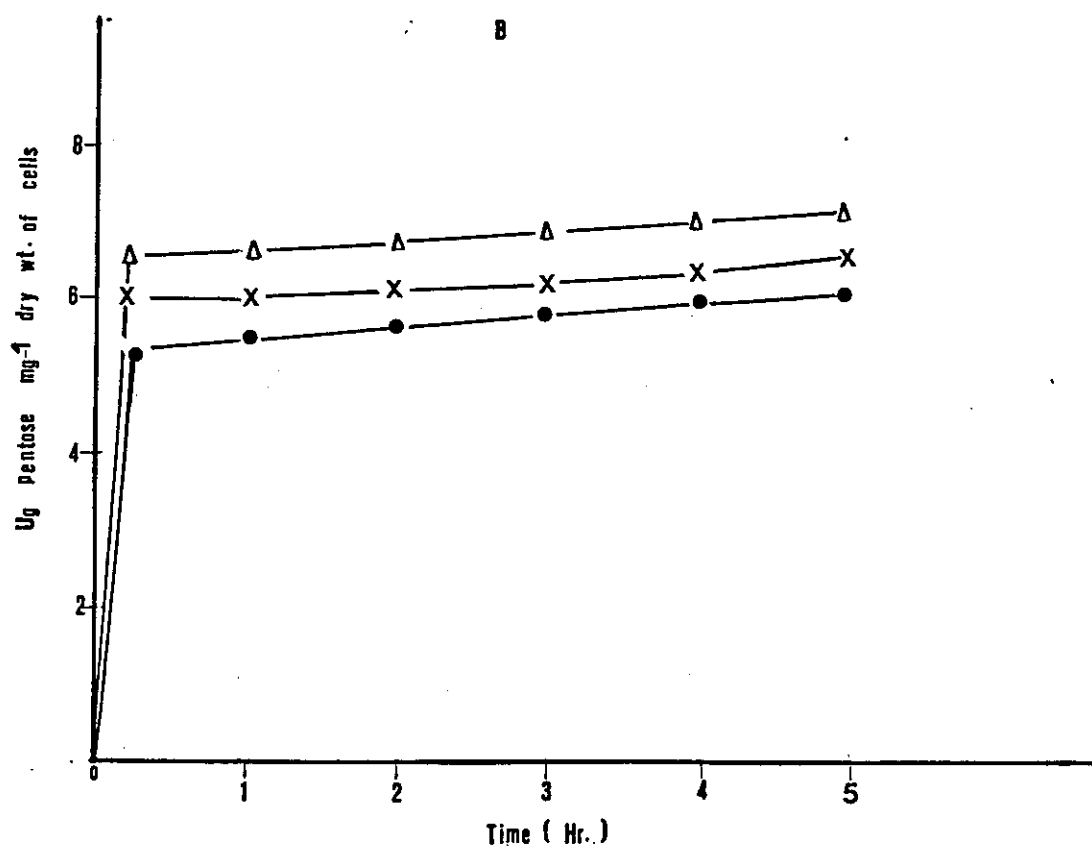
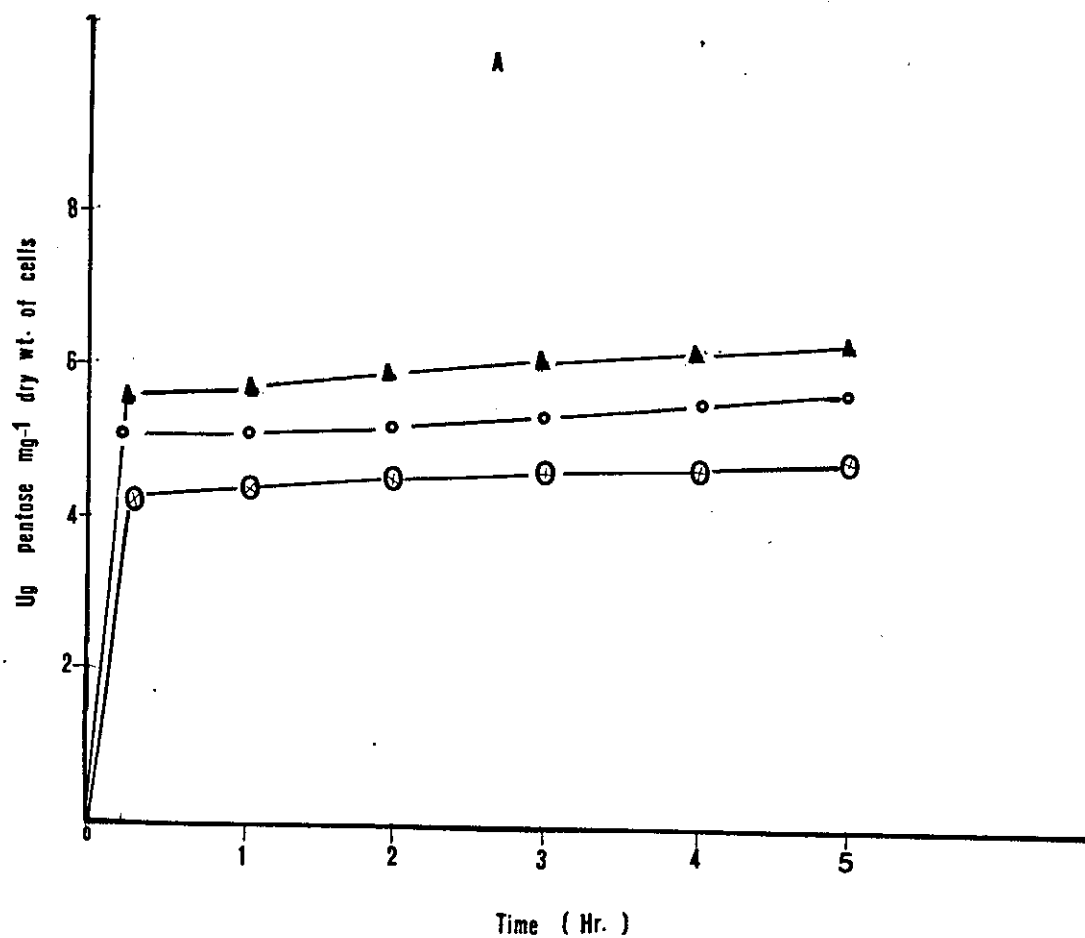


Fig. 3.67: Pentose release from cells of E.coli suspended in CFM initially at 0°C, for 1 hr and later at 37°C in the presence of fixed concentrations of β -blockers. Cell concentration time 4.2×10^9 cells.ml⁻¹.

(A)

O-O 5 mg.ml⁻¹ Propranolol

⊗-⊗ 10 mg.ml⁻¹ Propranolol

(B)

Δ-Δ 10 mg.ml⁻² Oxprenolol

●-● 20 mg.ml⁻¹ Oxprenolol

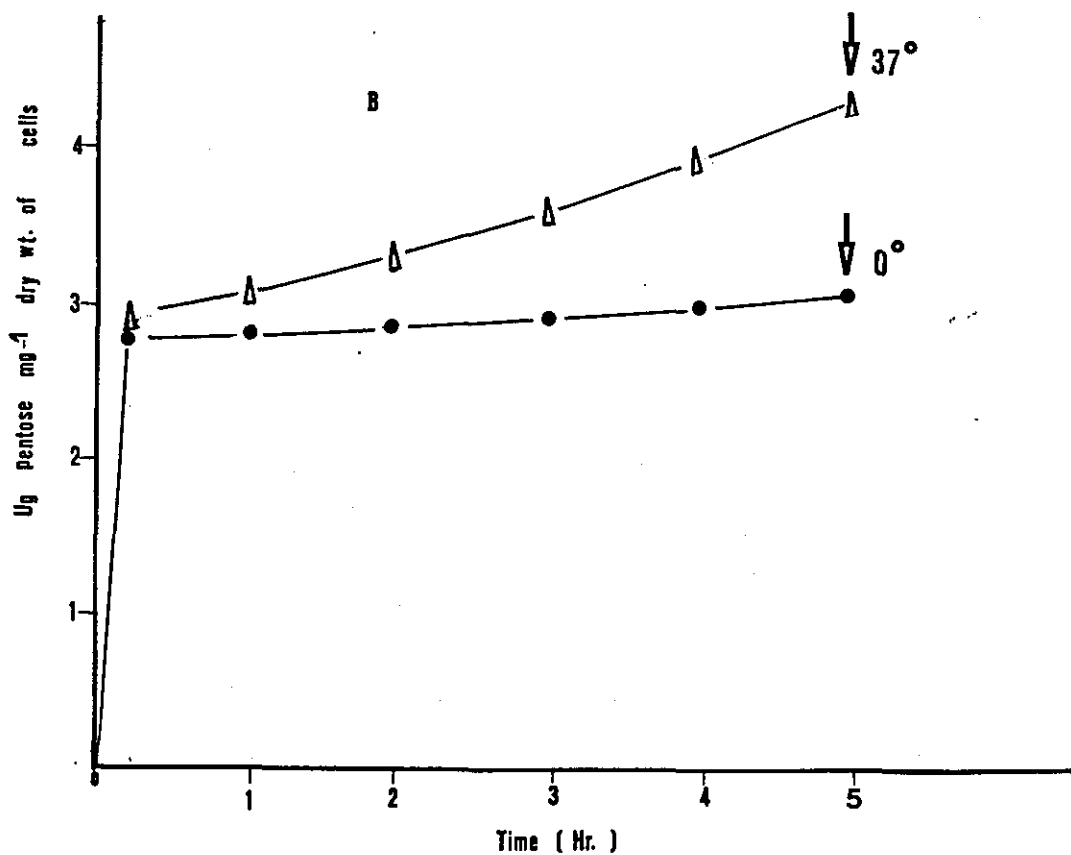
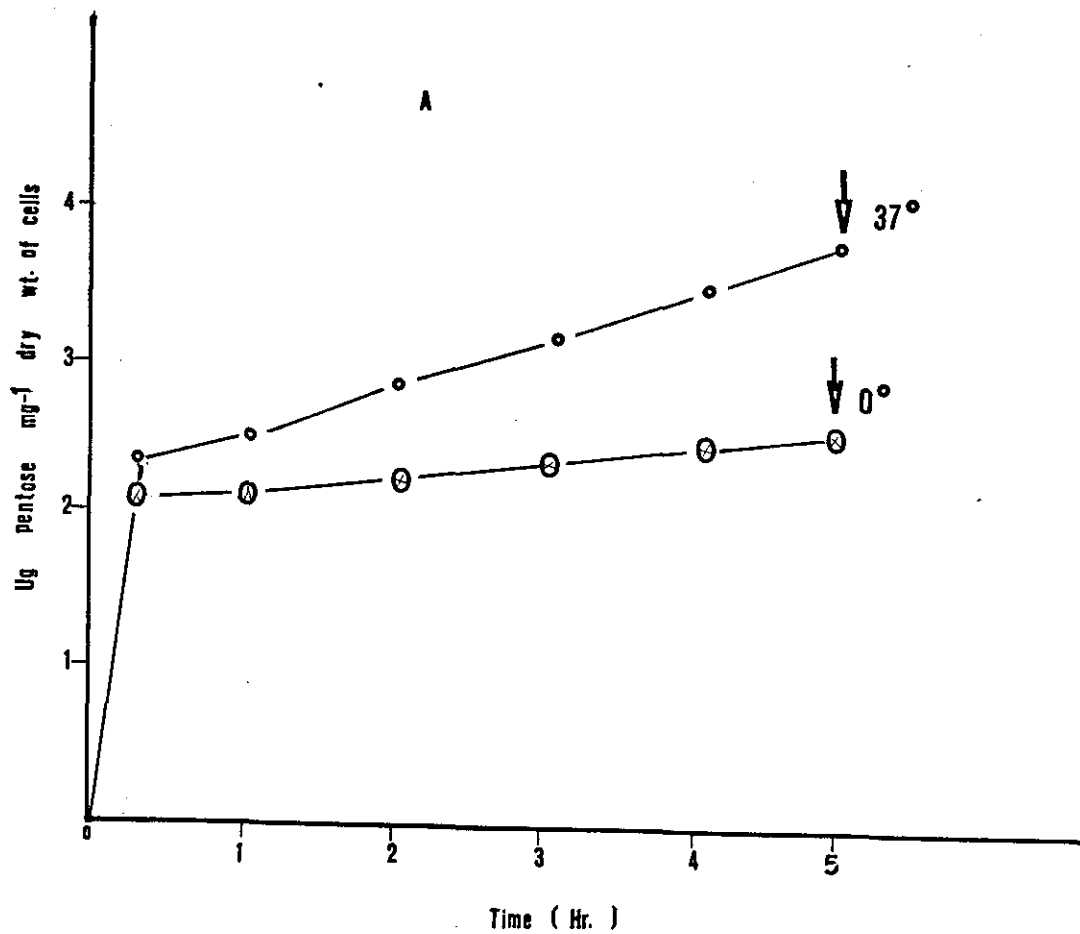


Fig. 3.68: K^+ release from cells of (A) E.coli and (B) C.albicans suspended in CFM at 22°C after treatment with different concentrations of β -blockers. Contact time 30 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

●-● Propranolol

□-□ Atenolol

--- release of K^+ from cells after boiling for 5 min.

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

O-O Propranolol

■-■ Atenolol

--- release of K^+ from cells after boiling for 5 min.

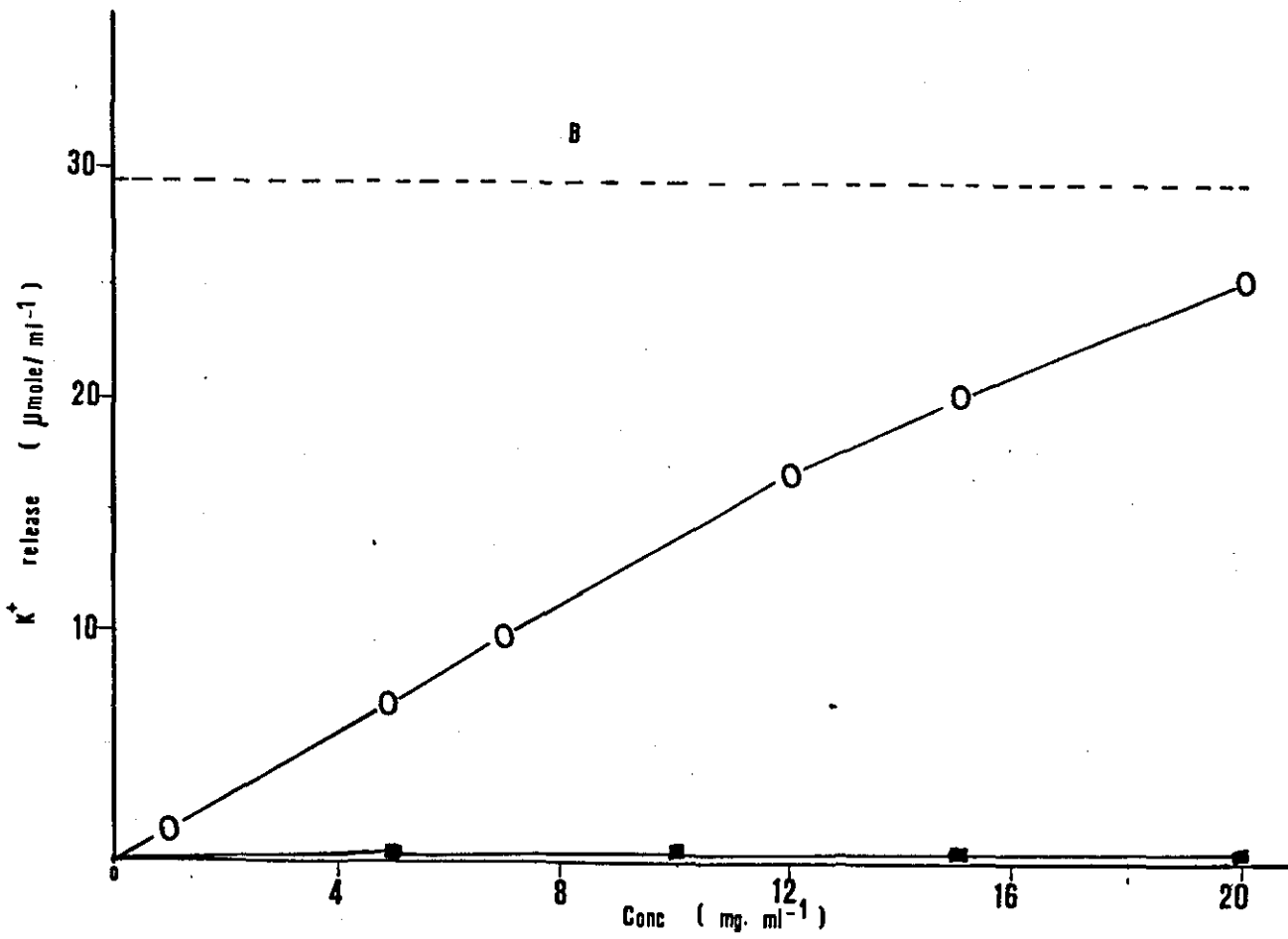
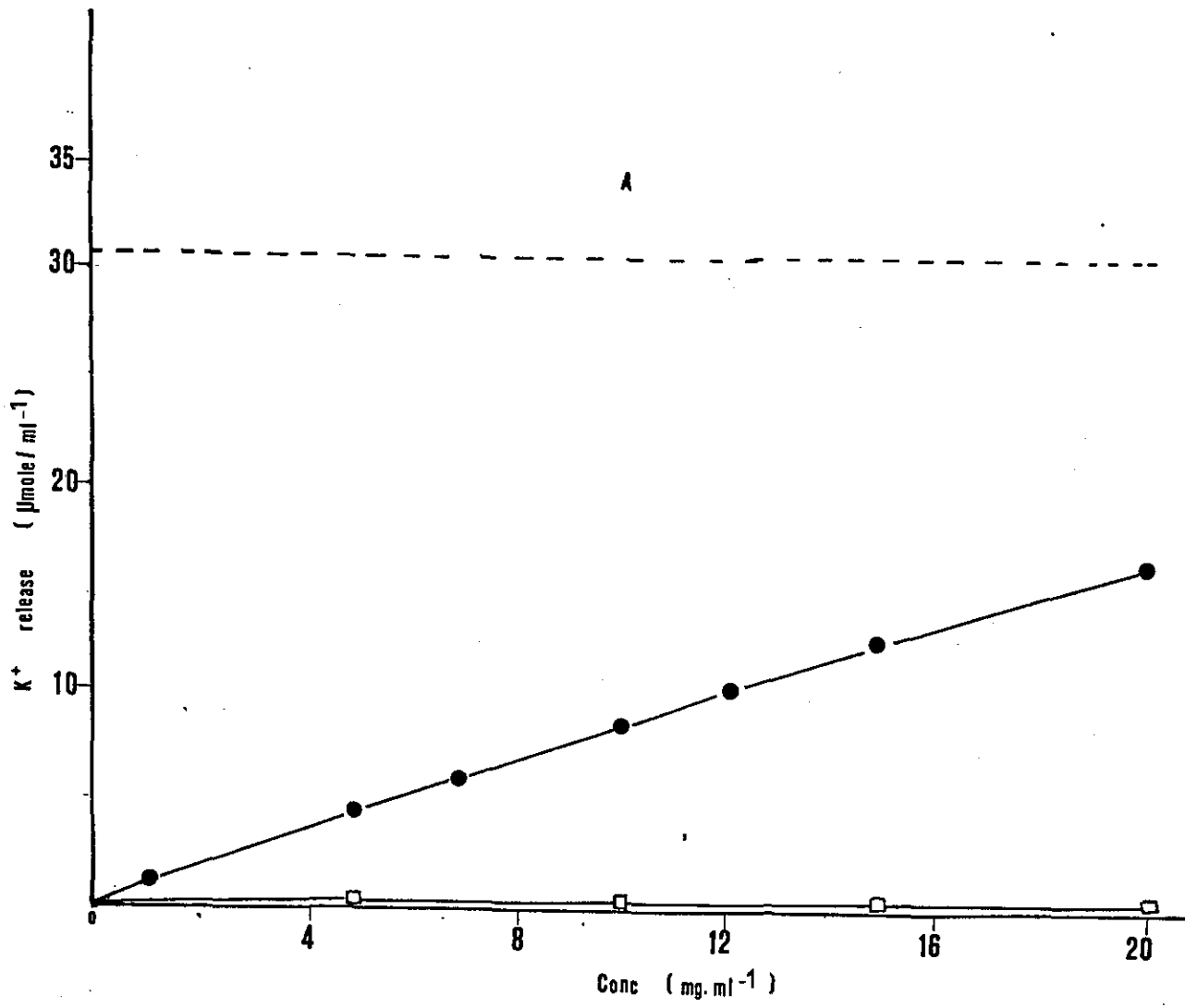


Fig. 3.69: K^+ release from cells of (A) E.coli and (B) C.albicans suspended in CFM at 22°C after treatment with β -blockers at various contact times.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

∇ - ∇ 0.5 mg.ml⁻¹ Propranolol
 $+$ - $+$ 1.0 mg.ml⁻¹ Propranolol
 Δ - Δ 5.0 mg.ml⁻¹ Propranolol
 $\blacksquare$ - $\blacksquare$ 15.0 mg.ml⁻¹ Propranolol
 $\bullet$ - $\bullet$ 20.0 mg.ml⁻¹ Propranolol
 --- release of K^+ from cells after
 boiling for 5 min.

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

x - x 0.5 mg.ml⁻¹ Propranolol
 o - o 1.0 mg.ml⁻¹ Propranolol
 $\square$ - $\square$ 5.0 mg.ml⁻¹ Propranolol
 $\otimes$ - $\otimes$ 15.0 mg.ml⁻¹ Propranolol
 --- release of K^+ from cells after
 boiling for 5 min.

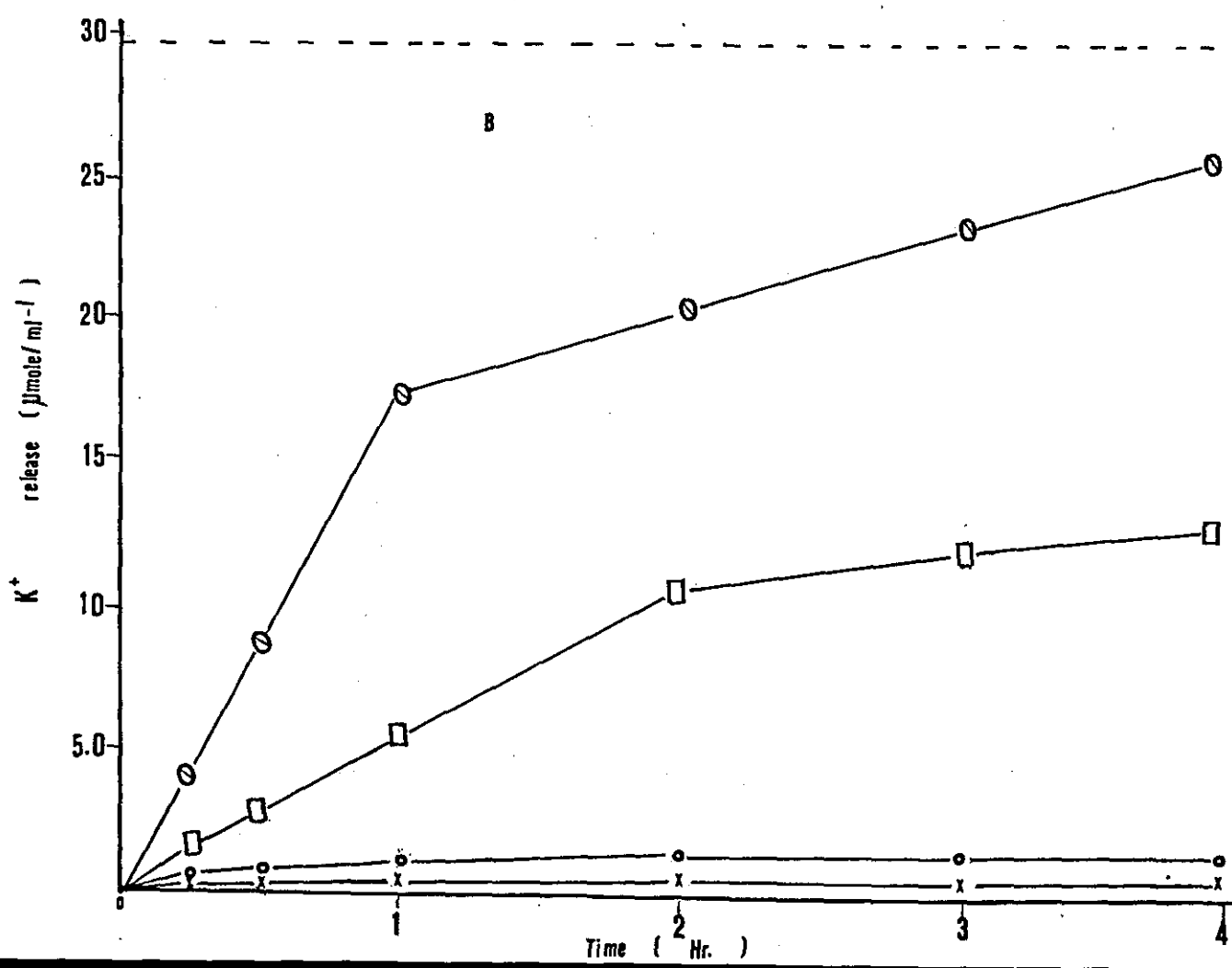
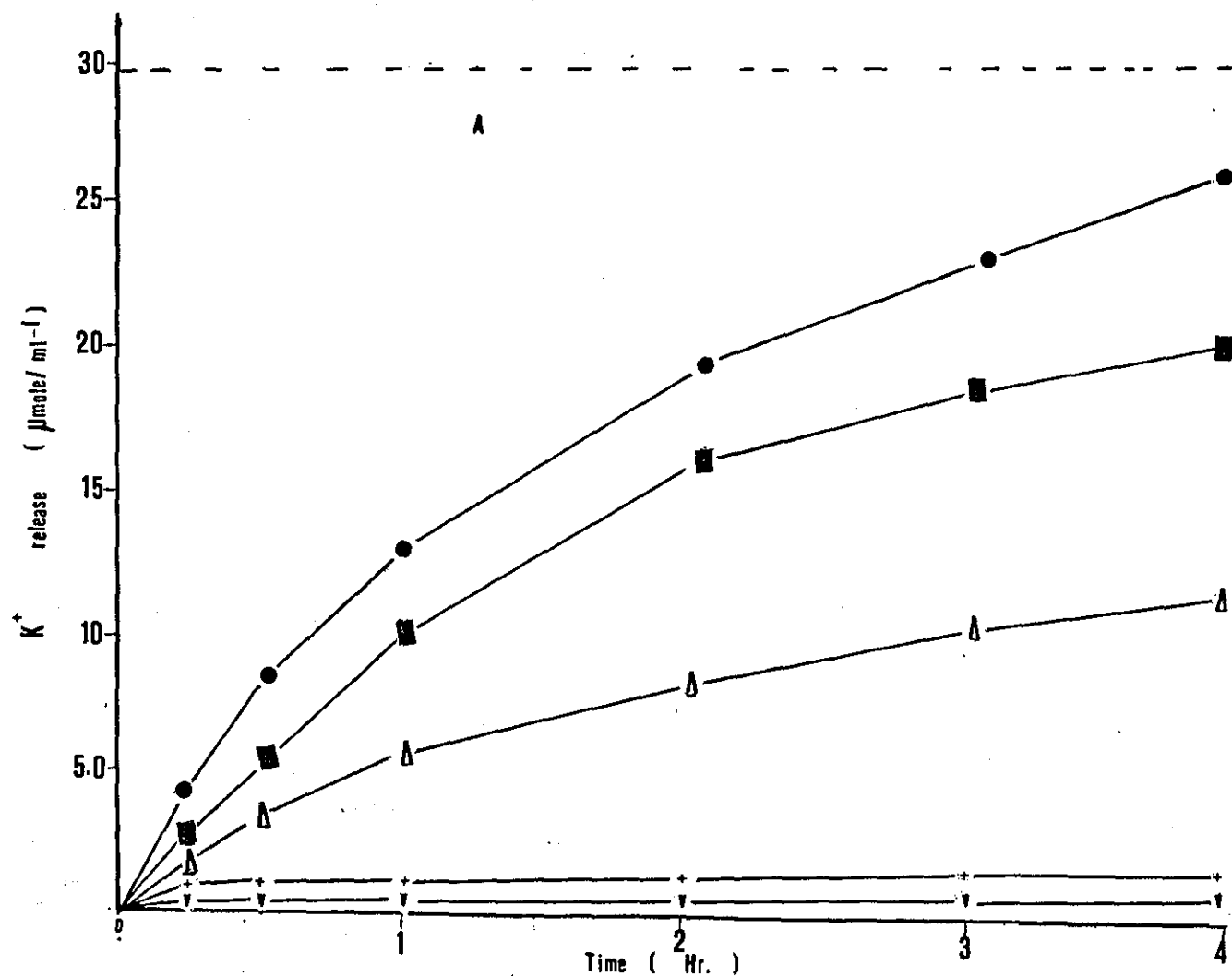


Fig. 3.70: Release of 260 nm from cells of (A) E.coli and (B) C.albicans suspended in CFM at 22°C after treatment with different concentrations of β -blockers. Contact time 15 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

●-● Propranolol

Δ-Δ Atenolol or Practolol

--- release of 260 nm from cells after boiling for 5 min.

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

⊗-⊗ Propranolol

○-○ Atenolol or Practolol

--- release of 260 nm from cells after boiling for 5 min.

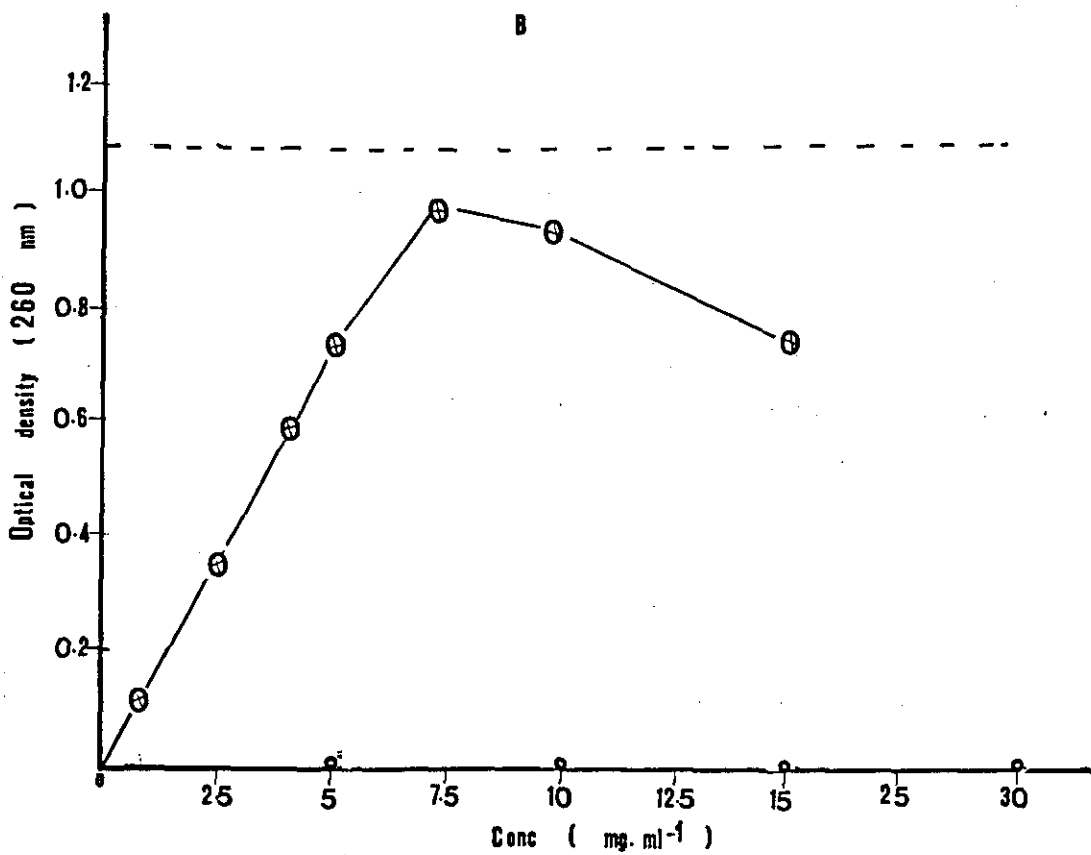
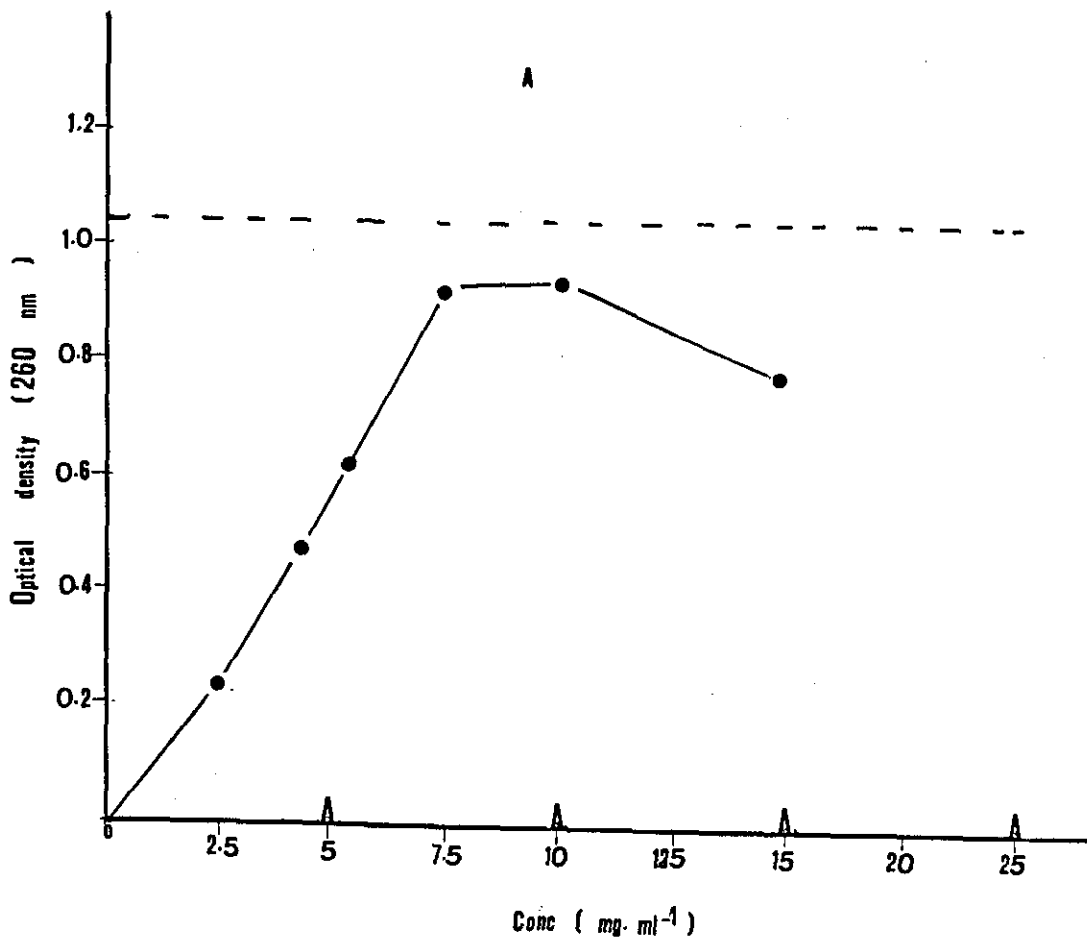


Fig. 3.71: Release of ^{32}P -labelled cells of E.coli suspended in CFM at 22°C after treatment with different concentrations of β -blockers. Cell concentration $4.2 \times 10^9 \text{ cells.ml}^{-1}$. Contact time 15 min.

(A)

O-O Propranolol

Δ - Δ Practolol

--- release of Pentose from cells after treatment with 5% trichloroacetic acid.

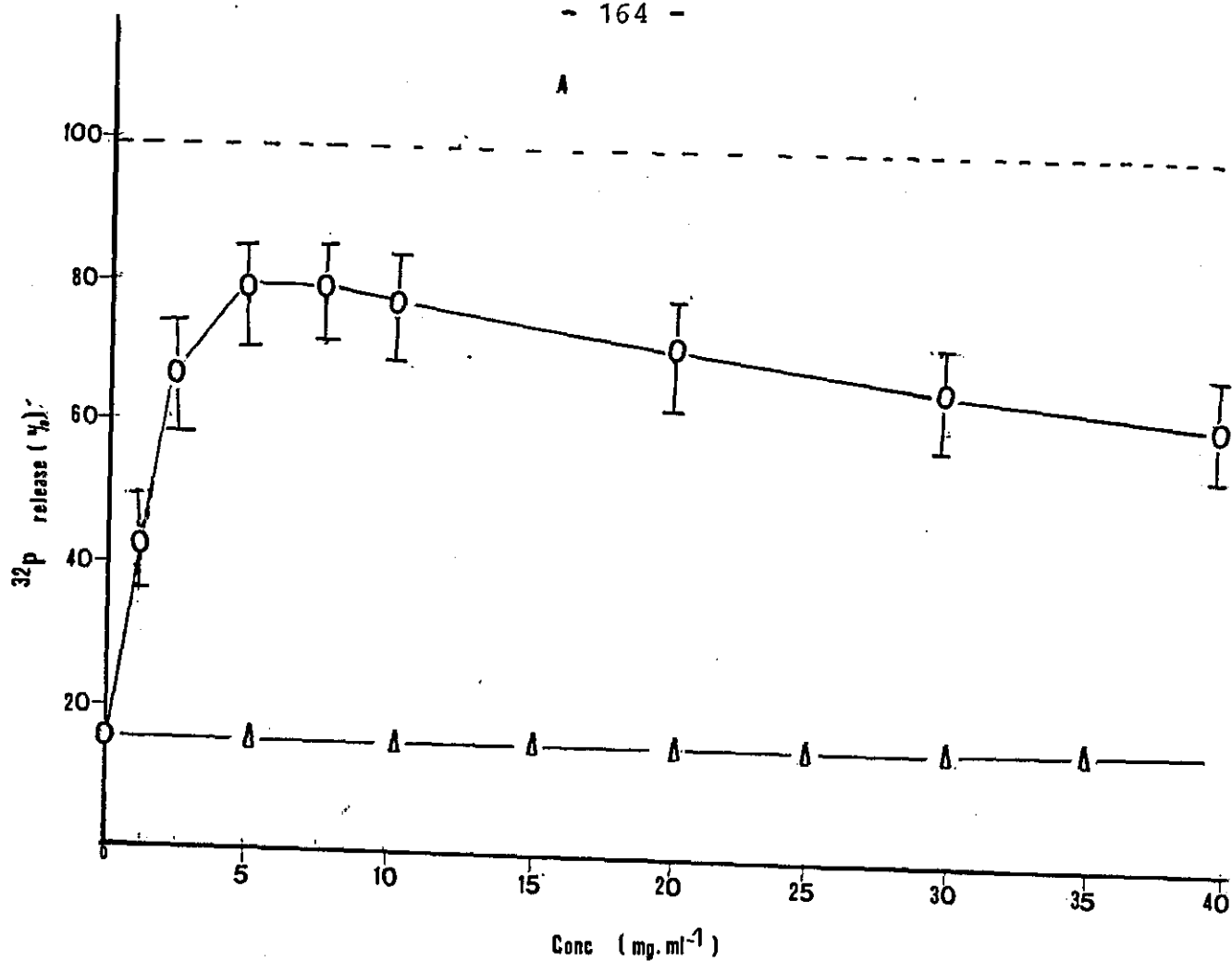
(B)

●-● Oxprenolol

$\blacktriangle$ - $\blacktriangle$ Atenolol

--- release of Pentose from cells after treatment with 5% Trichloroacetic acid.

A



B

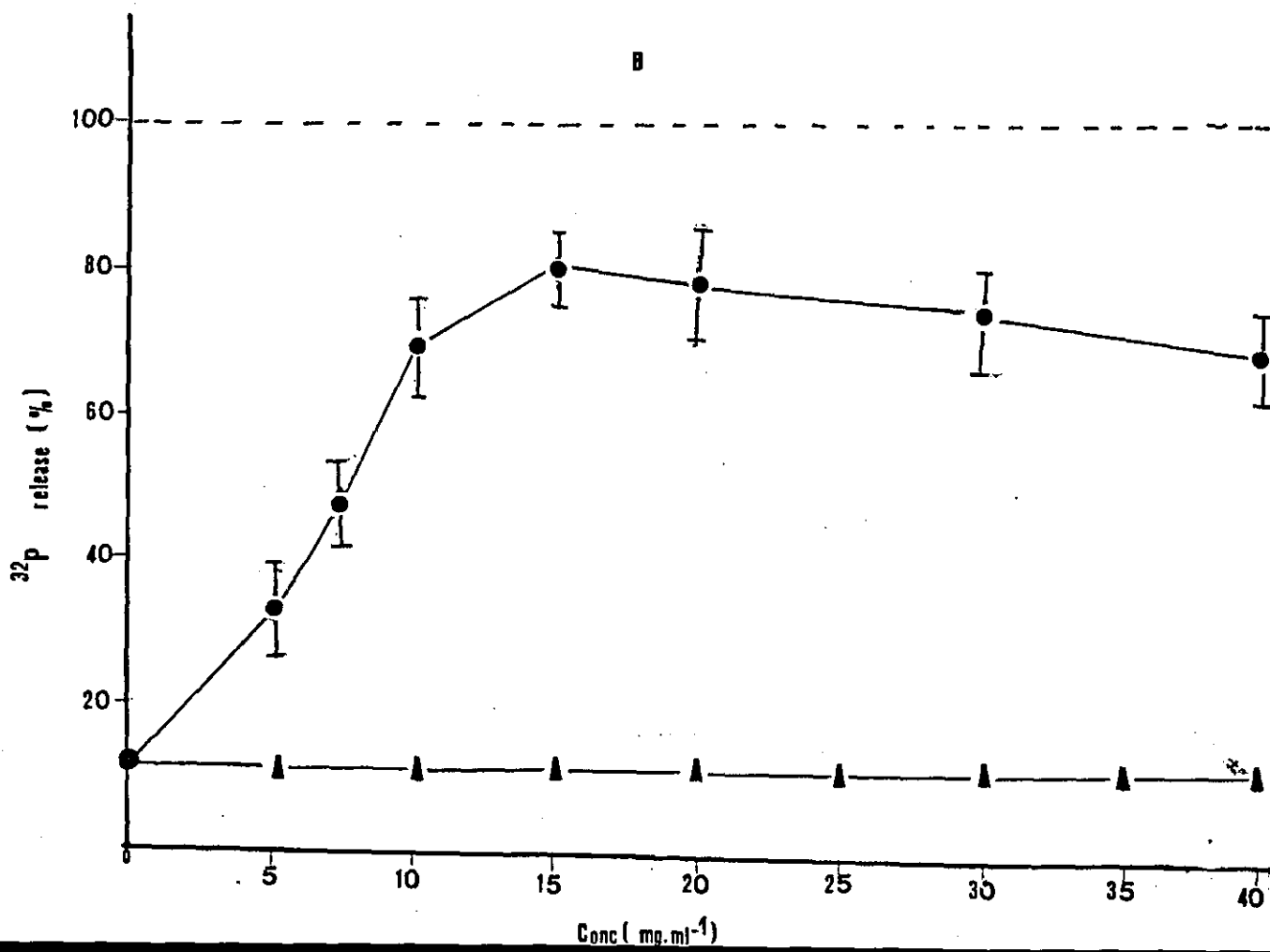


Fig. 3.72: Release of ^{32}P -labelled cells of C.albicans suspended in CFM at 22°C after treatment with different concentration of β -blockers. Cell concentration $3.8 \times 10^5 \text{ cells.ml}^{-1}$. Contact time 15 min.

(A)

O-O Propranolol

Δ - Δ Practolol

--- ^{32}P release from cells after 5% treatment with 5% Trichloroacetic acid.

(B)

●-● Oxprenolol

$\blacktriangle$ - $\blacktriangle$ Atenolol

--- ^{32}P release from cells after 5% treatment with 5% Trichloroacetic acid.

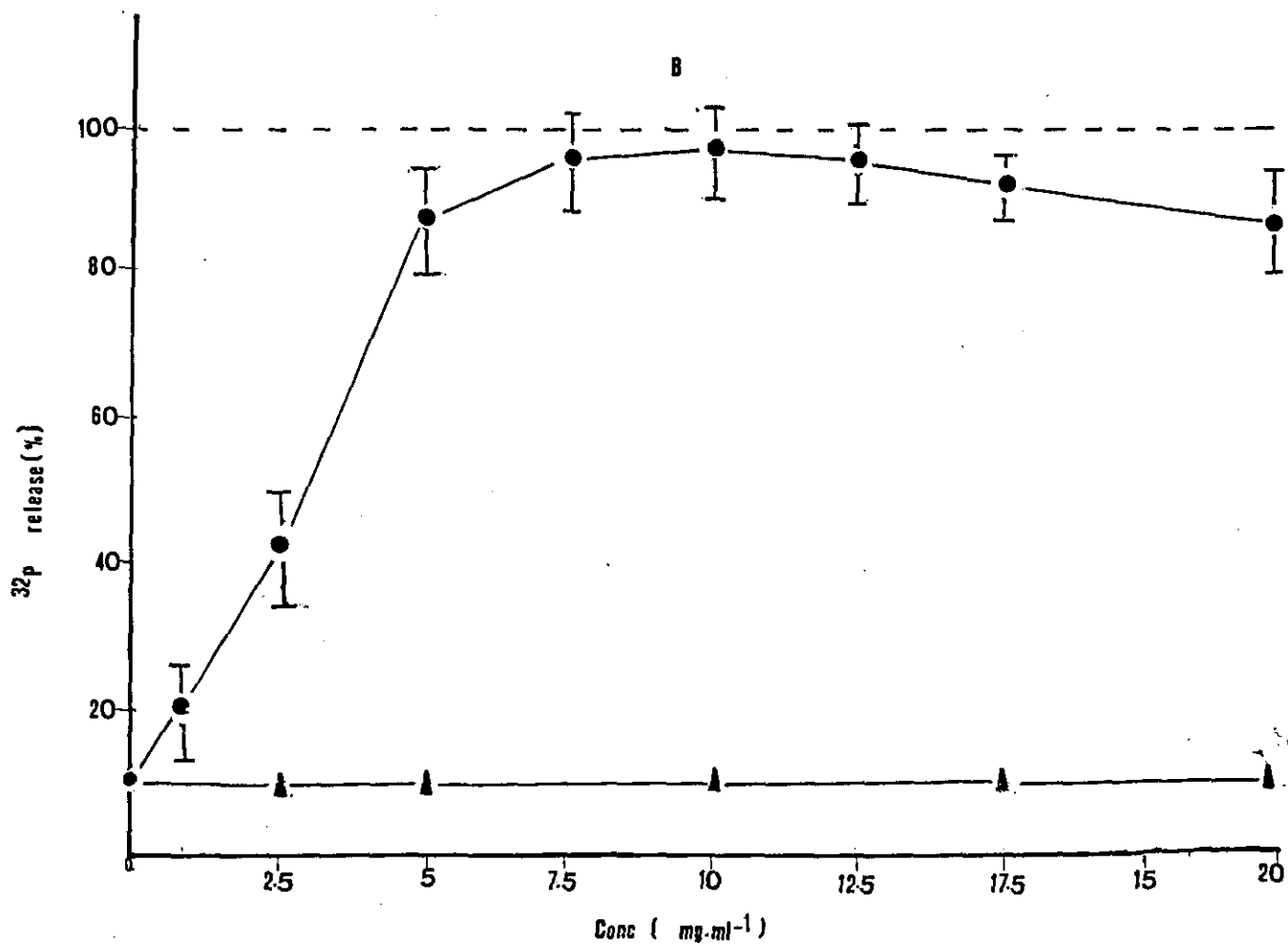
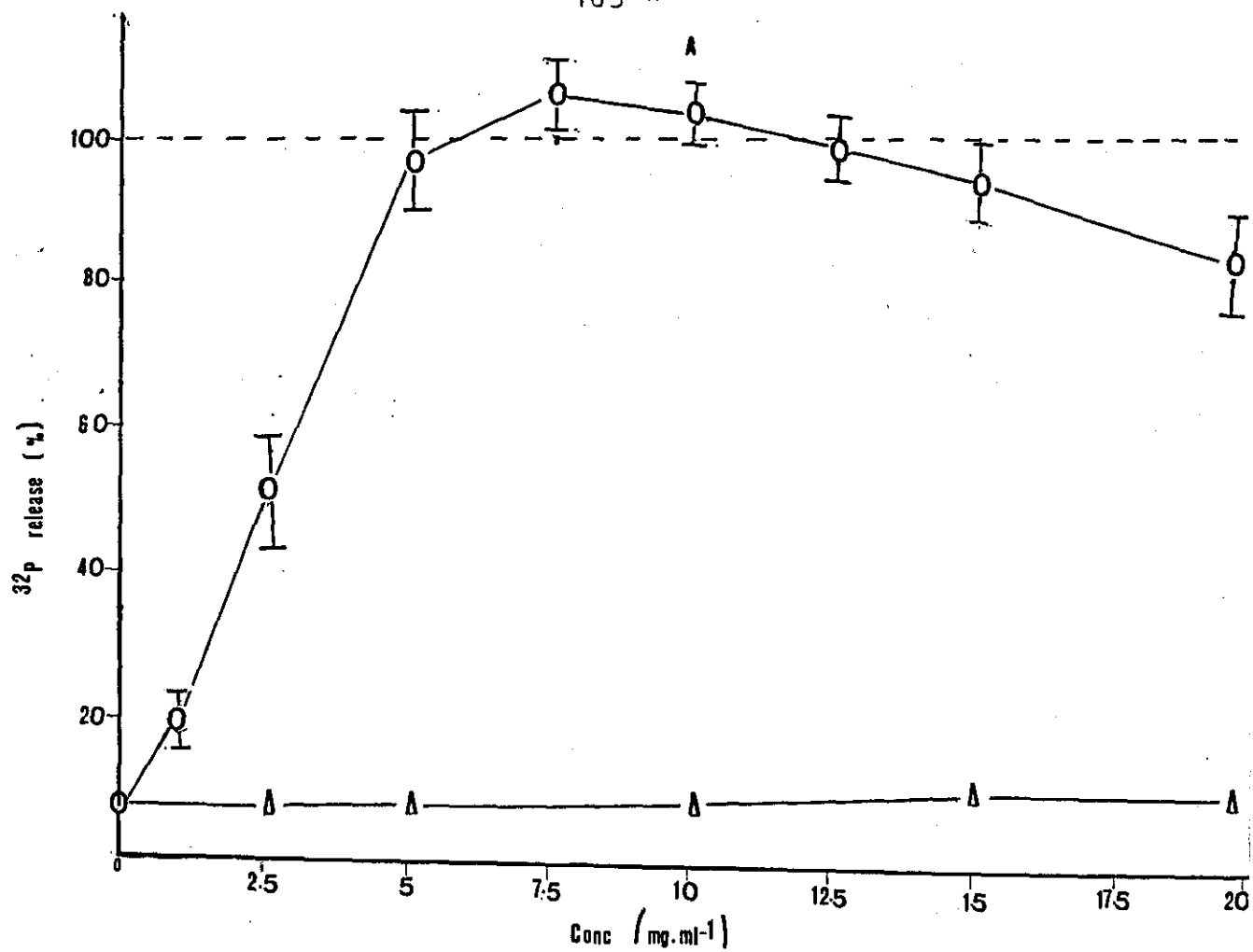


Fig. 3.73: Release of ^{32}P -labelled cells of E.coli suspended in CFM at 22°C after treatment with fixed concentrations of Propranolol (2.5mg.ml^{-1}) O-O, and Oxprenolol (5.0mg.ml^{-1}) O-O. Cell concentration $4.2 \times 10^9 \text{ cells.ml}^{-1}$.

Fig. 3.74: Release of ^{32}P -labelled cells of C.albicans suspended in CFM at 22°C after treatment with fixed concentrations of Propranolol (2.5mg.ml^{-1}) O-O, and Oxprenolol (5.0mg.ml^{-1}) O-O. Cell concentration $3.8 \times 10^5 \text{ cells.ml}^{-1}$.

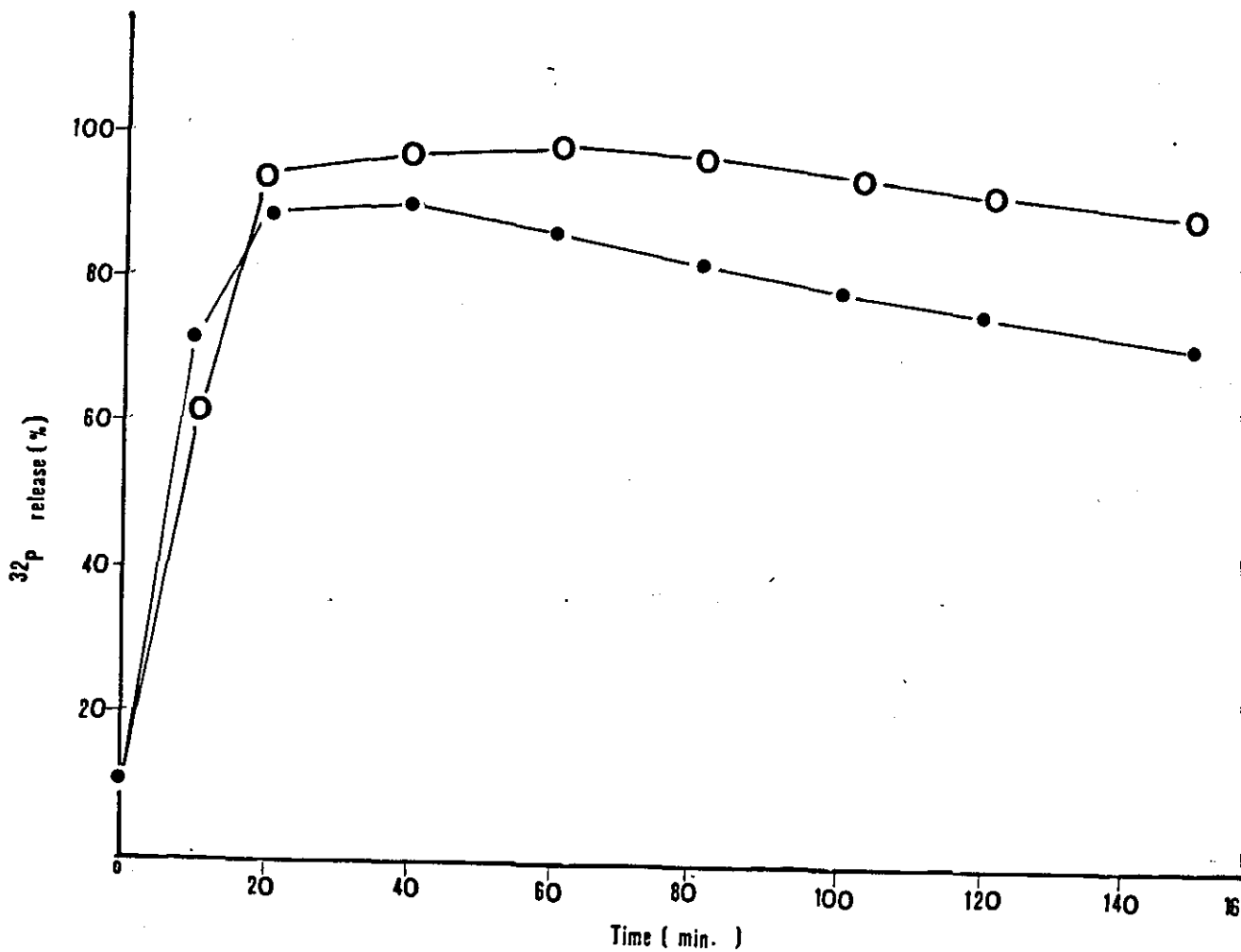
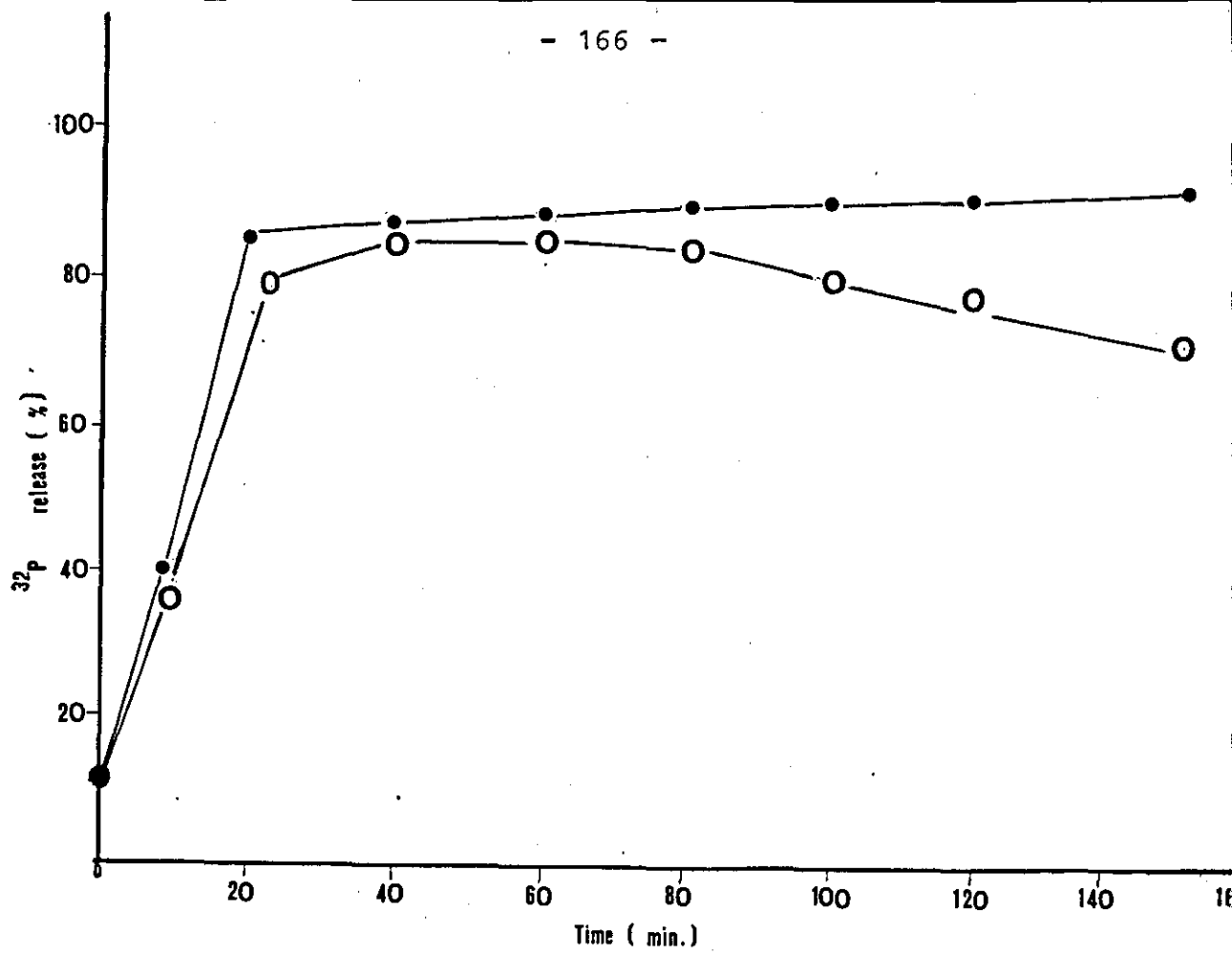


Fig. 3.75: Uptake of dansyl propranolol and atenolol by cells (A) E.coli and (B) C.albicans suspended in CFM. Contact time 30 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹
Temperature 37°C

O-O Propranolol (dansyl derivative)

■-■ Atenolol (dansyl derivative)

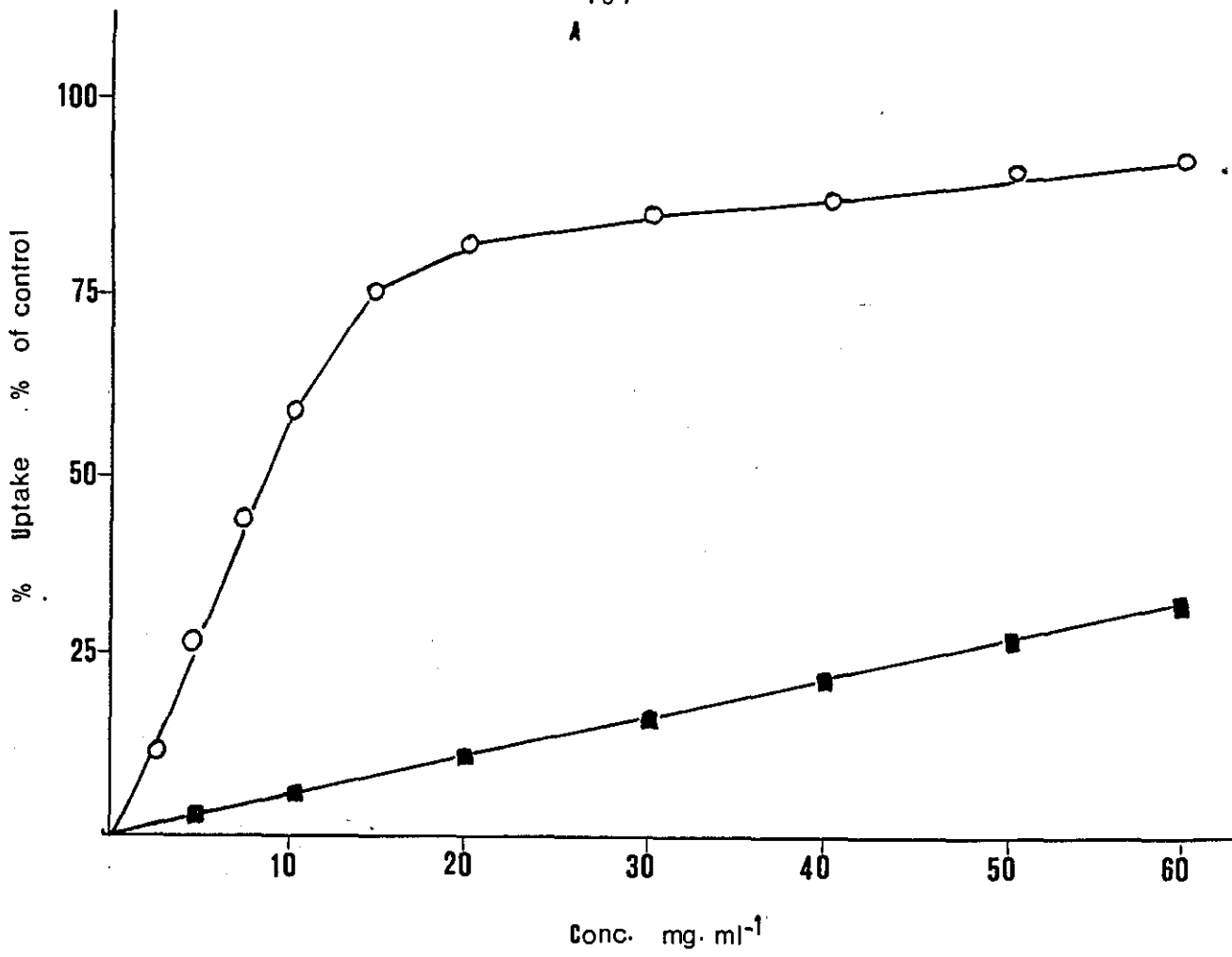
(B)

Cell concentration 3.8×10^5 cells.ml⁻¹
Temperature 30°C

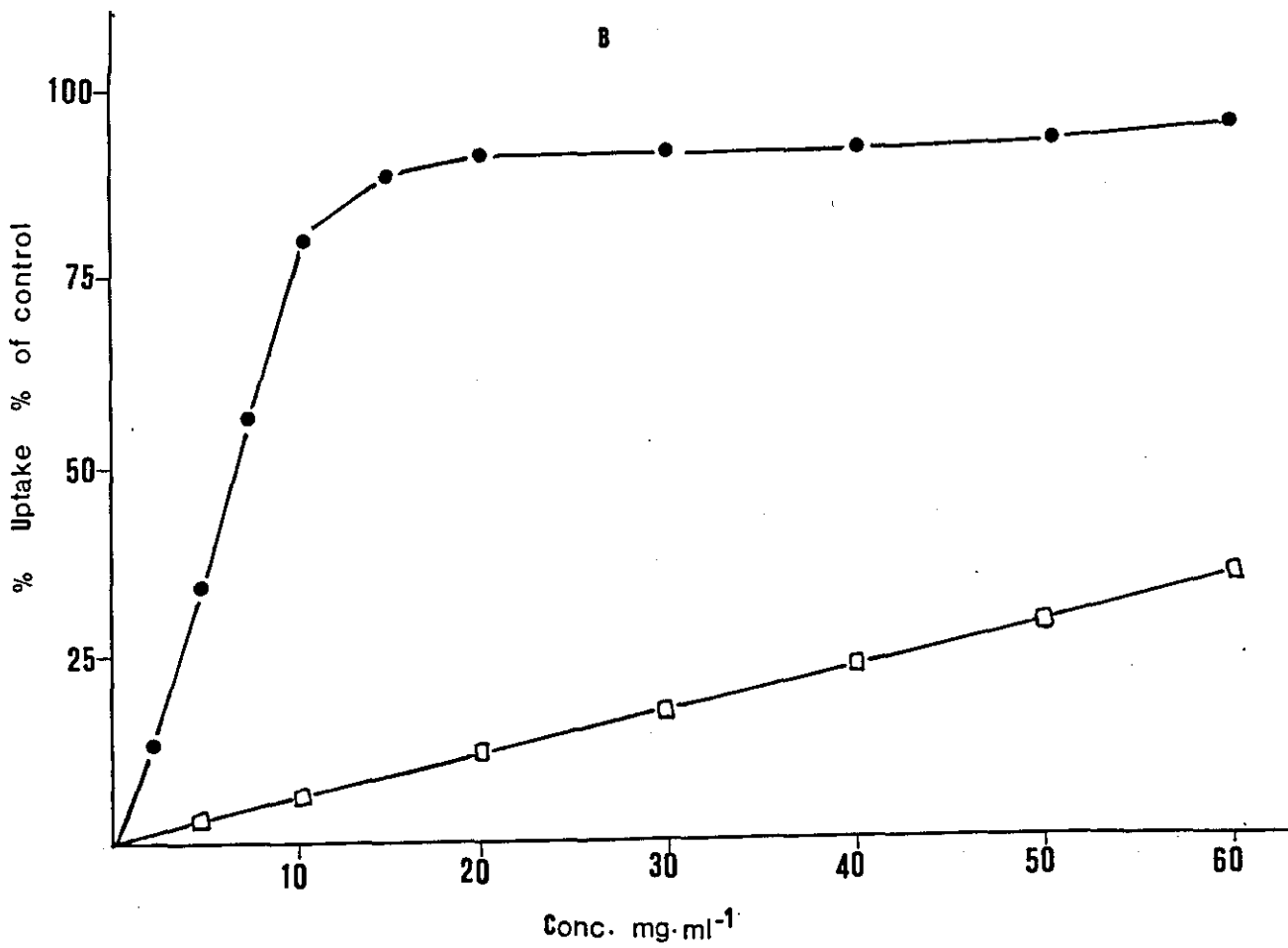
●-● Propranolol (dansyl derivative)

□-□ Atenolol (dansyl derivative)

A



B

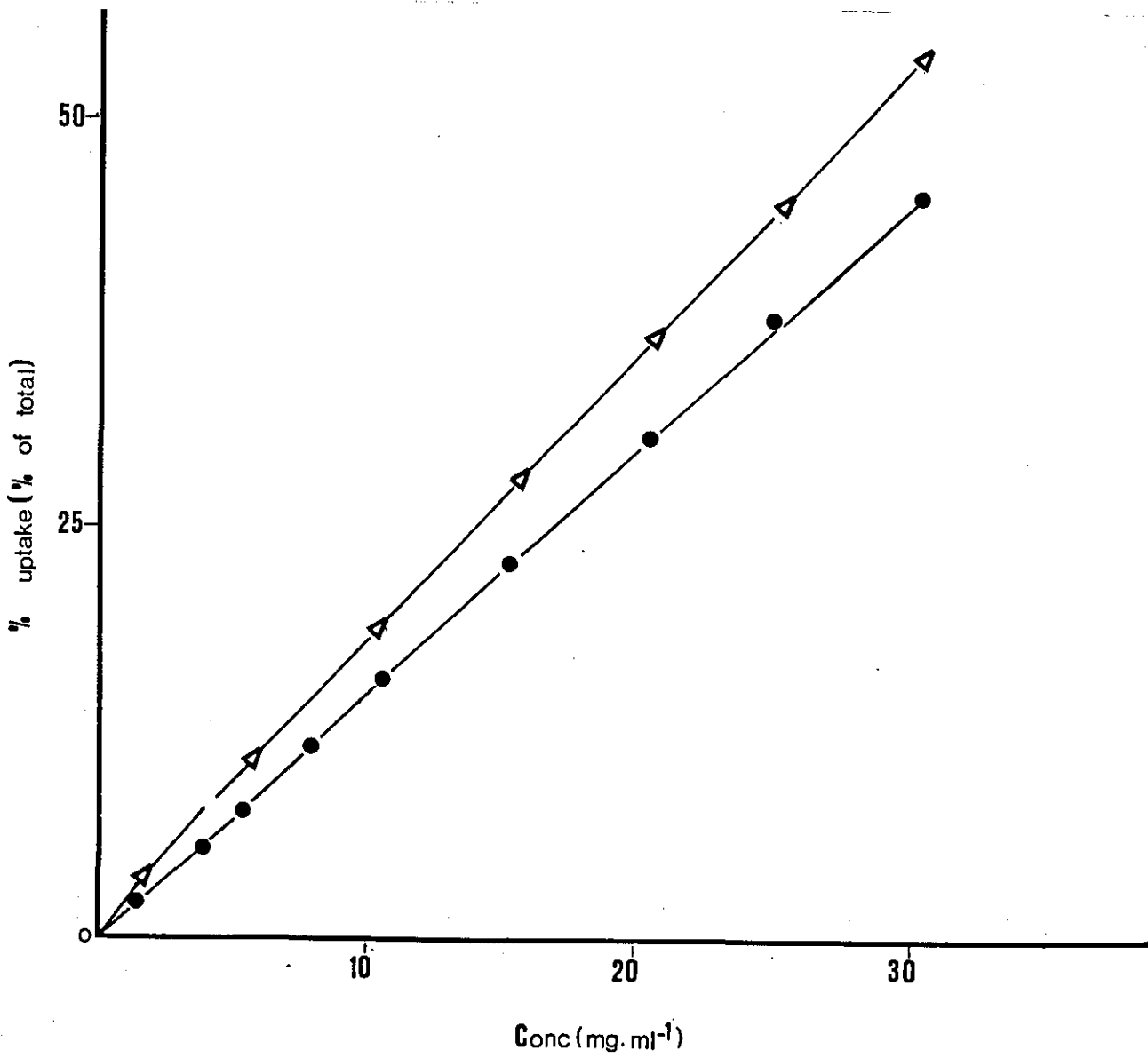
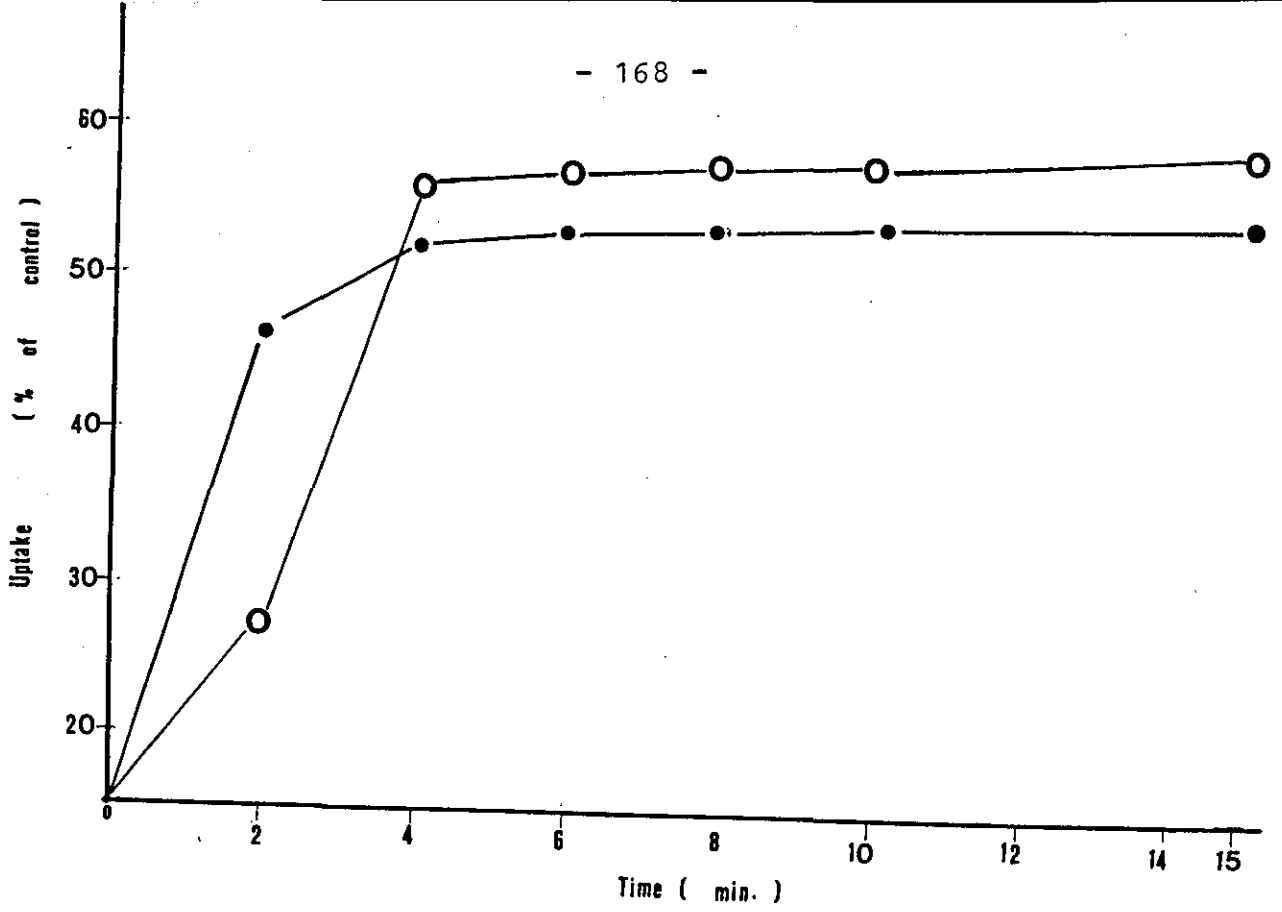


(A)

Fig.3.76: Uptake of ^3H -propranolol by cells of E.coli ●-●, cell concentration 4.2×10^9 cells.ml $^{-1}$ and C.albicans, o-o, cell concentration 3.8×10^5 cells.ml $^{-1}$ at 37° and 30°C respectively.

(B)

Fig. 3.76: Uptake of ^3H -propranolol by cells of E.coli o-o, cell concentration 4.2×10^9 cells.ml $^{-1}$ and C.albicans Δ-Δ, cell concentration 3.8×10^8 cells.ml $^{-1}$ at 37° and 30°C respectively. Contact time 15 min.



A. Fig. 3.77: Solubilization of Sudan Black B by β -blockers and local anaesthetics in CFM at 22°C.

●-● Propranolol

⊗-⊗ Tetracaine

x-x Atenolol

▼-▼ Procaine

B. Solubilization of Sudan Black B by β -blockers and local anaesthetics in the presence of cells of E.coli suspended in CFM at 22°C.

O-O Propranolol

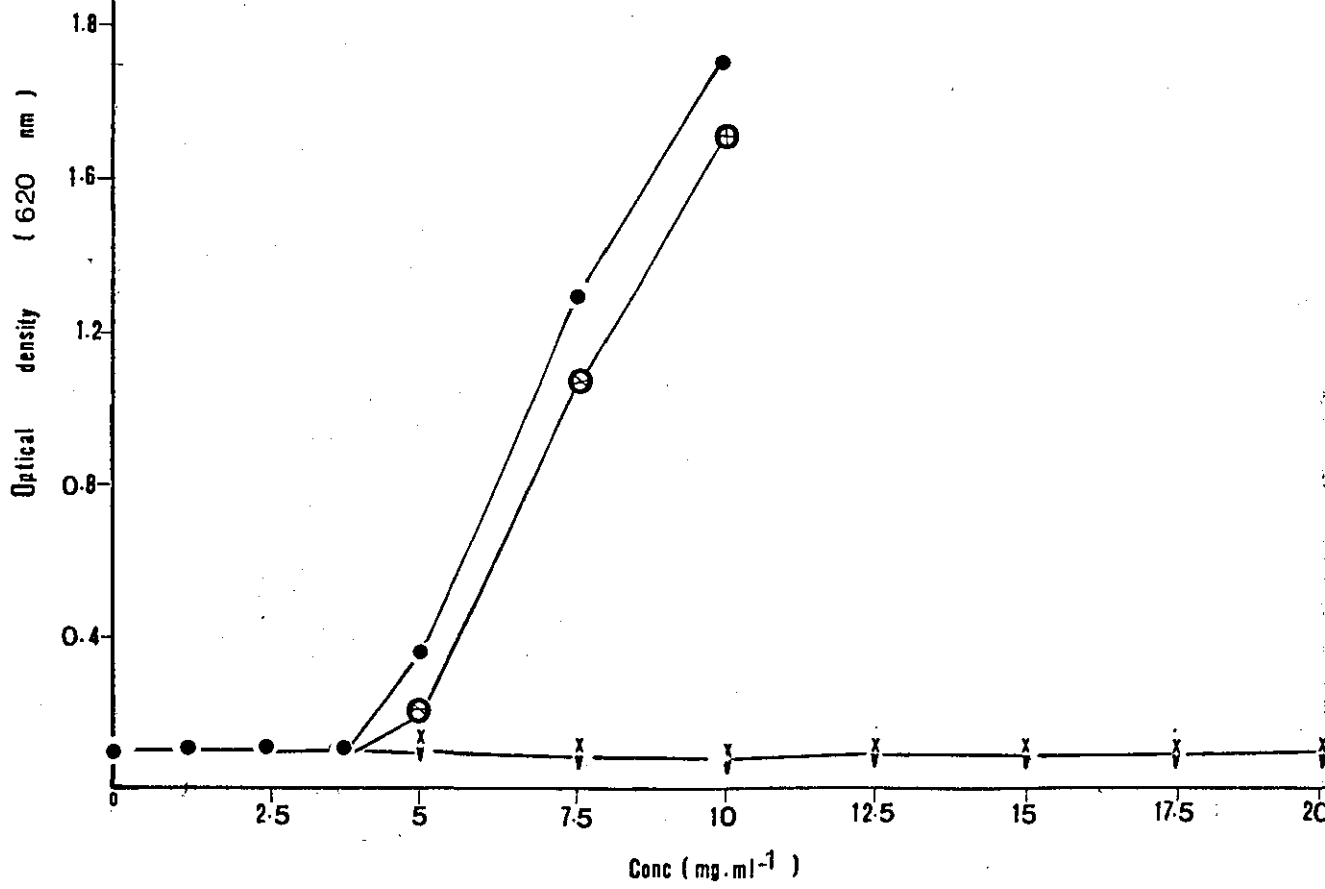
▲-▲ Tetracaine

□-□ Atenolol

+--+ Procaine

A

- 169 -



B

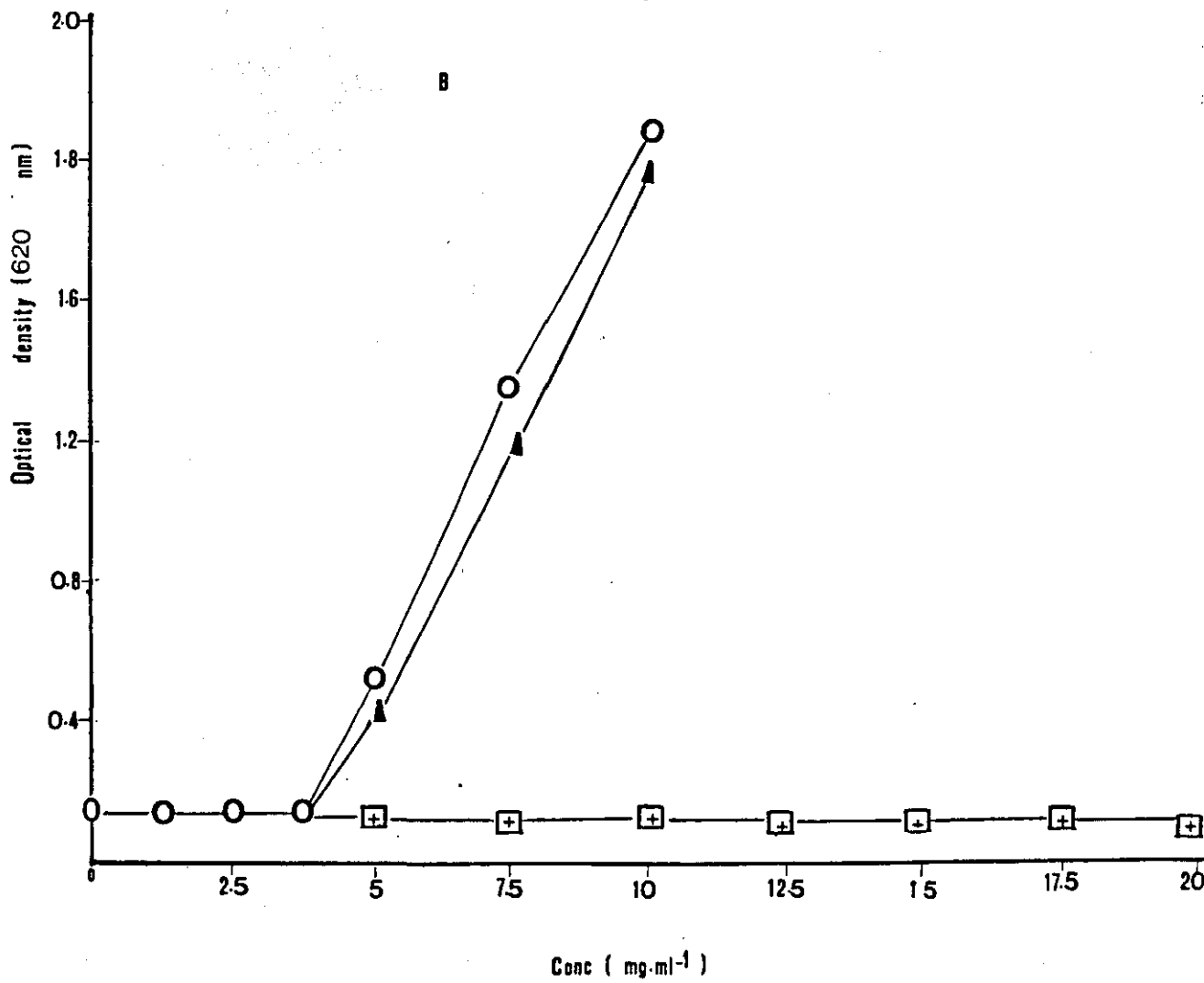


Fig. 3.78: Surface tension measurement for β -blockers
at air-liquid interface at 22°C.

●-● Propranolol

O-O Atenolol

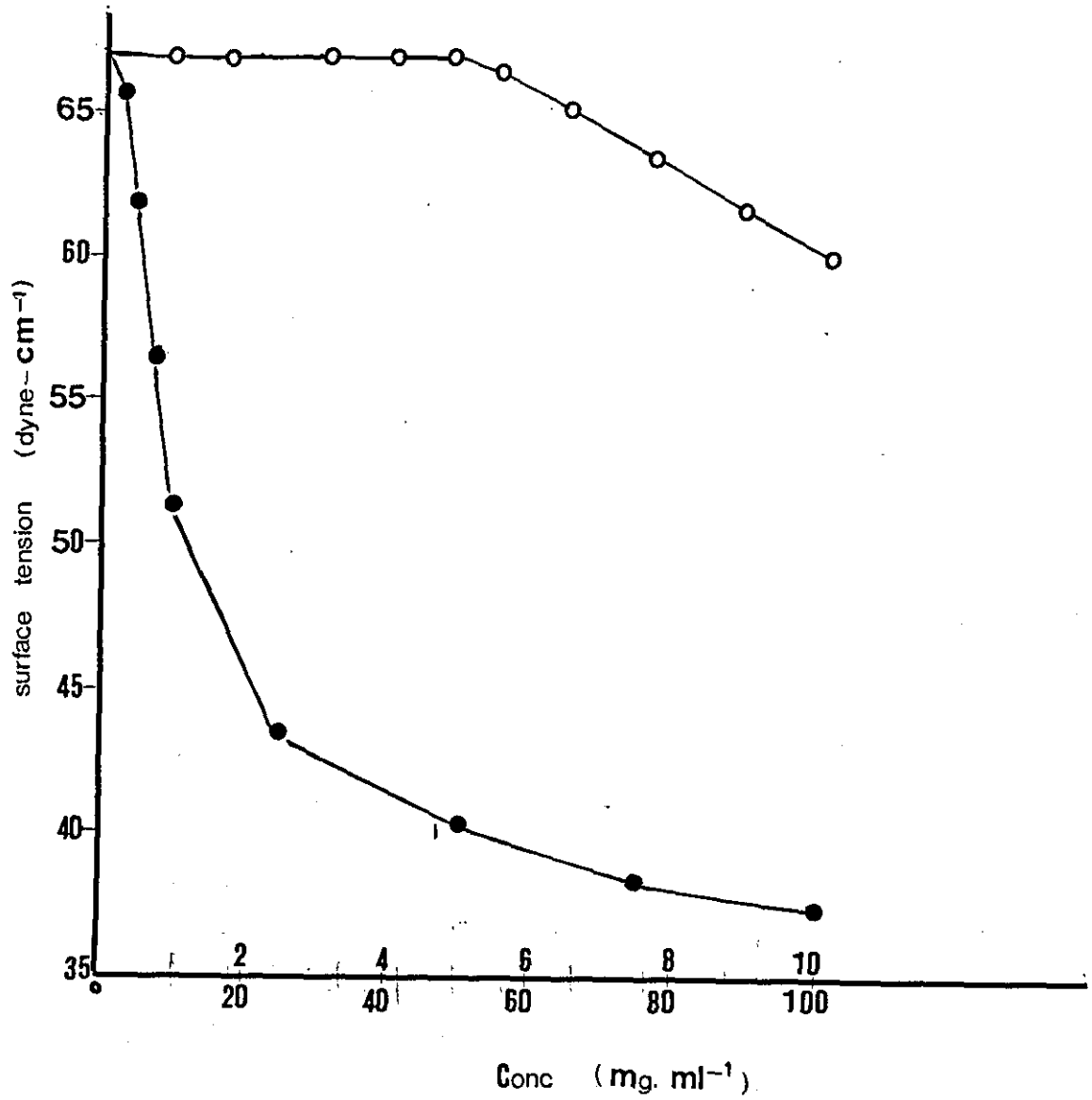


Fig. 3.79: Stabilizing and lytic effects of β -blockers and HTAB on rabbit erythrocytes suspended in hypotonic saline and phosphate buffer 0.05M pH 7.4 at 22°C.

Cell concentration 4.7×10^7 cells.ml⁻¹

Drug concentrations

O-O	mg.ml ⁻¹	Atenolol
▲-▲	mg.ml ⁻¹	Propranolol
●-●	mg.μl ⁻¹	HTAB

Haemolysis (543 nm)

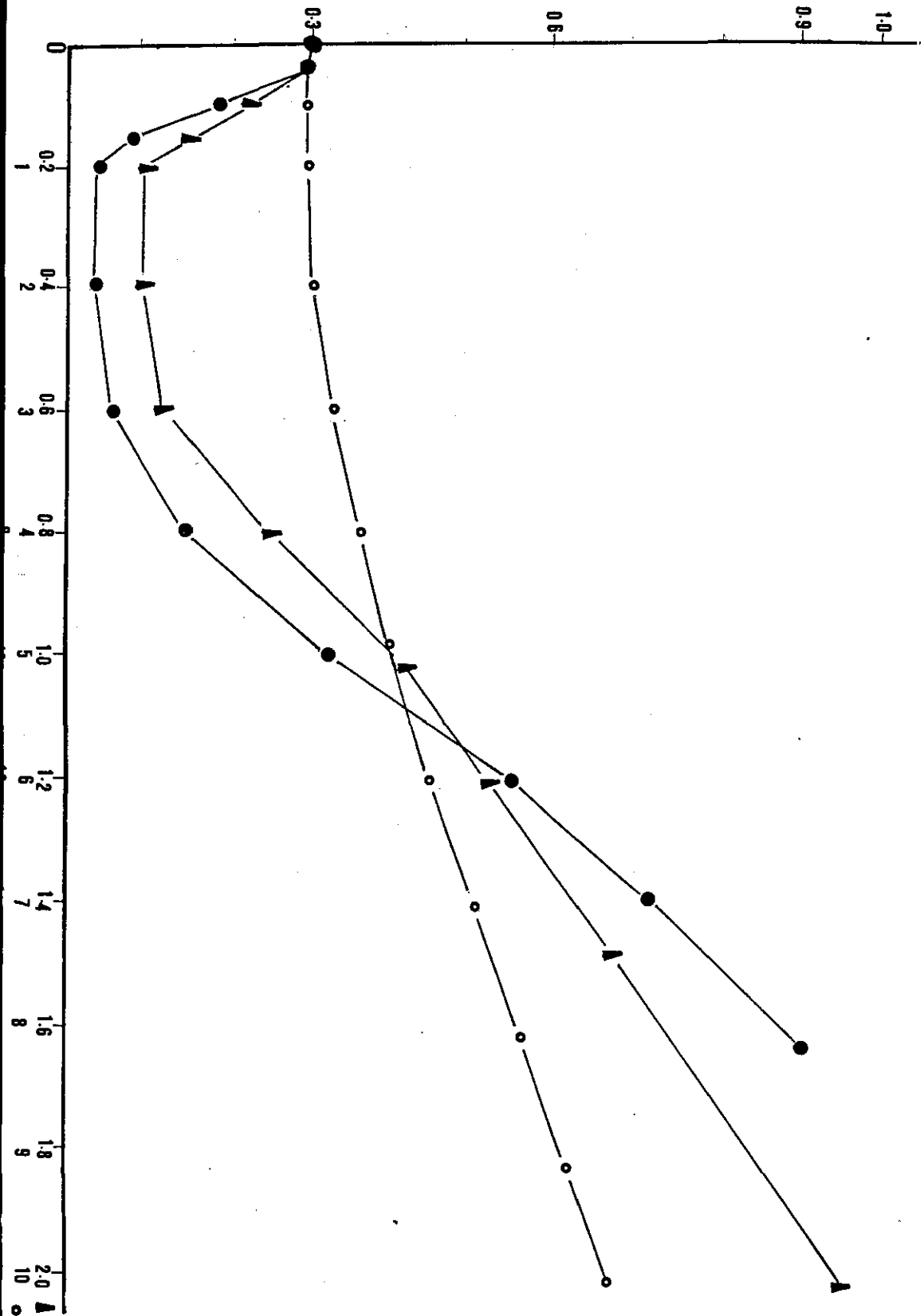


Fig. 3.80: Stabilizing and lytic effects of local anaesthetics on rabbit erythrocytes suspended in hypotonic saline and phosphate buffer 0.05M pH 7.4 at 22°C. Cell concentration 4.7×10^7 cells.ml⁻¹. Drug Concentrations

●-● mg.ml⁻¹ Tetracaine
o-o mg.ml⁻¹ Procaine :

Haemolysis 543 nm

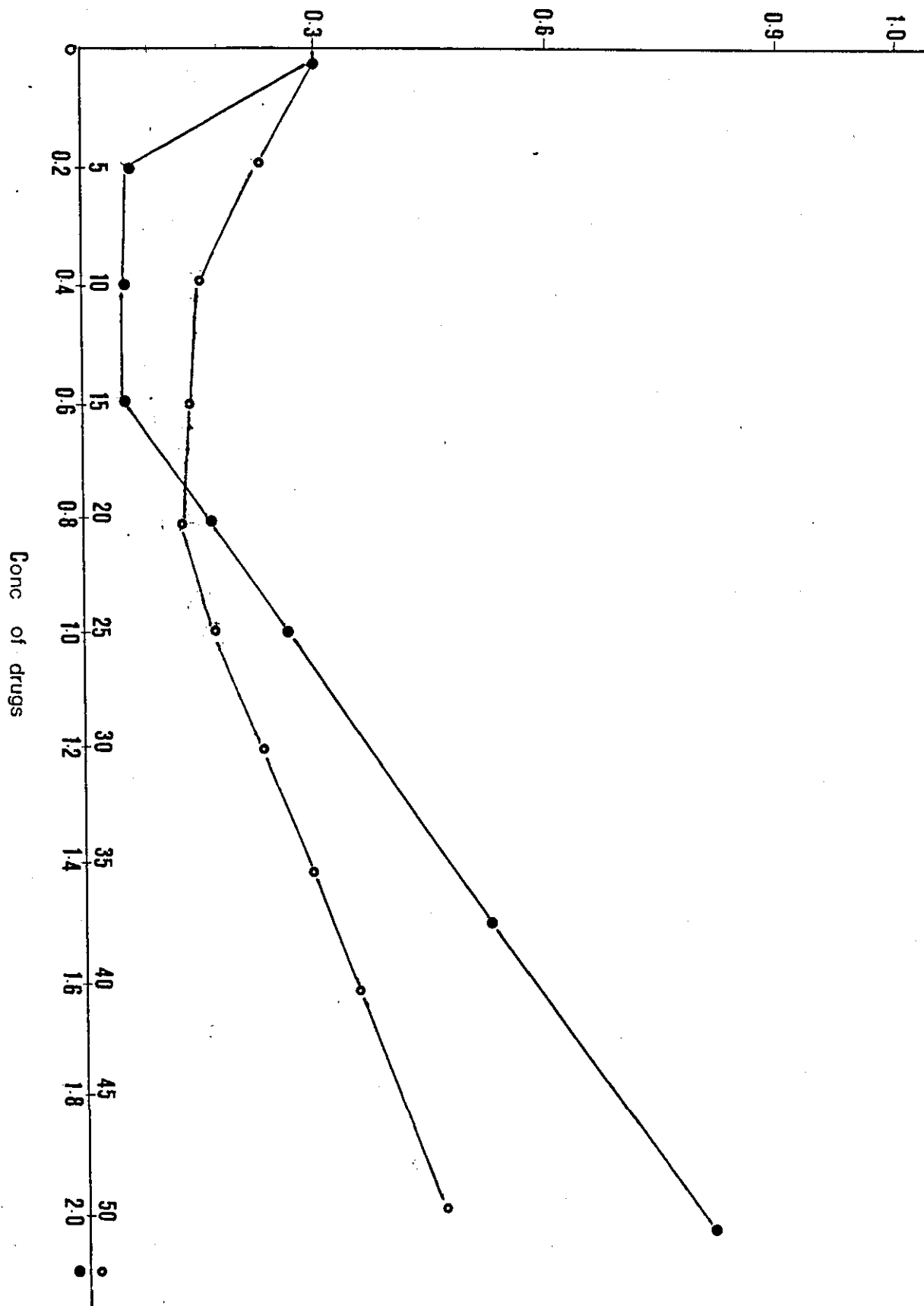


Fig. 3.81: The relationship between stabilization of rabbit erythrocyte membrane against hypotonic haemolysis and the retention of both haemoglobin and K^+ in the presence of Propranolol.

Temperature 22°C .

Cell concentration 4.7×10^7 cells.ml⁻¹.

●-● retention and release of haemoglobin

O-O retention and release of K^+

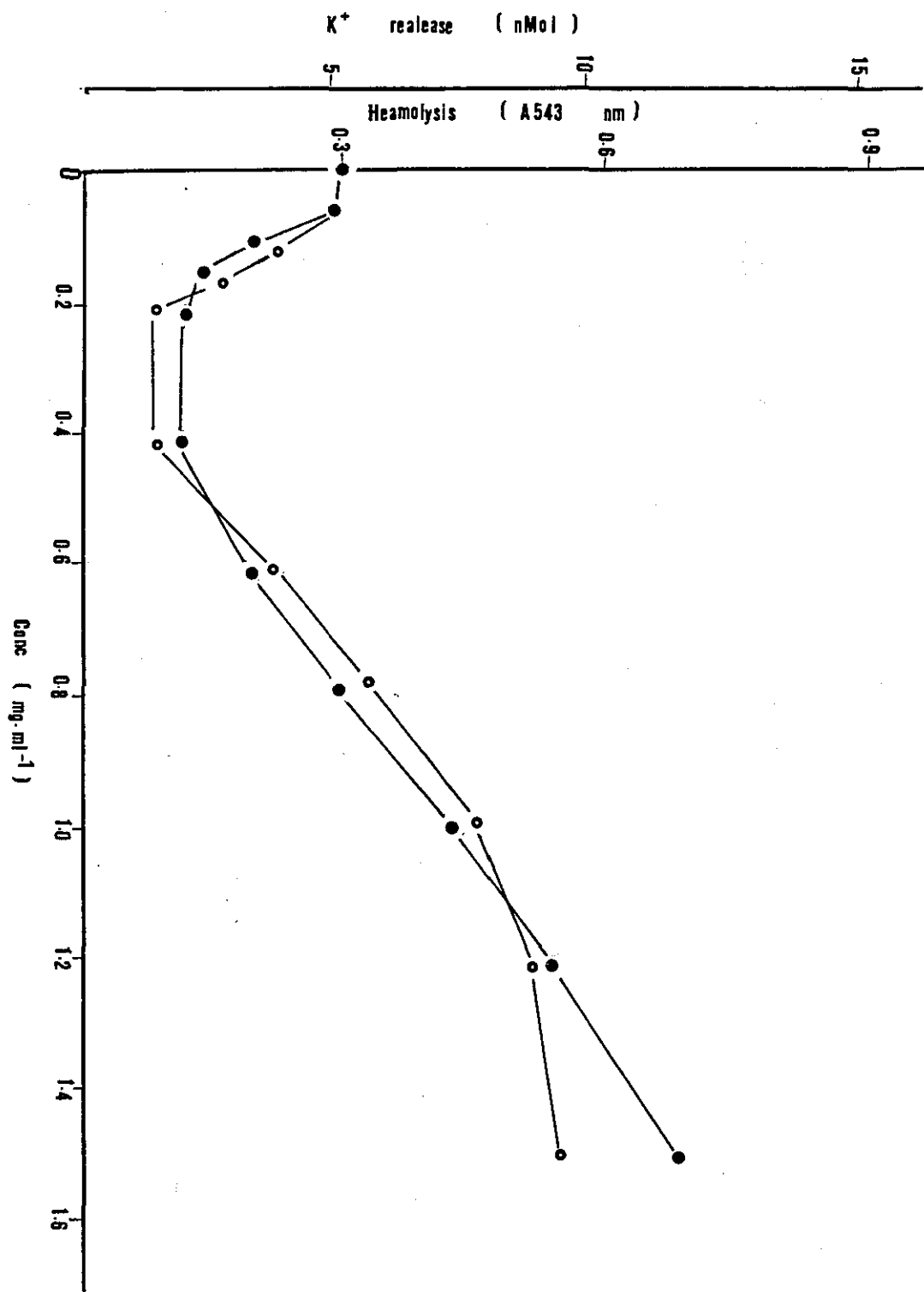


Fig. 3.82: Protection against the lytic effects of Propranolol by Mg^{2+} on rabbit erythrocyte membrane suspended in hypotonic saline and phosphate buffer 0.05M pH 7.4 at 22°C.

Cell concentration 4.7×10^7 cells.ml⁻¹
Cation concentration

●-● Propranolol in the absence of Mg^{2+}

□-□ 0.5 mM Mg^{2+}

▲-▲ 2.5 mM Mg^{2+}

x-x 5.0 mM Mg^{2+}

○-○ 10.0 mM Mg^{2+}

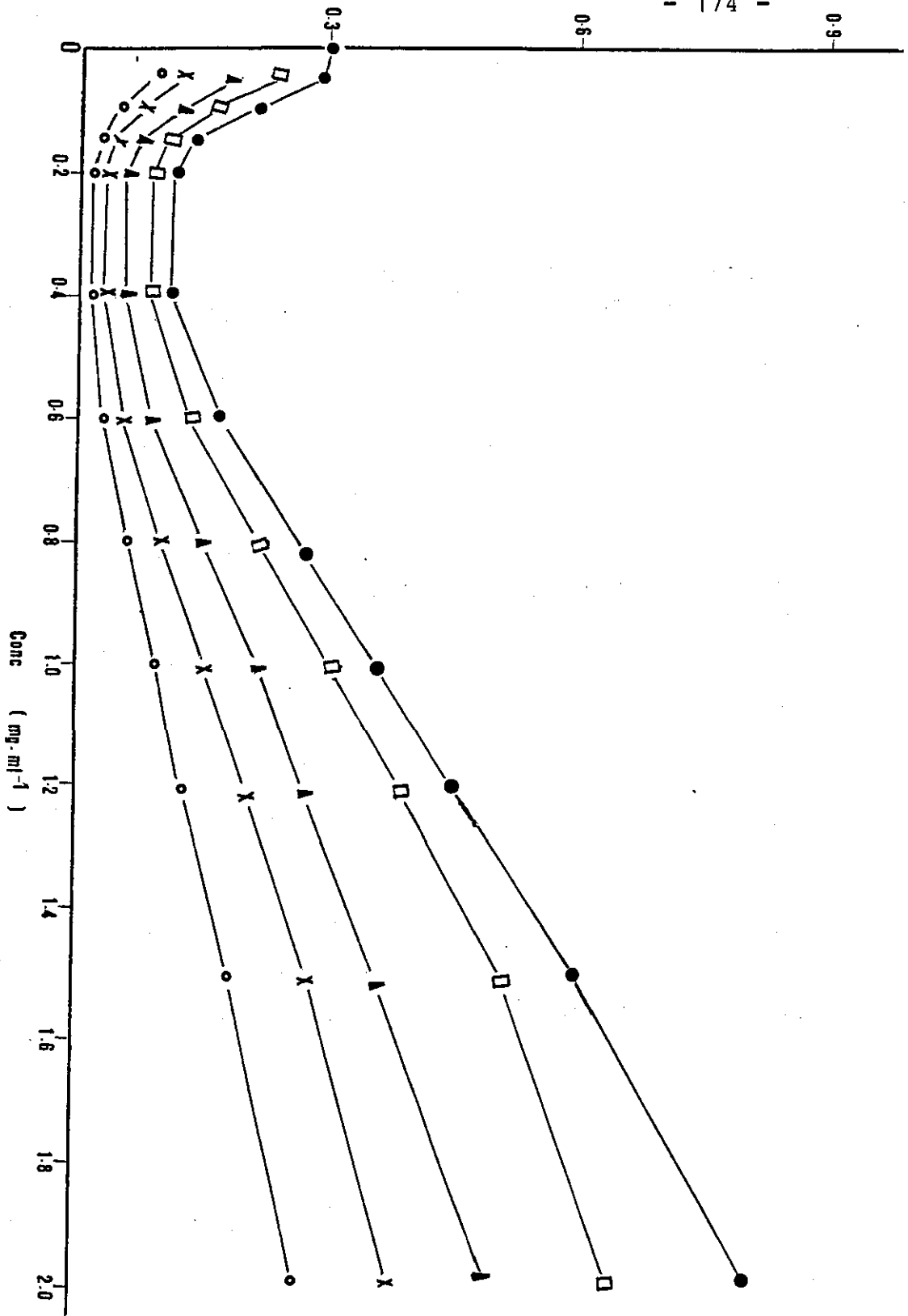


Fig. 3.83: Protection against the lytic effects of Propranolol by Ca^{2+} on rabbit erythrocyte suspended in hypotonic saline and phosphate buffer 0.05M pH 7.4 at 22°C.

Cell concentration 4.7×10^7 cells.ml⁻¹

Cation concentration 4.7×10^7 cells.ml⁻¹.

●-● Propranolol in the absence of Ca^{2+}

□ □ 0.5 mM Ca^{2+}

▲-▲ 2.5 mM Ca^{2+}

x-x 5.0 mM Ca^{2+}

O-O 10.0 mM Ca^{2+}

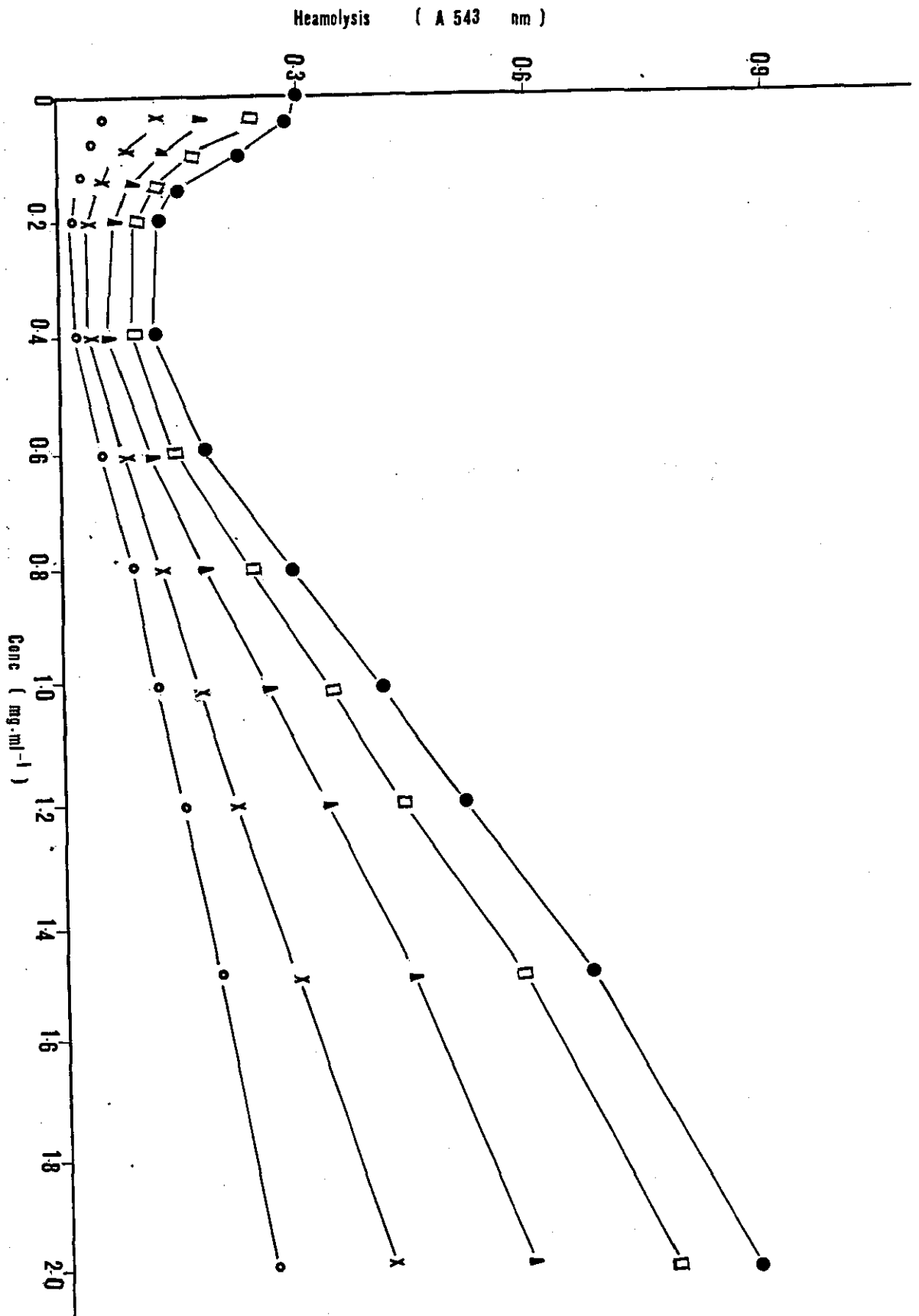


Fig. 3.84: Stabilizing and lytic effects of varying concentrations of saline on rabbit erythrocyte membrane suspended in phosphate buffer 0.05M pH 7.4 at 22°C.

Cell concentration 4.7×10^7 cells.ml⁻¹

●-● Stabilization and lysis by saline.

O-O Stabilization and lysis in the presence of saline and Ca²⁺ ion (2.5 mM).

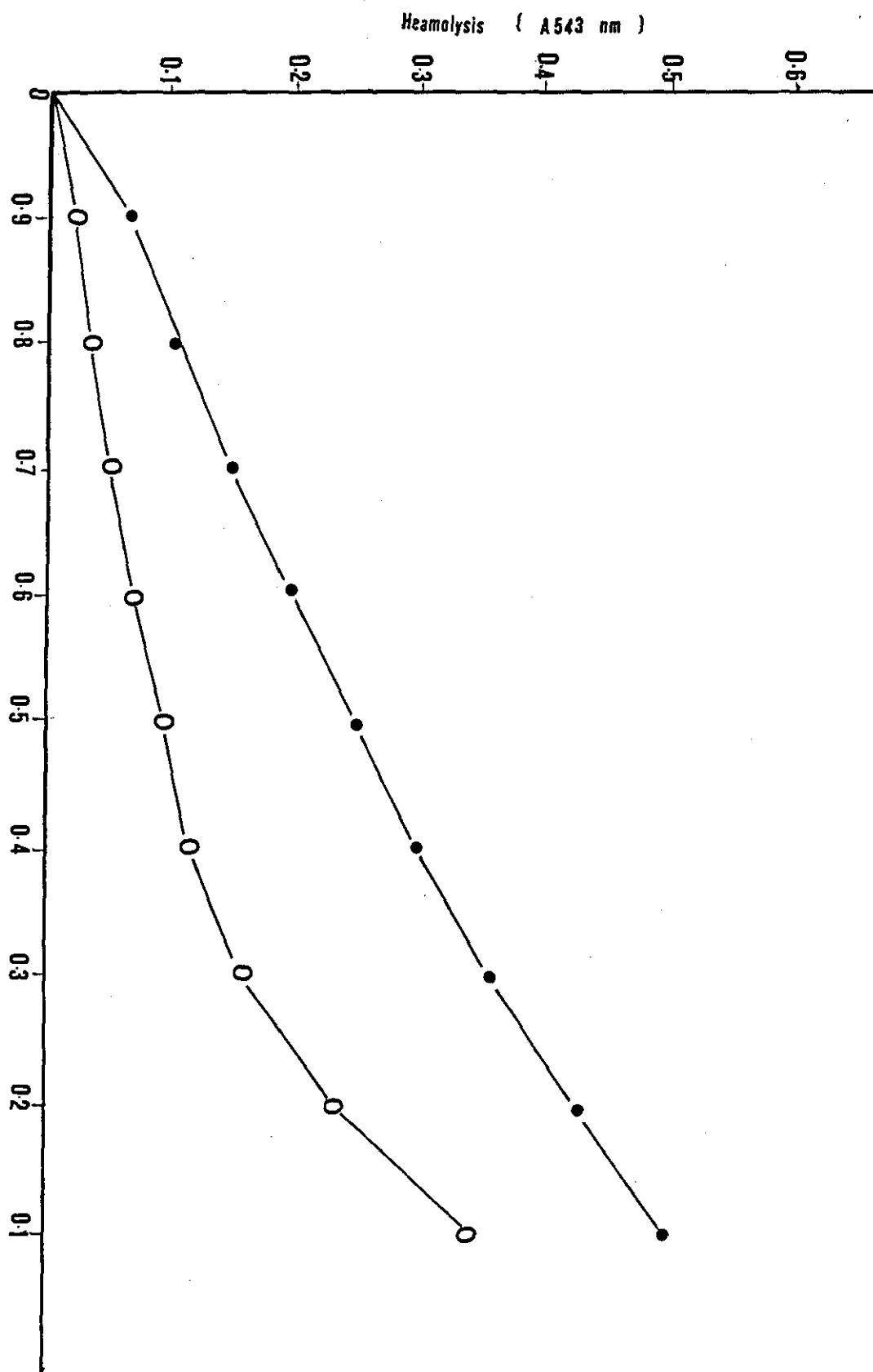


Fig. 3.85: The effect of HTAB on rabbit erythrocyte stability in hypotonic saline containing Propranolol (0.2 mg.ml^{-1}).

Temperature 22°C . Cell concentration $4.0 \times 10^7 \text{ cells.ml}^{-1}$.

O-O HTAB alone

O-O HTAB + 0.2 mg.ml^{-1} propranolol.

Haemolysis 543 nm

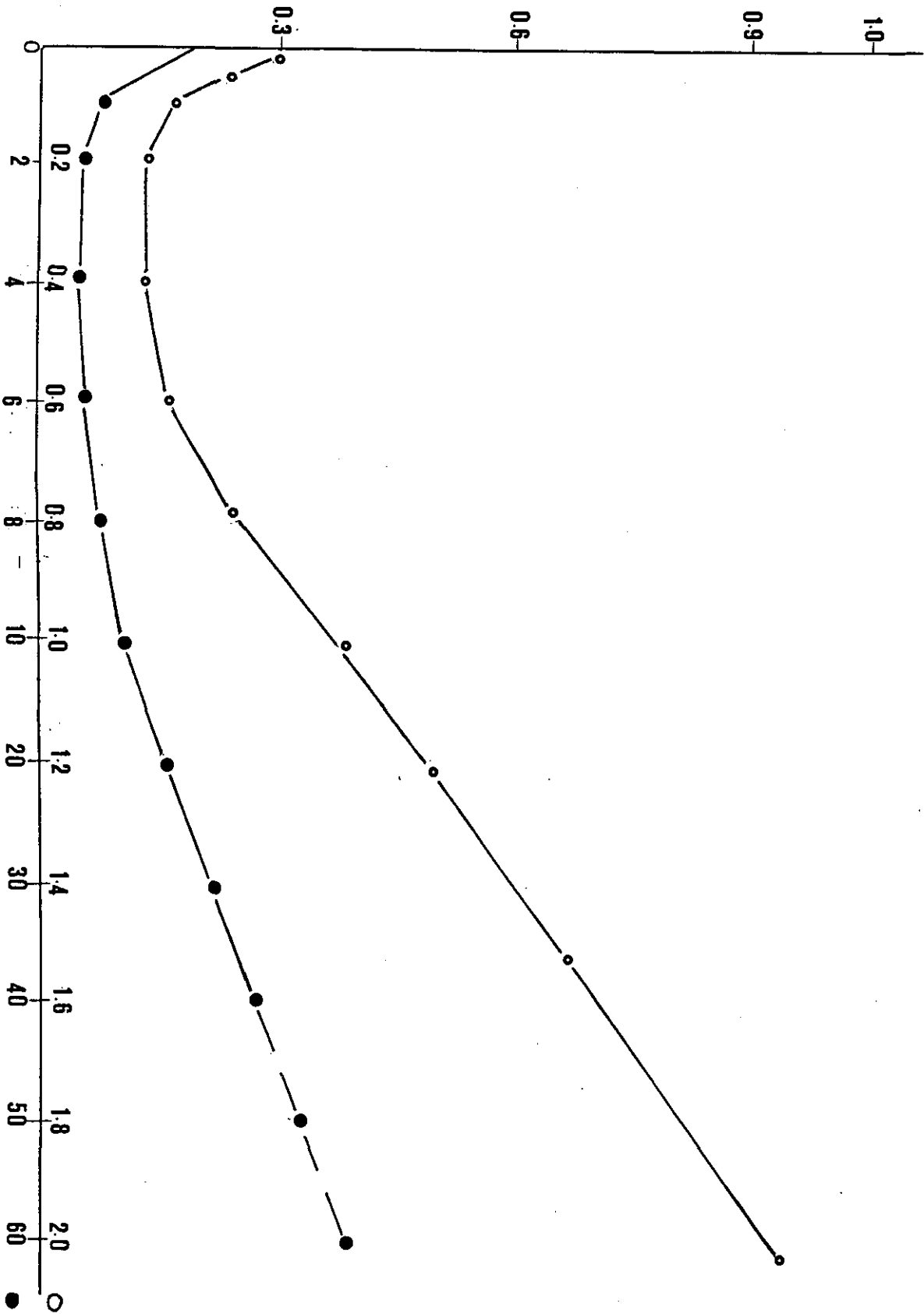


Fig. 3.86: Stabilizing and lytic effects of β -blockers on lysosomes suspended in solution containing 0.4% glycogen, 0.45M sucrose and 0.02M Tris-HCL buffer ph 7.4 at 37°C.

(A) Arylsulphatase

O-O Propranolol

●-● Atenolol

(B) β -glucuronidase

O-O Propranolol

●-● Atenolol

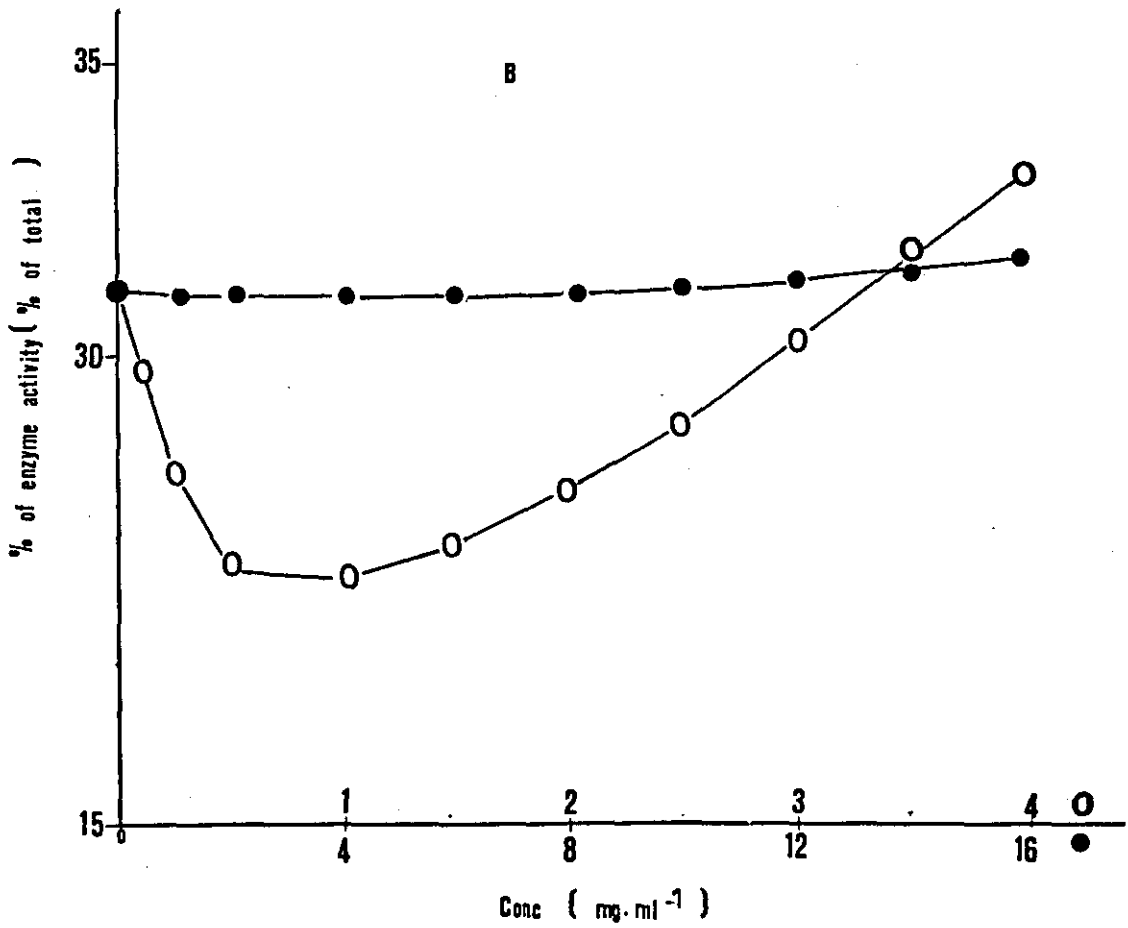
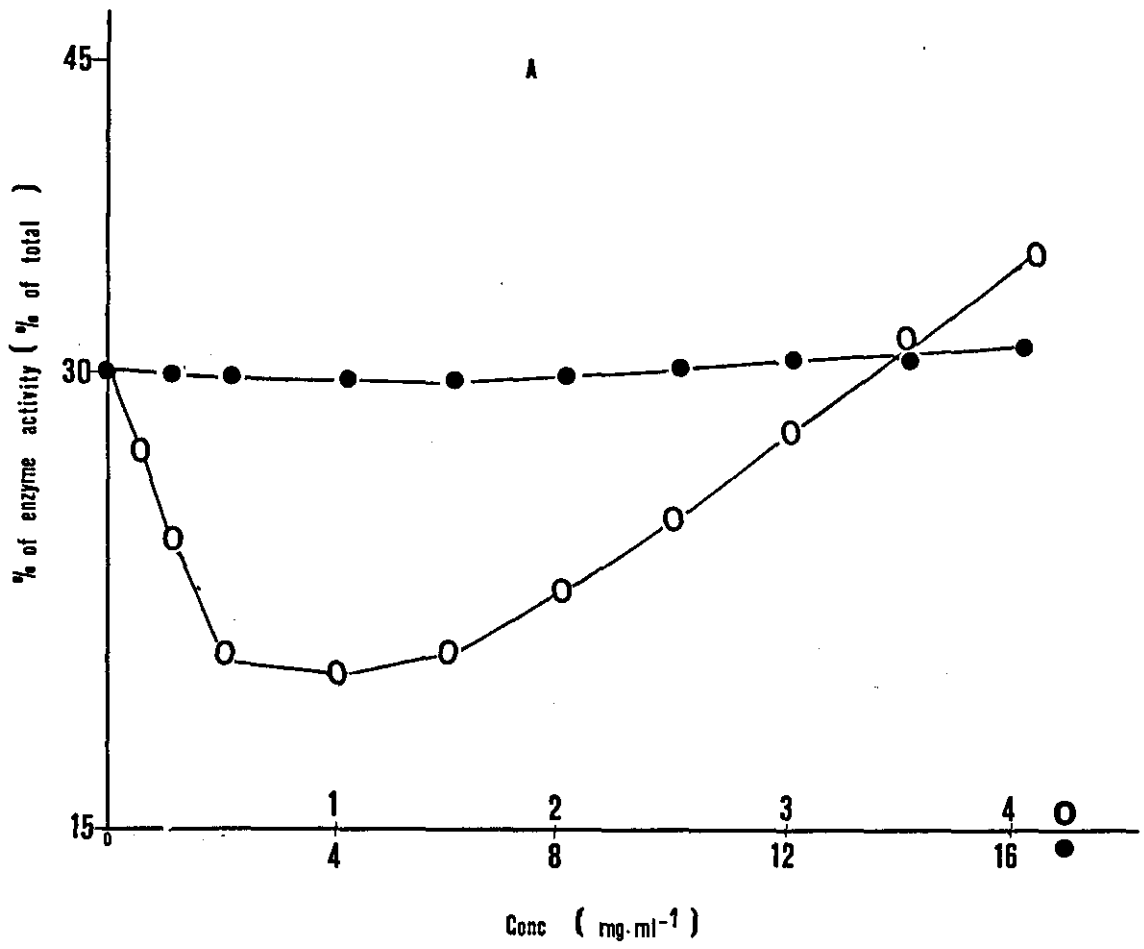


Fig. 3.87: Stabilizing and lytic effects of β -blockers on acid phosphatase enzyme suspended in solution containing 0.4% glycogen, 0.45M sucrose and 0.02M Tris-HCL buffer pH 7.4 at 37°C.

Drug concentration

●-● Atenolol

O-O Propranolol

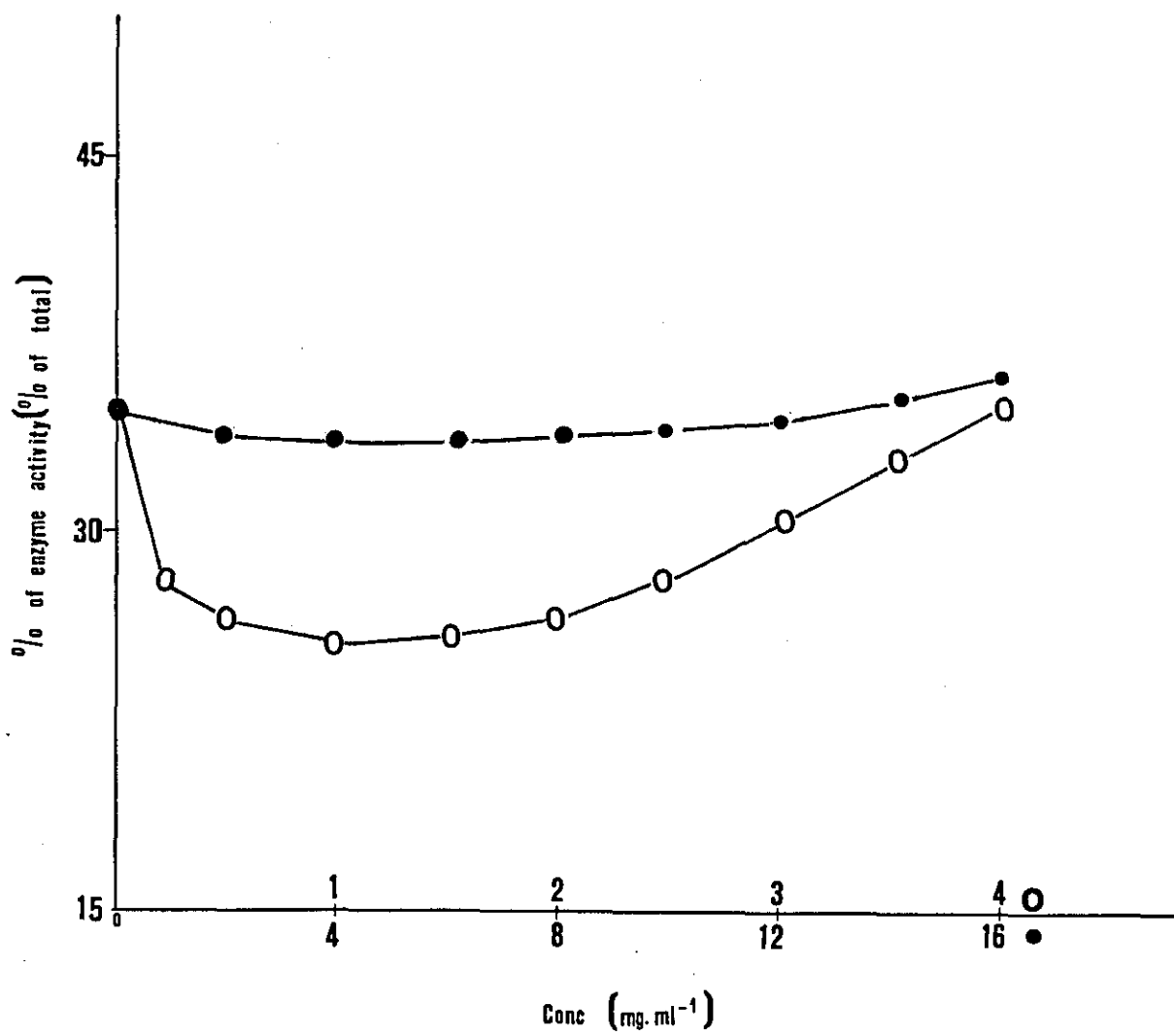


Fig. 3.88: Changes in the turbidity of cells of E.coli suspended in CFM containing different concentrations of β -blockers at 22°C. Cell concentration 4.2×10^9 cells.ml⁻¹. Contact time 10 min.

(A)

●-● Propranolol

Δ-Δ Atenolol

O-O .Practolol

(B)

●-● Oxprenolol

Δ-Δ Metaprolol

o-o Acebutolol

- 180 -

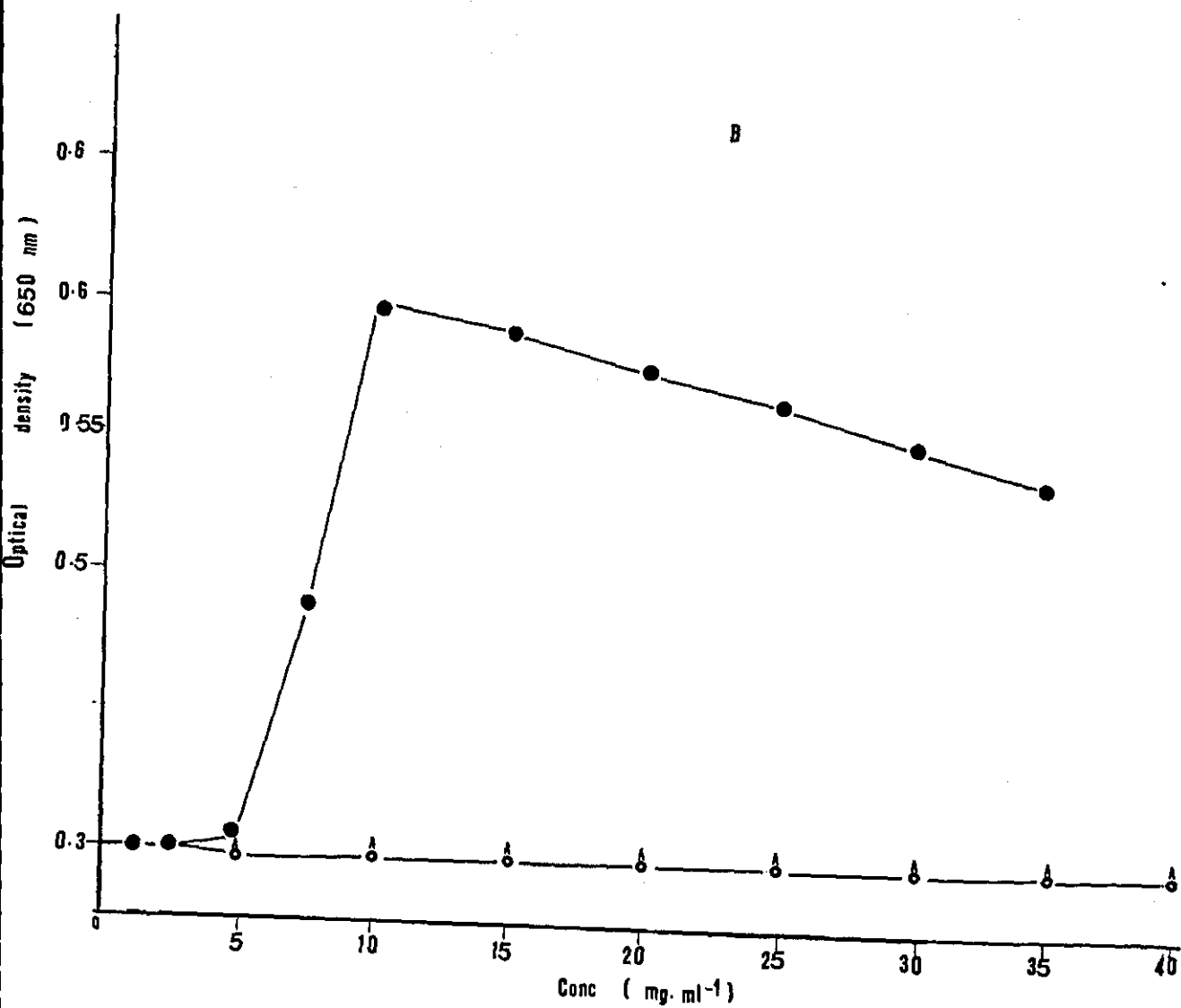
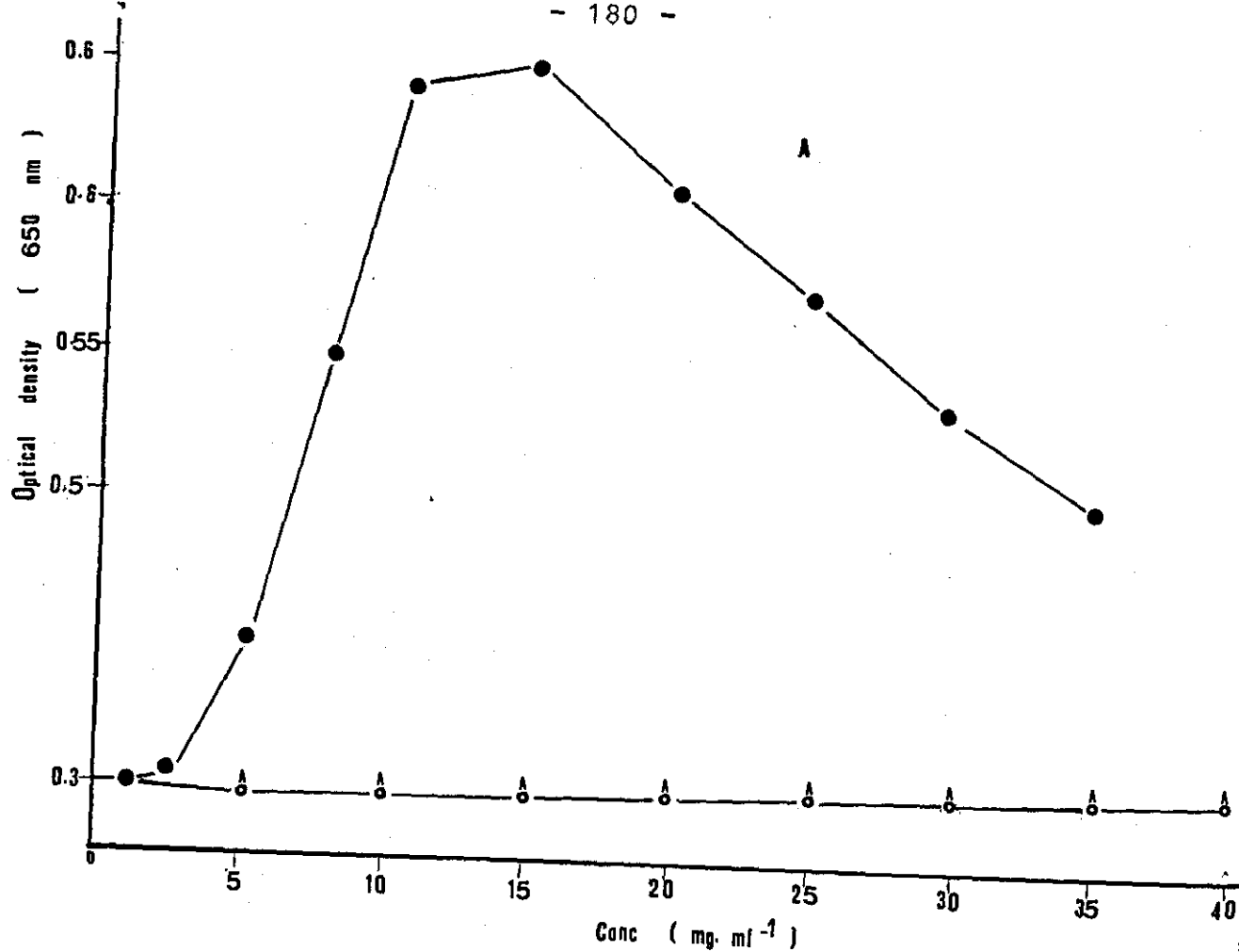


Fig. 3.89: Changes in the turbidity of cells of (A) E.coli and (B) C.albicans suspended in CFM containing different concentrations of local anaesthetics and β -blockers at 22°C. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

●-● Tetracaine
Δ-Δ Procaine
o-o Procainamide

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

●-● Propranolol
Δ-Δ Atenolol
o-o Practolol

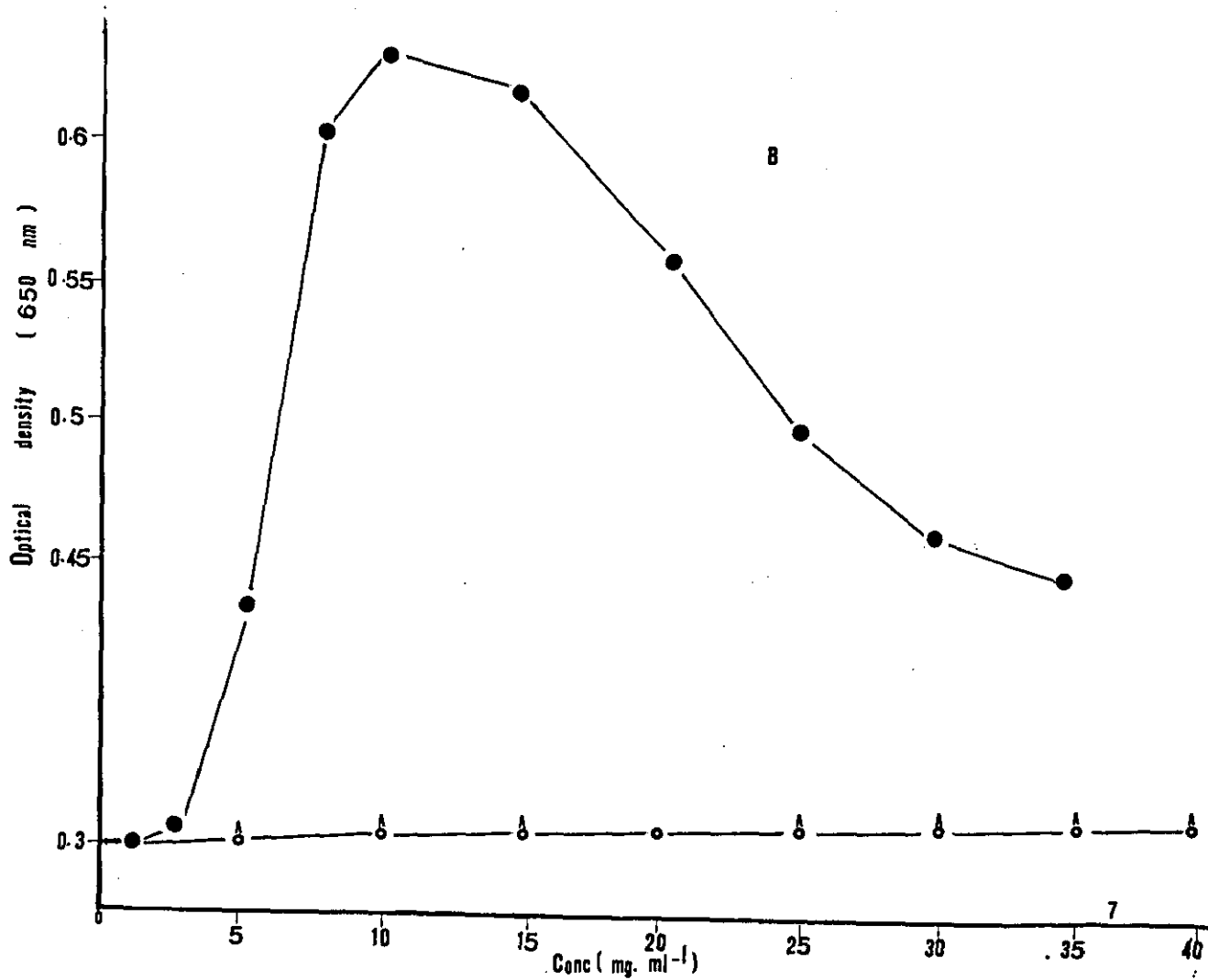
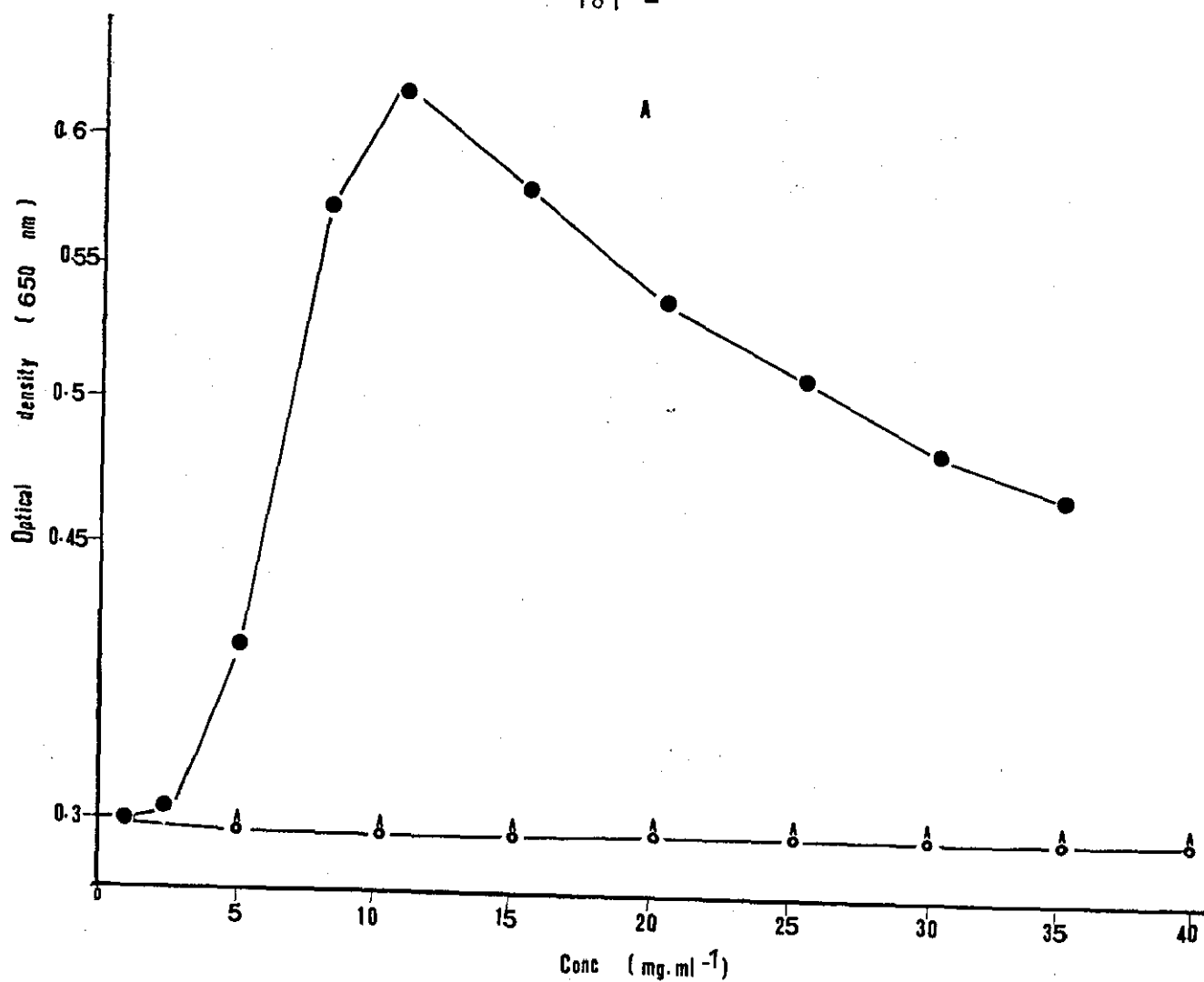


Fig. 3.90: Changes in the turbidity of cells of C.albicans suspended in CFM containing different concentrations of local anaesthetics and β -blockers at 22°C. Cell concentration 3.8×10^5 cells.ml⁻¹.

(A)

●-● Oxprenolol
Δ-Δ Metoprolol
o-o Acebutolol

(B)

●-● Tetracaine
Δ-Δ Procaine
o-o Procainamide

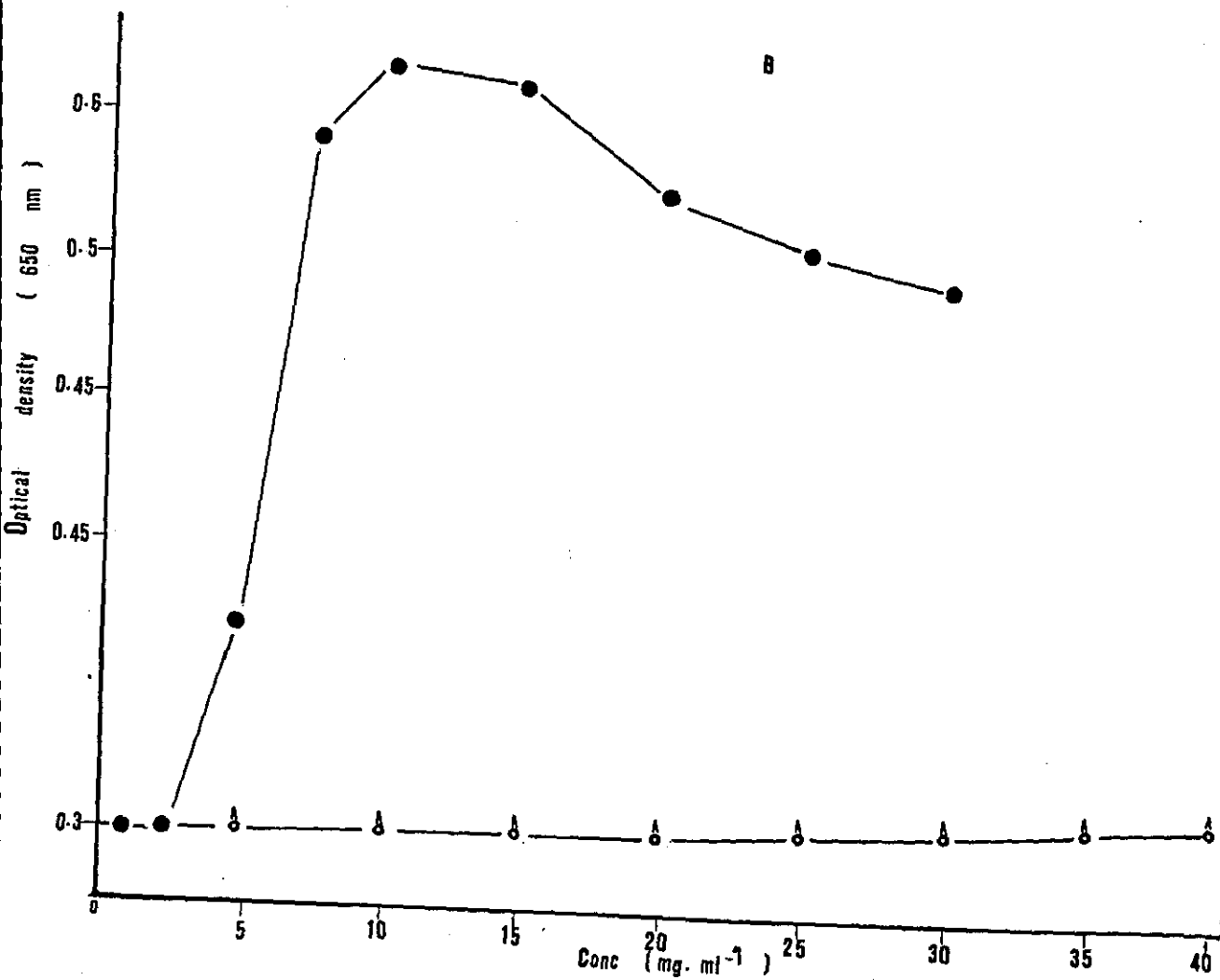
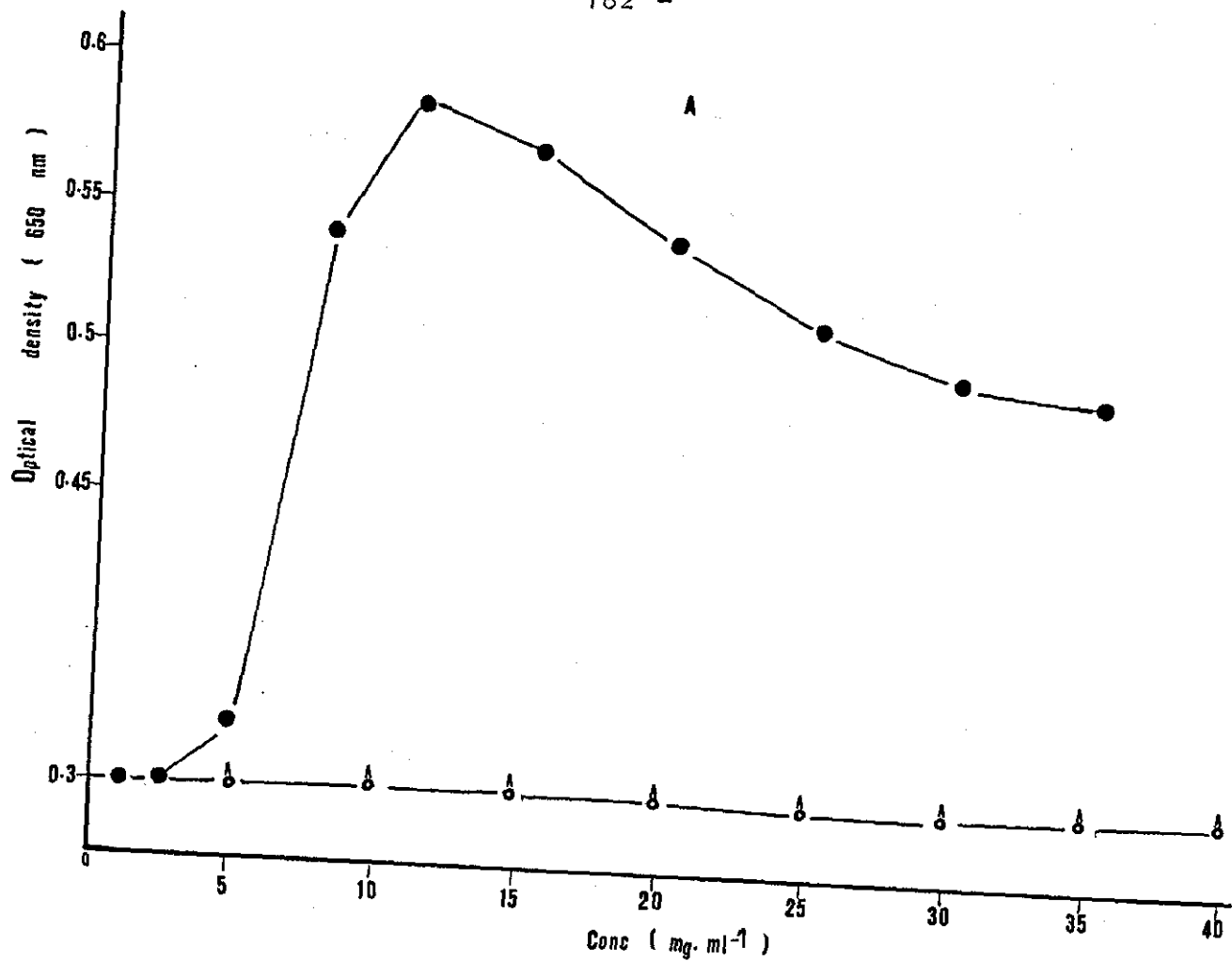


Fig. 3.91: Changes in the turbidity of cells of Ps.aeruginosa suspended in CFM containing different concentrations of β -blockers at 22°C. Cell concentration 3.9×10^8 cells.ml⁻¹. Contact time 10 min.

(A)

●-● Propranolol
Δ-Δ Atenolol
o-o Practolol

(B)

●-● Oxprenolol
Δ Δ Metoprolol
o-o Acebutolol

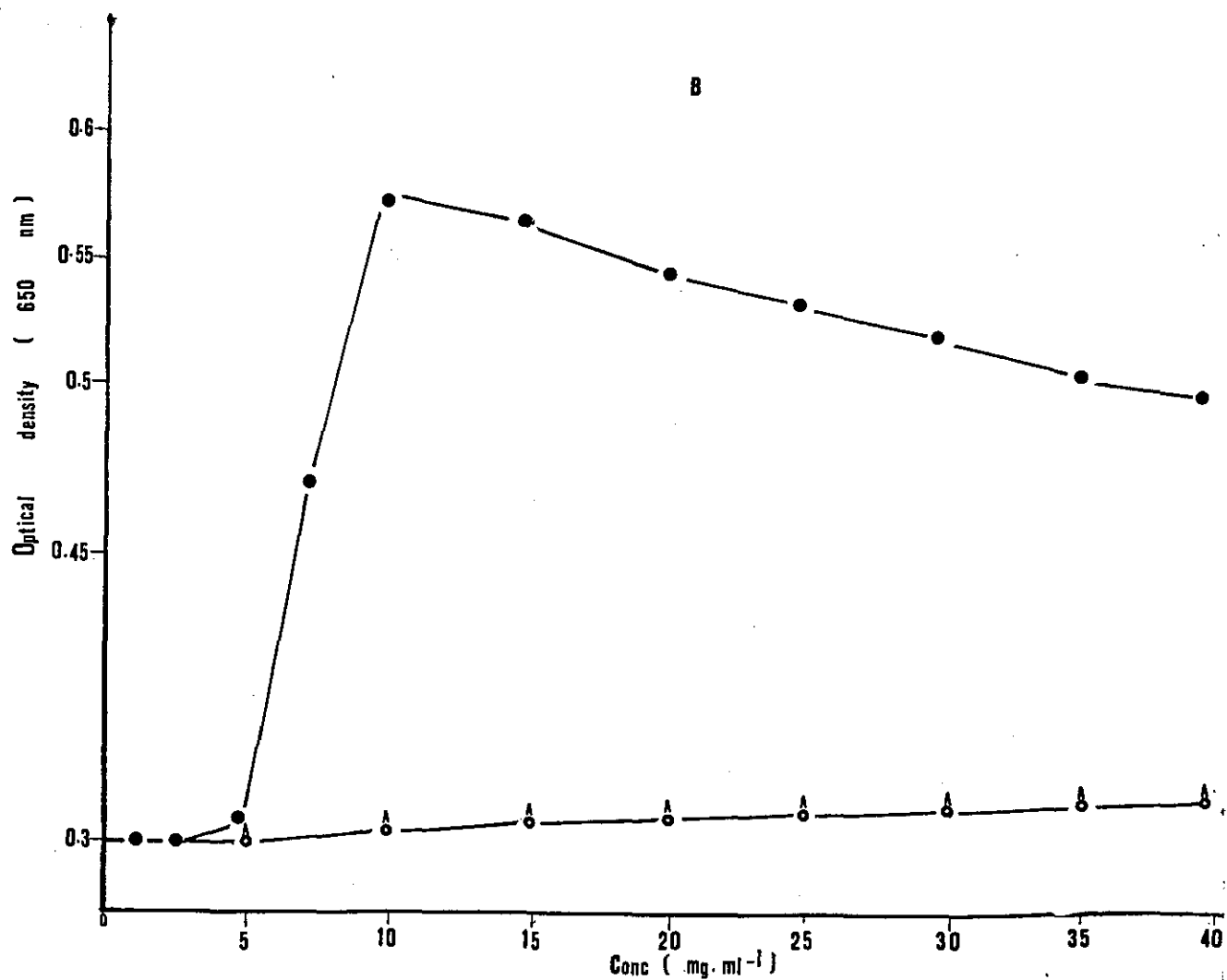
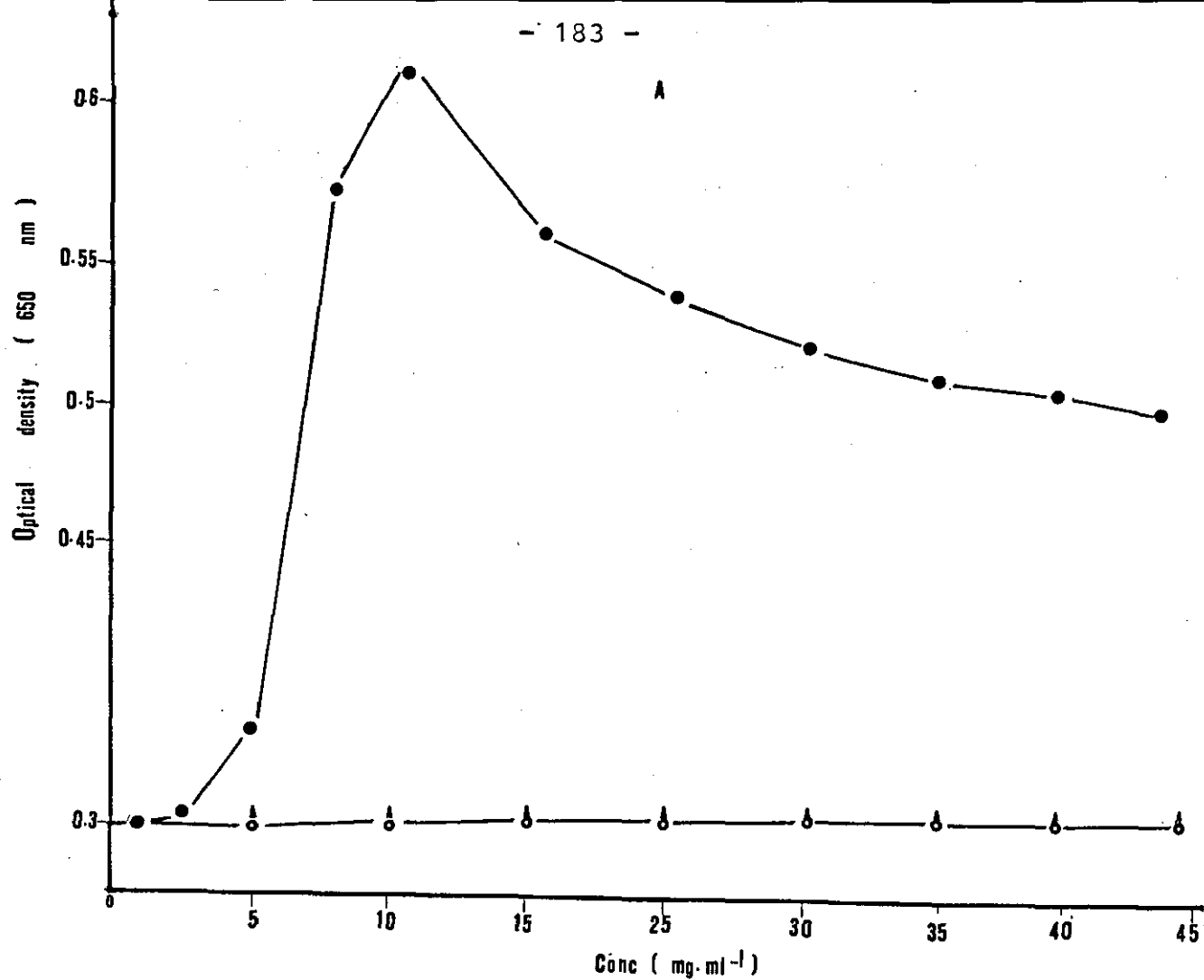


Fig. 3.92: Changes in the turbidity of cells of (A) Ps.aeruginosa and (B) B.cereus suspended in CFM containing different concentrations of local anaesthetics and β -blockers. Contact time 10 min.

(A)

Cell concentrations 3.9×10^8 cells.ml⁻¹

●-● Tetracaine
Δ Δ Procaine
o-o Procainamide

(B)

Cell concentrations 4.0×10^9 cells.ml⁻¹

●-● Propranolol
Δ-Δ Atenolol
o-o Procainamide

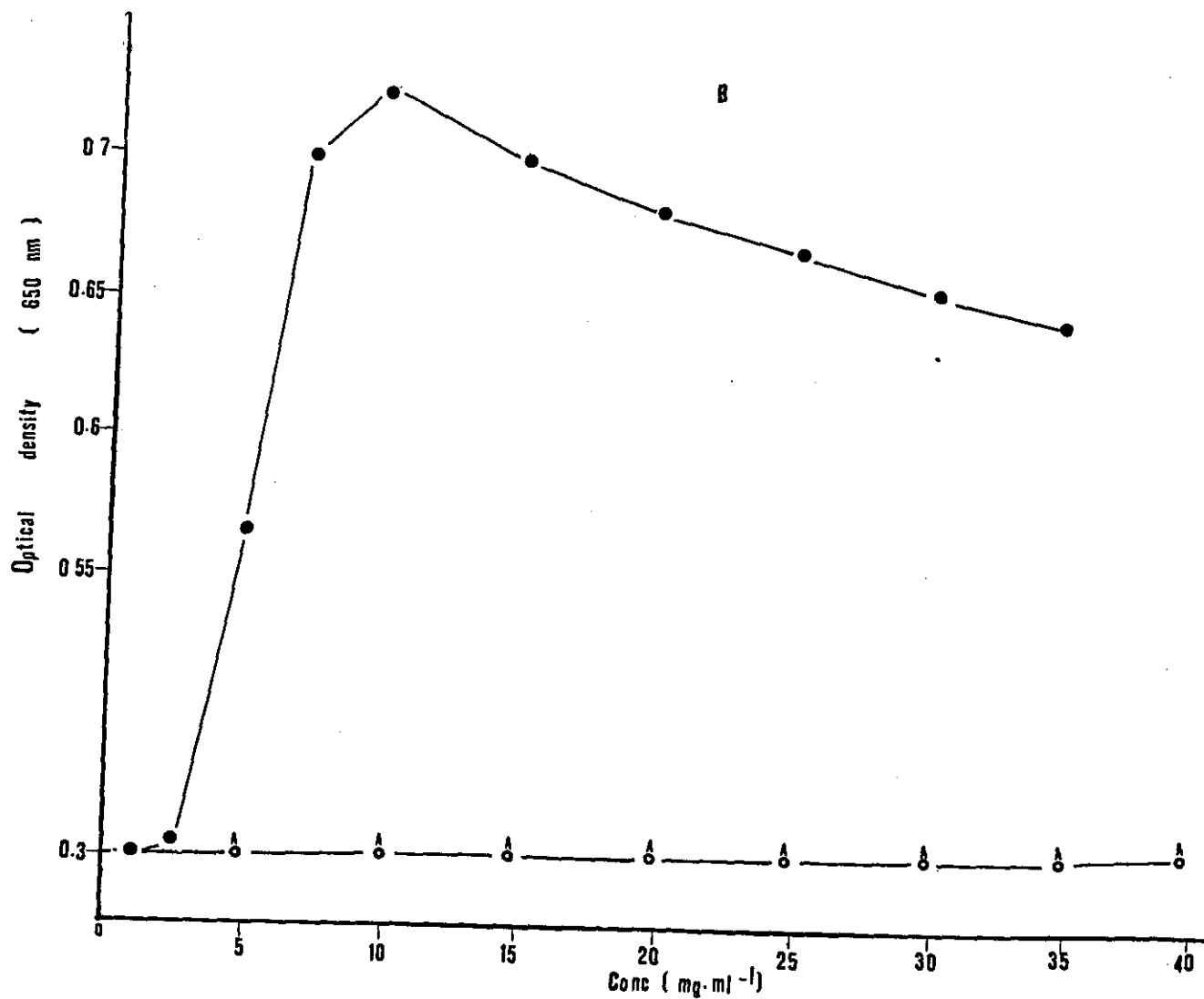
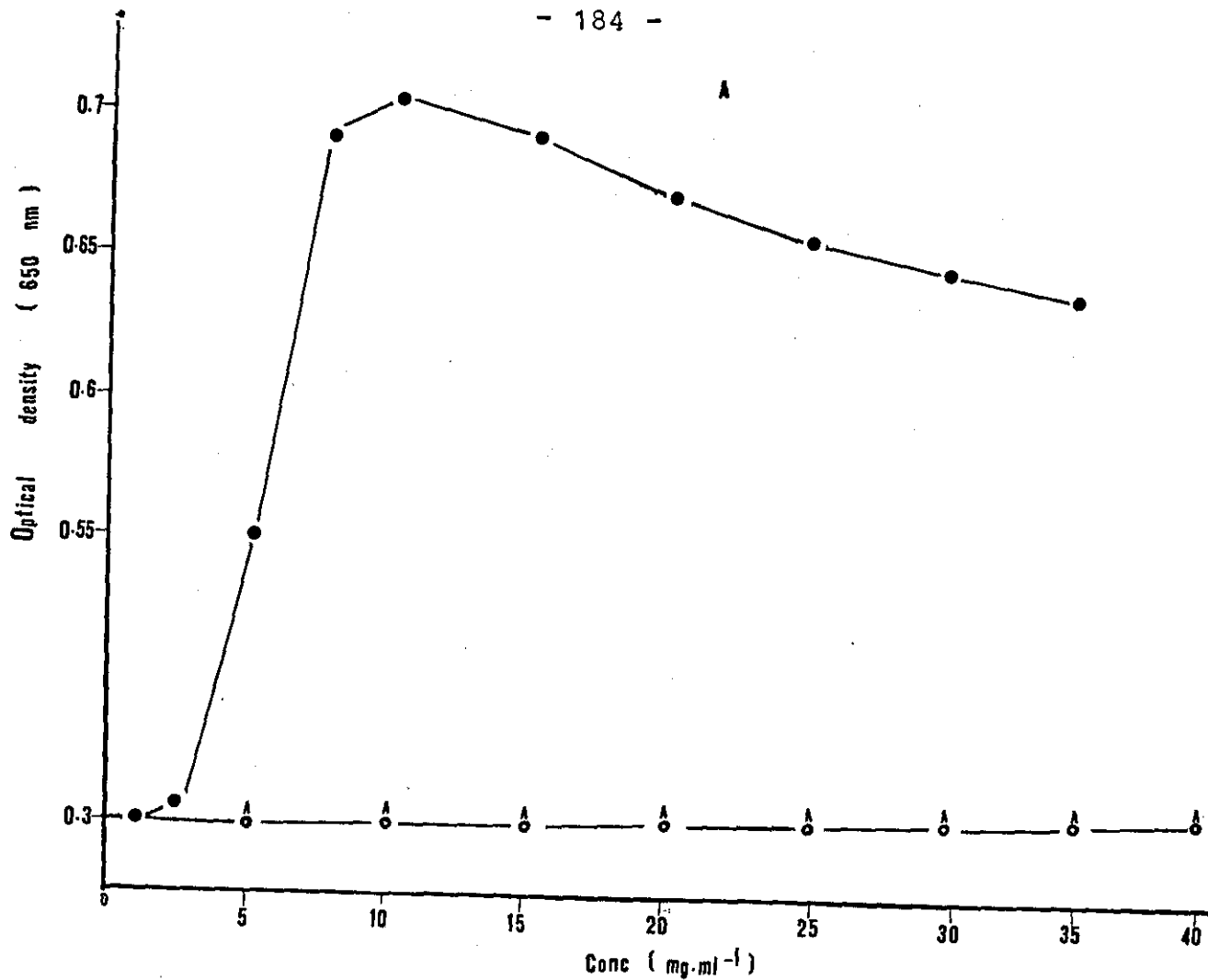


Fig. 3.93: Changes in the turbidity of cells of B.cereus suspended in CFM containing different concentrations of β -blockers at 22°C. Cell concentration 4.0×10^9 cells.ml⁻¹.

(A)

●-● Oxprenolol
Δ-Δ Metoprolol
o-o Acebutolol

(B)

●-● Tetracaine
Δ-Δ Procaine
o-o Procainamide

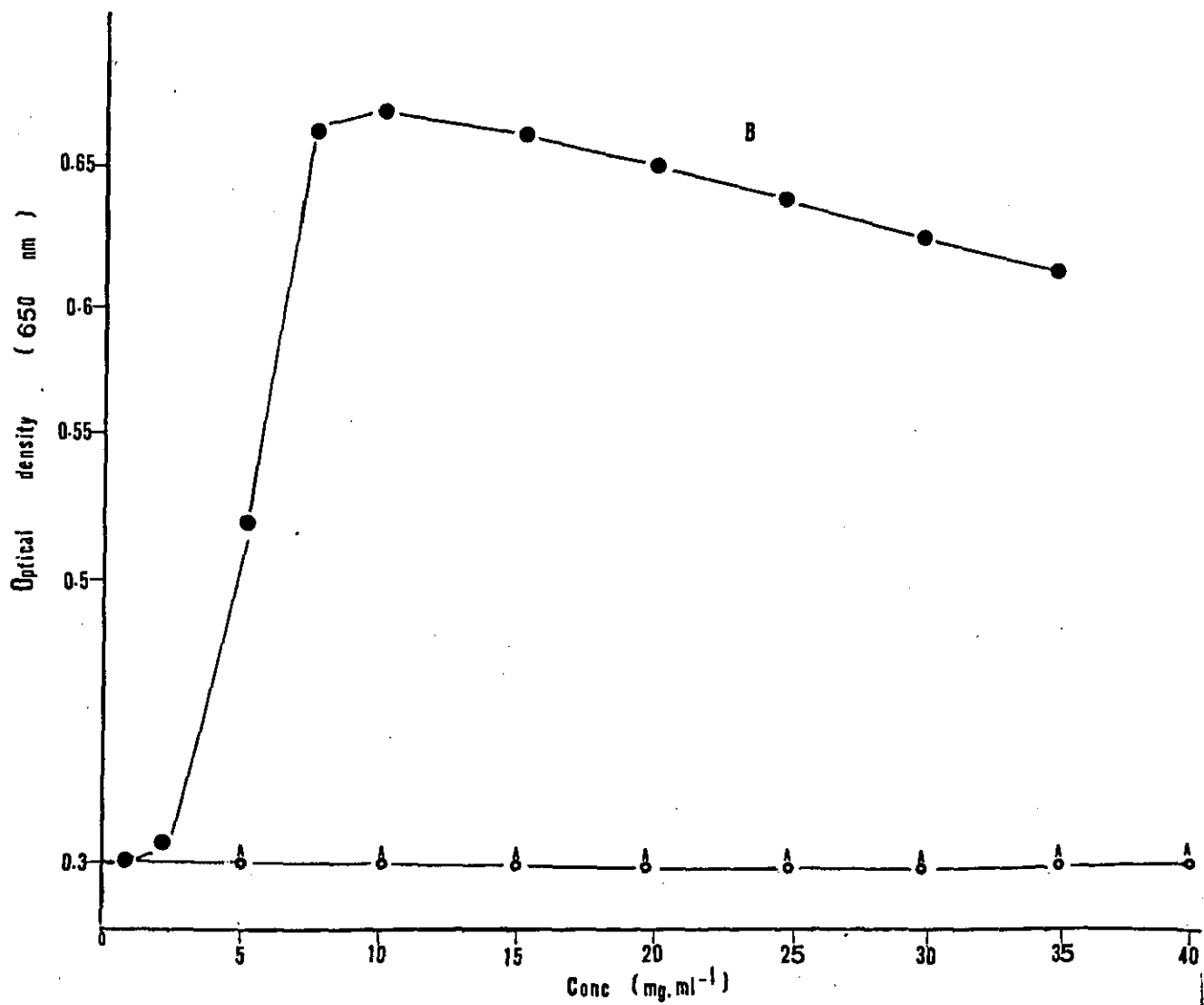
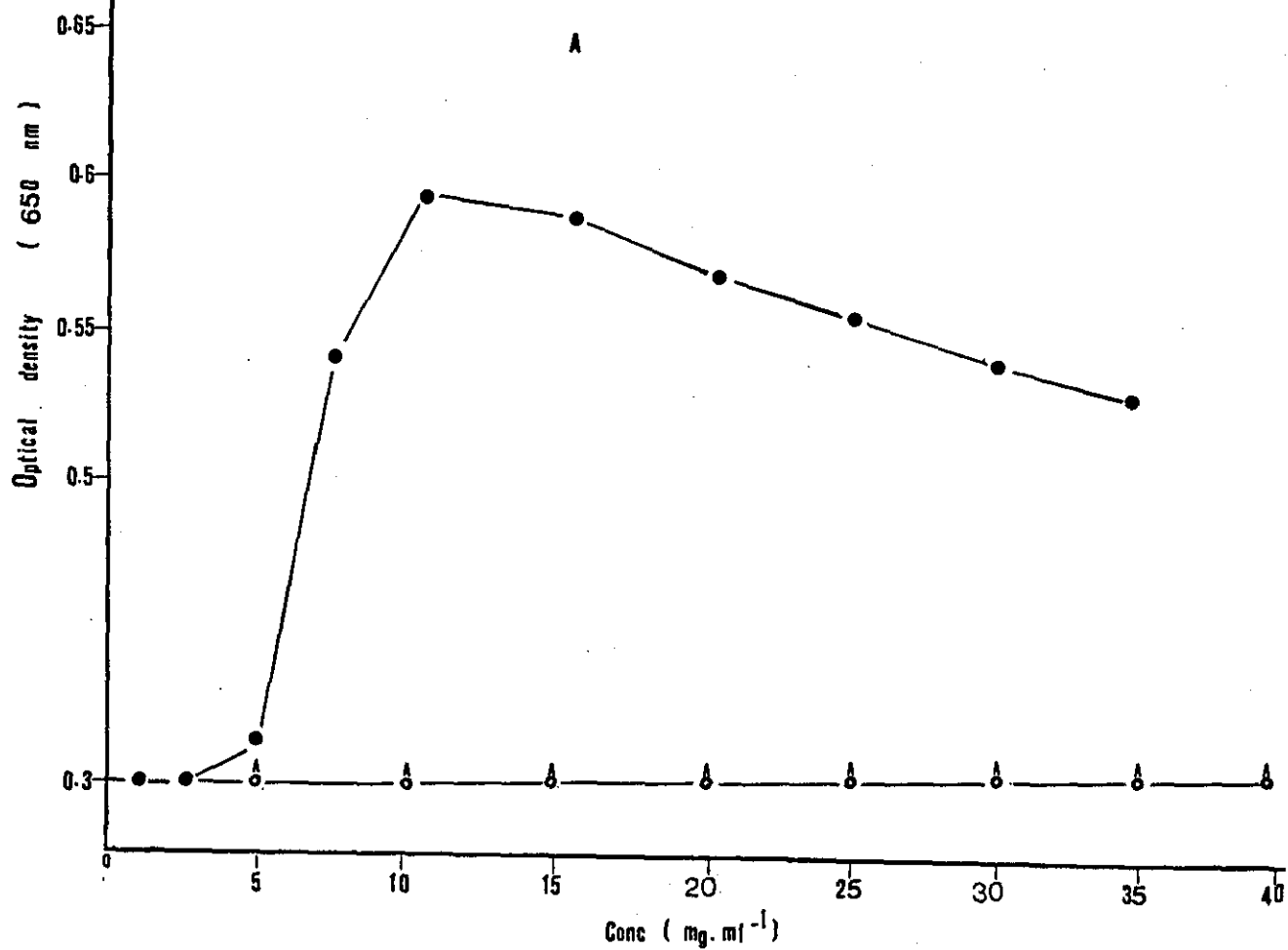


Fig. 3.94: The effect of time on the turbidity changes in cells of (A) E.coli and (B) C.albicans suspended in CFM containing different concentrations of propranolol, practolol and atenolol at 22°C. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

●-● Propranolol (7.5 mg.ml⁻¹)

Δ-Δ Atenolol (20 mg.ml⁻¹)

o-o Practolol (20.0 mg.ml⁻¹)

(B)

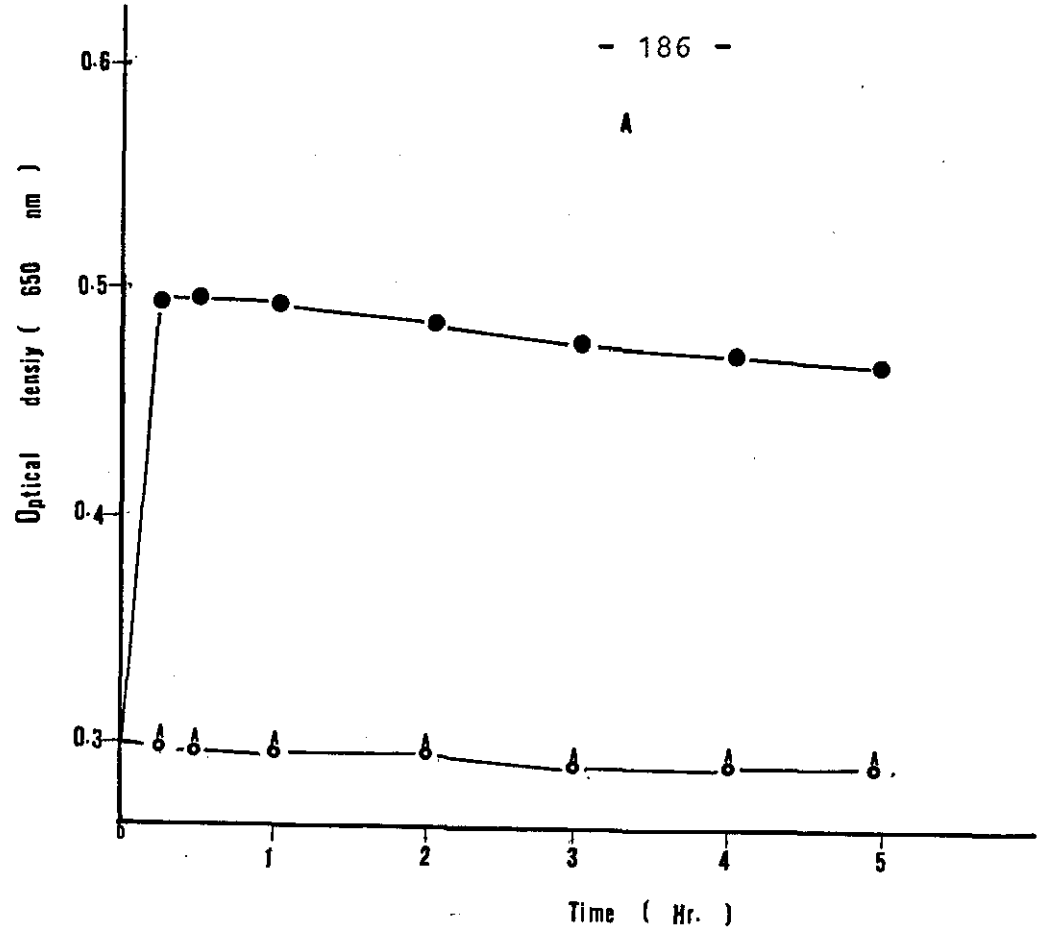
Cell concentration 3.8×10^5 cells.ml⁻¹

●-● Propranolol (7.5 mg.ml⁻¹)

Δ-Δ Atenolol (20.0 mg.ml⁻¹)

o-o Practolol (20.0 mg.ml⁻¹)

A



B

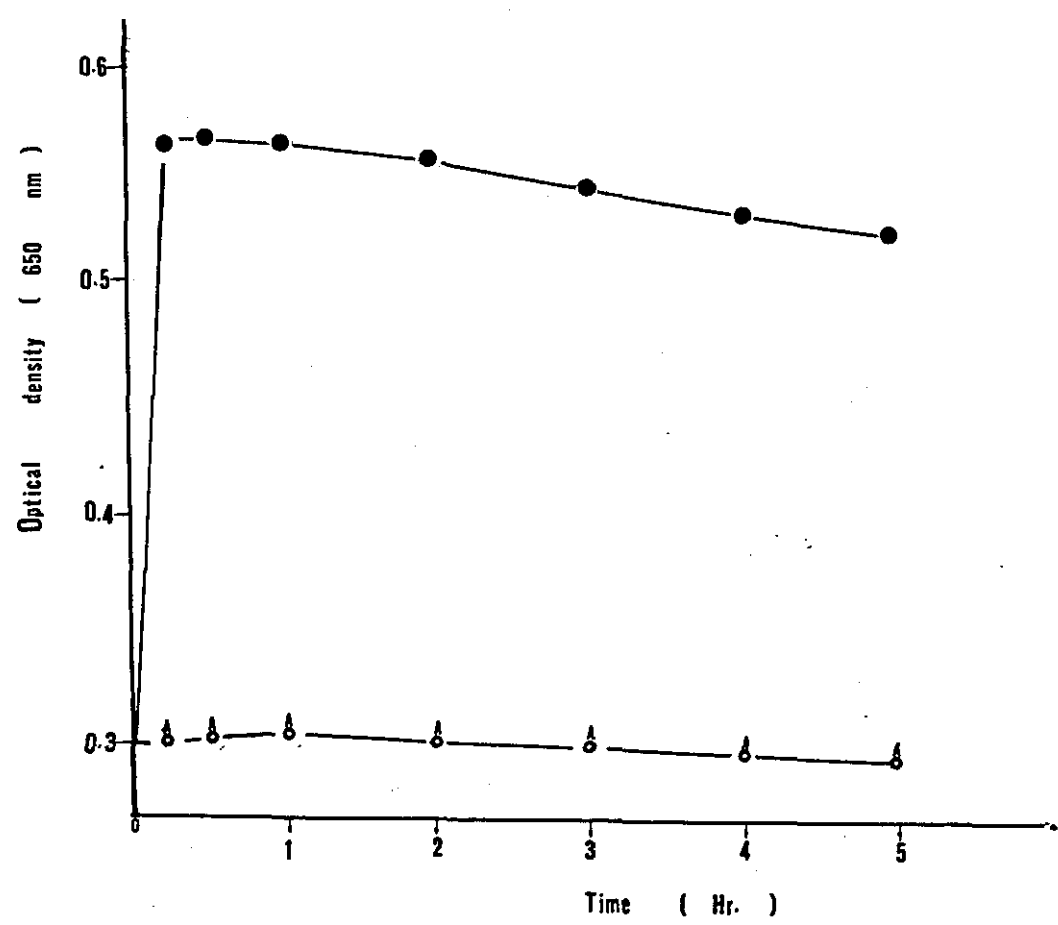


Fig. 3.95: Changes in the turbidity of cells of (A) E.coli and (B) C.albicans suspended in (0.2M) phosphate buffer pH 7.4 containing different concentrations of Propranolol. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

0-0 Propranolol

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

●-● Propranolol

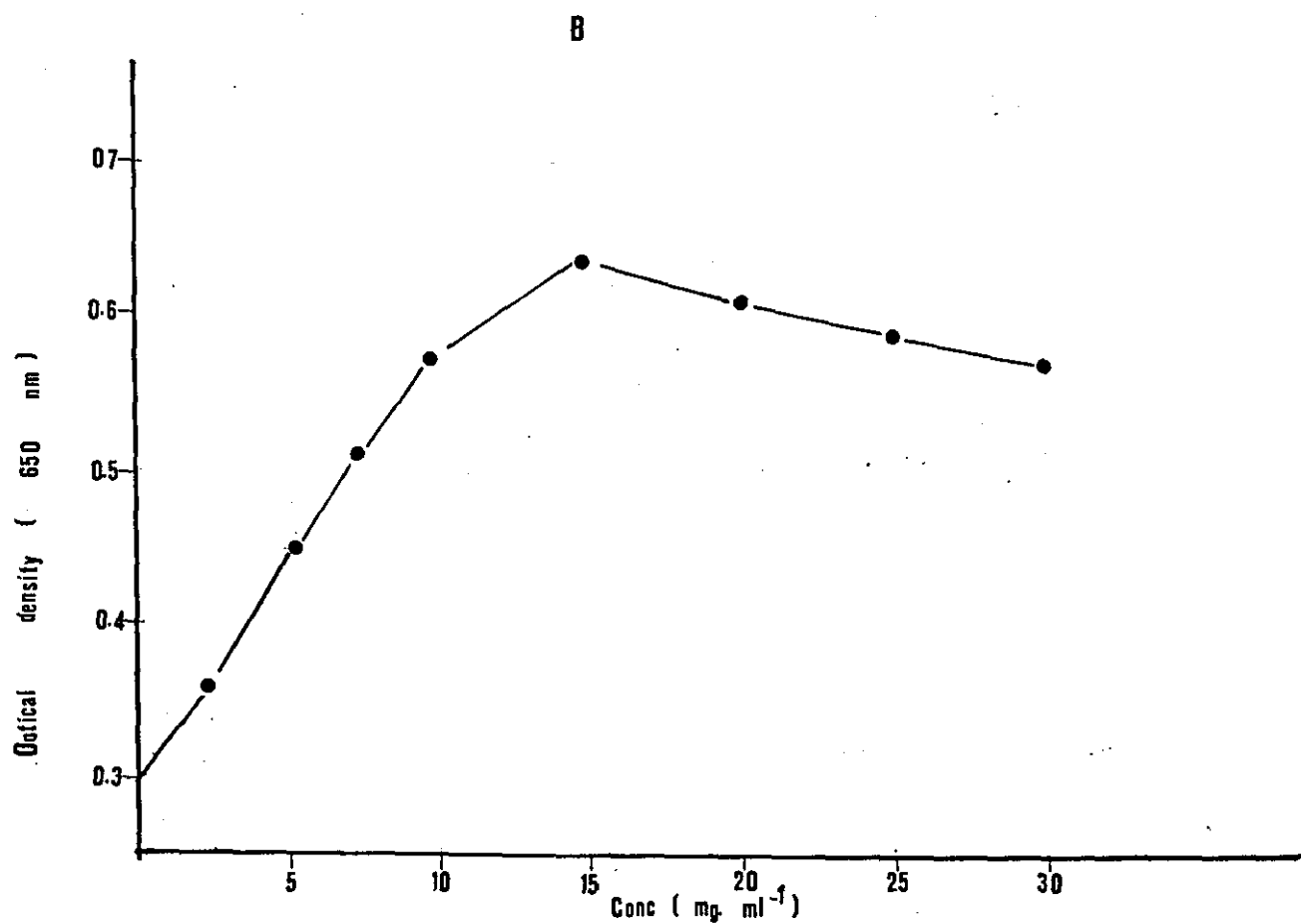
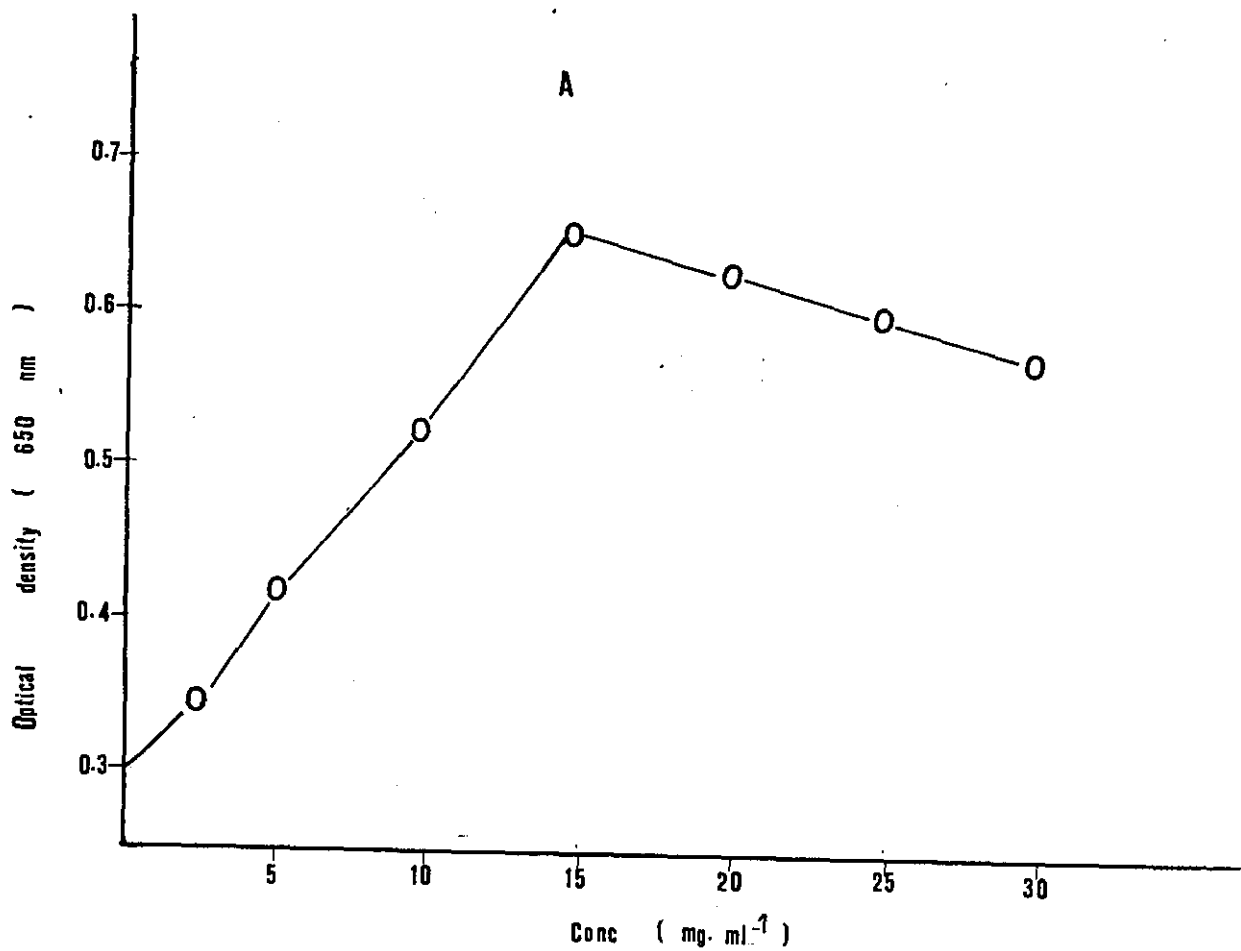


Fig. 3.96: Changes in the turbidity of cells of (A) E.coli and (B) C.albicans suspended in water containing different concentrations of Propranolol. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

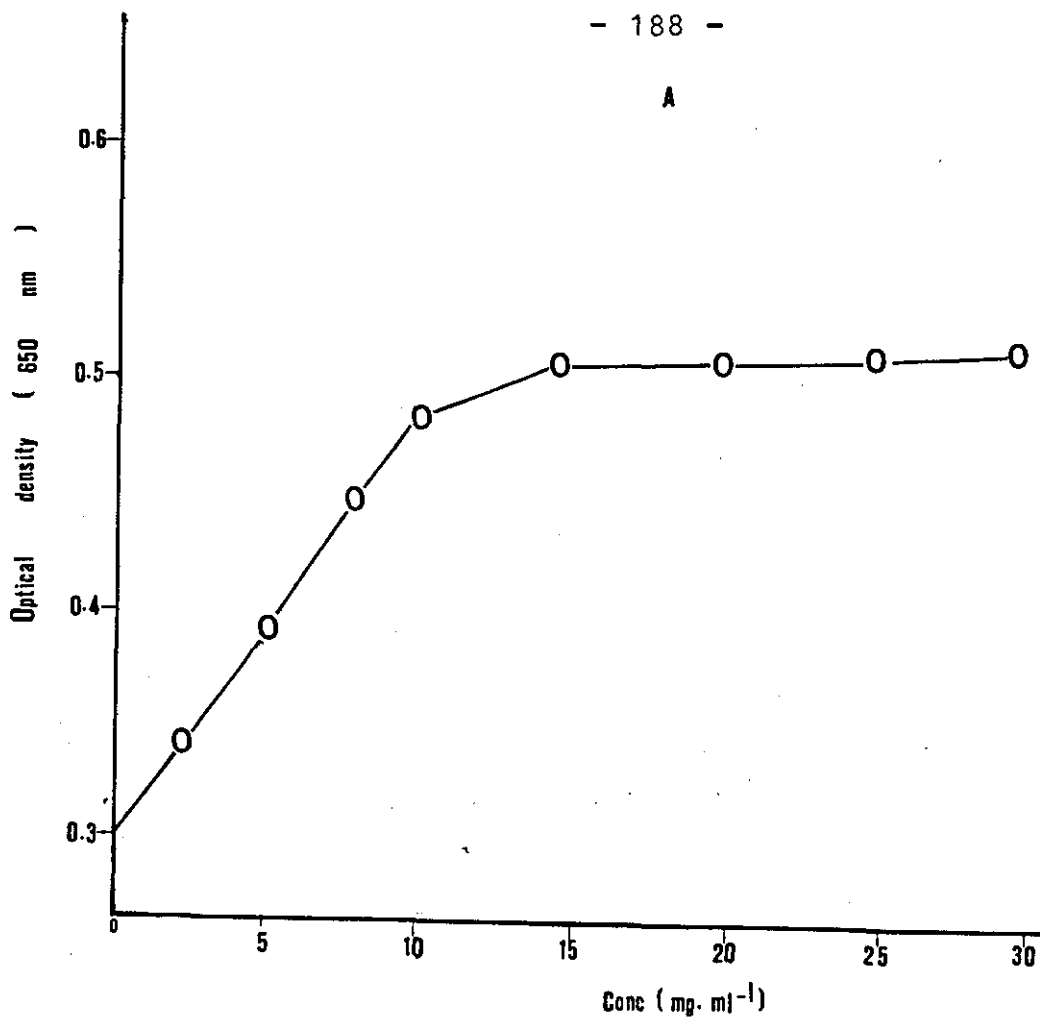
O-O Propranolol

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

●-● Propranolol

A



B

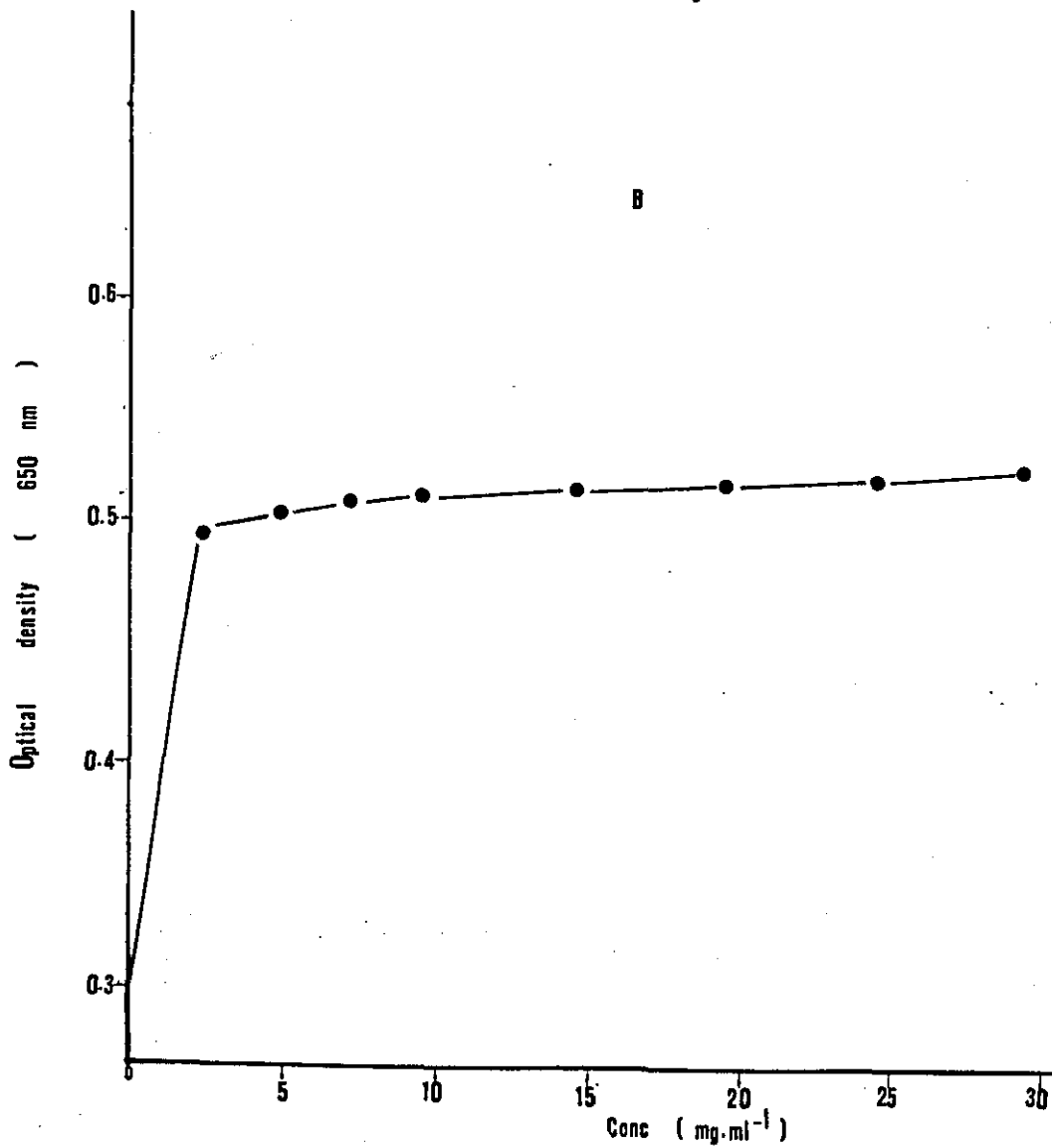


Fig. 3.97: The influence of divalent cations on the changes of turbidity of cells of (A) E.coli and (B) C.albicans suspended in CFM containing different concentrations of Propranolol at 22°C. Concentration of divalent cations 0.001M. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

⊗-⊗ Propranolol in the absence of divalent cation

Δ-Δ Propranolol in the presence of Mg²⁺

x-x Propranolol in the presence of Ca²⁺

o-o Propranolol in the presence of Zn²⁺

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

●-● Propranolol in the absence of divalent cation

▲-▲ Propranolol in the presence of Mg²⁺

x-x Propranolol in the presence of Ca²⁺

o-o Propranolol in the presence of Zn²⁺

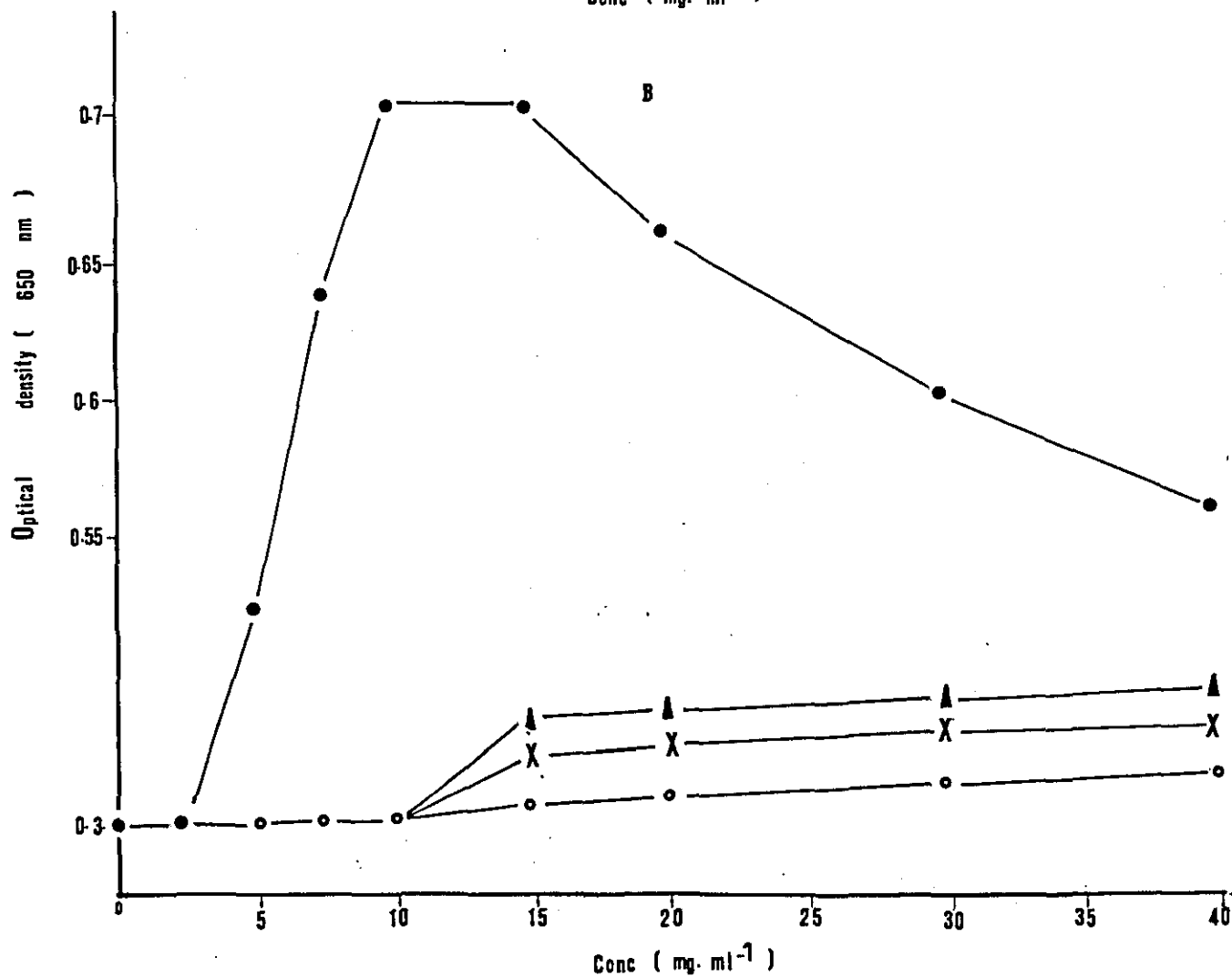
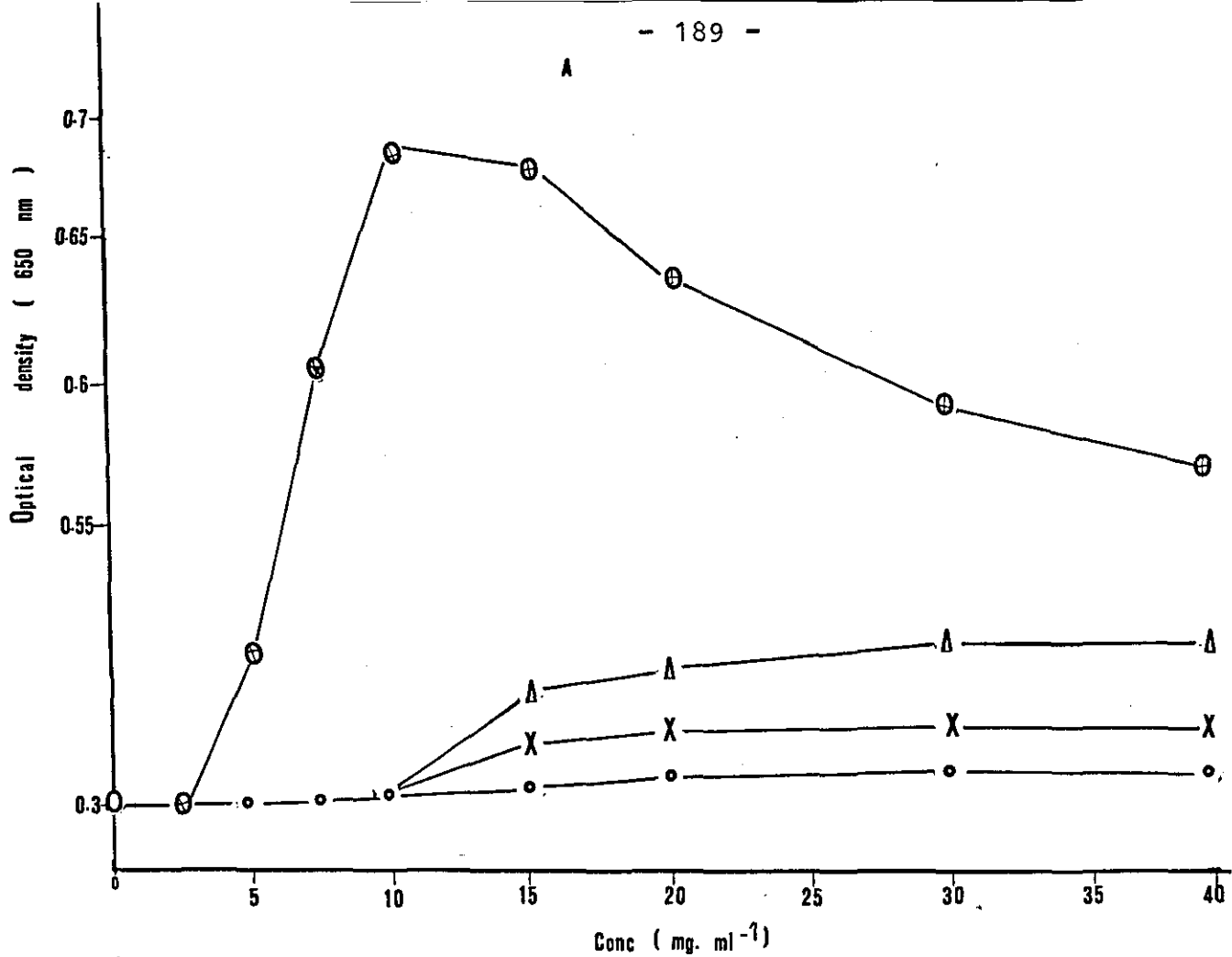


Fig. 3.98: The effect of different concentrations of divalent cations on the turbidity increase caused by 7.5 mg.ml^{-1} Propranolol on cells of (A) E.coli and (B) C.albicans suspended in CFM at 22°C . Contact time 10 min.

(A)

Cell concentration $4.2 \times 10^9 \text{ cells.ml}^{-1}$

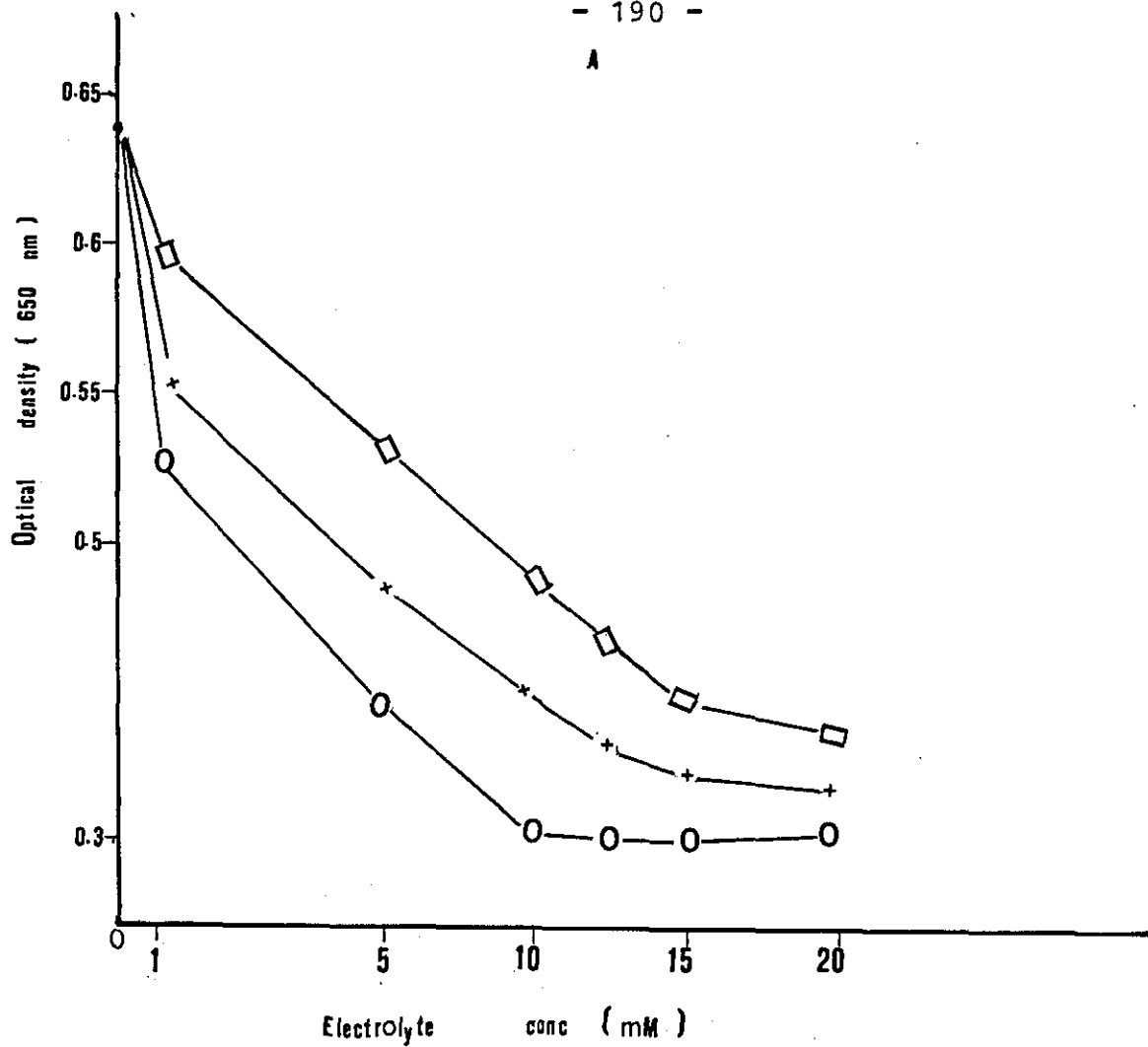
□-□ Mg^{2+}
 +-+ Ca^{2+}
 O-O Zn^{2+}

(B)

Cell concentration $3.8 \times 10^5 \text{ cells.ml}^{-1}$

▲-▲ Mg^{2+}
 O-O Ca^{2+}
 ●-● Zn^{2+}

A



B

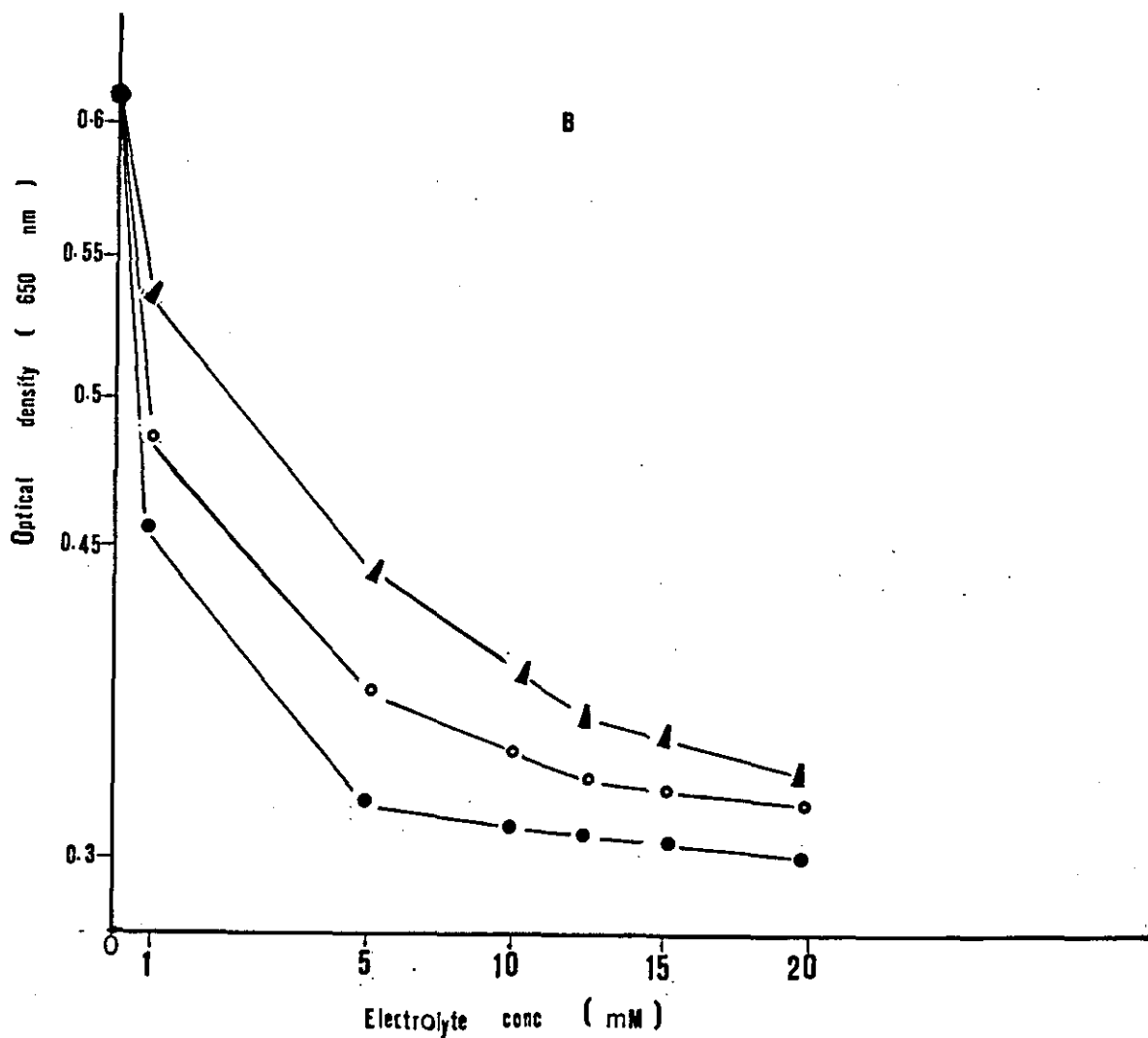


Fig. 3.99: The effect of Propranolol and Atenolol on the turbidity changes of cell envelope and intracellular preparation of (A) E.coli and (B) C.albicans suspended in CFM at 22°C. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

- Intracellular materials exposed to Propranolol
- Cell envelope exposed to Propranolol
- ... Intracellular materials exposed to Atenolol
- Δ-Δ Cell envelope exposed to Atenolol

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

- Intracellular materials exposed to Propranolol
- o-o Cell envelope exposed to Propranolol
- +--+ Intracellular materials exposed to Atenolol
- ▲-▲ Cell envelope exposed to Atenolol.

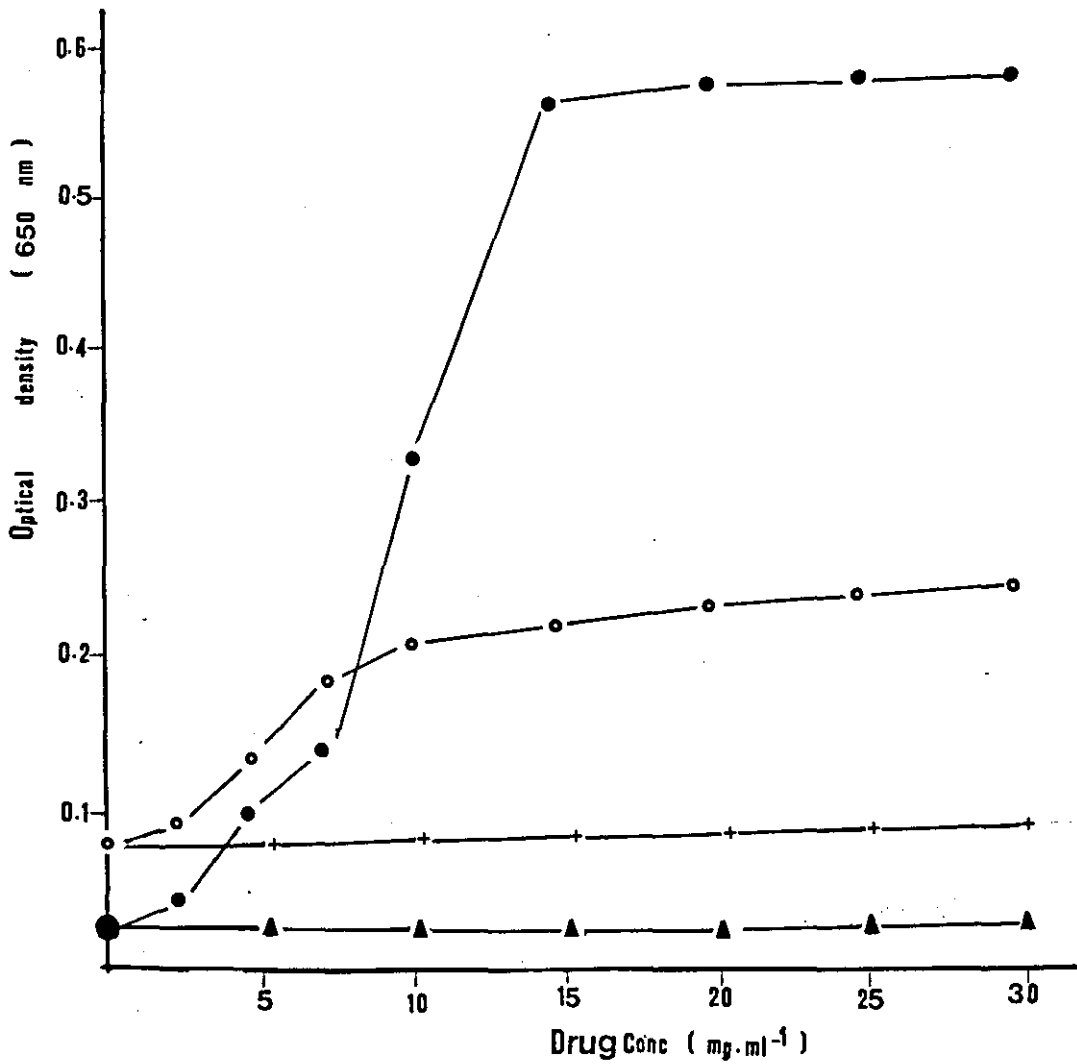
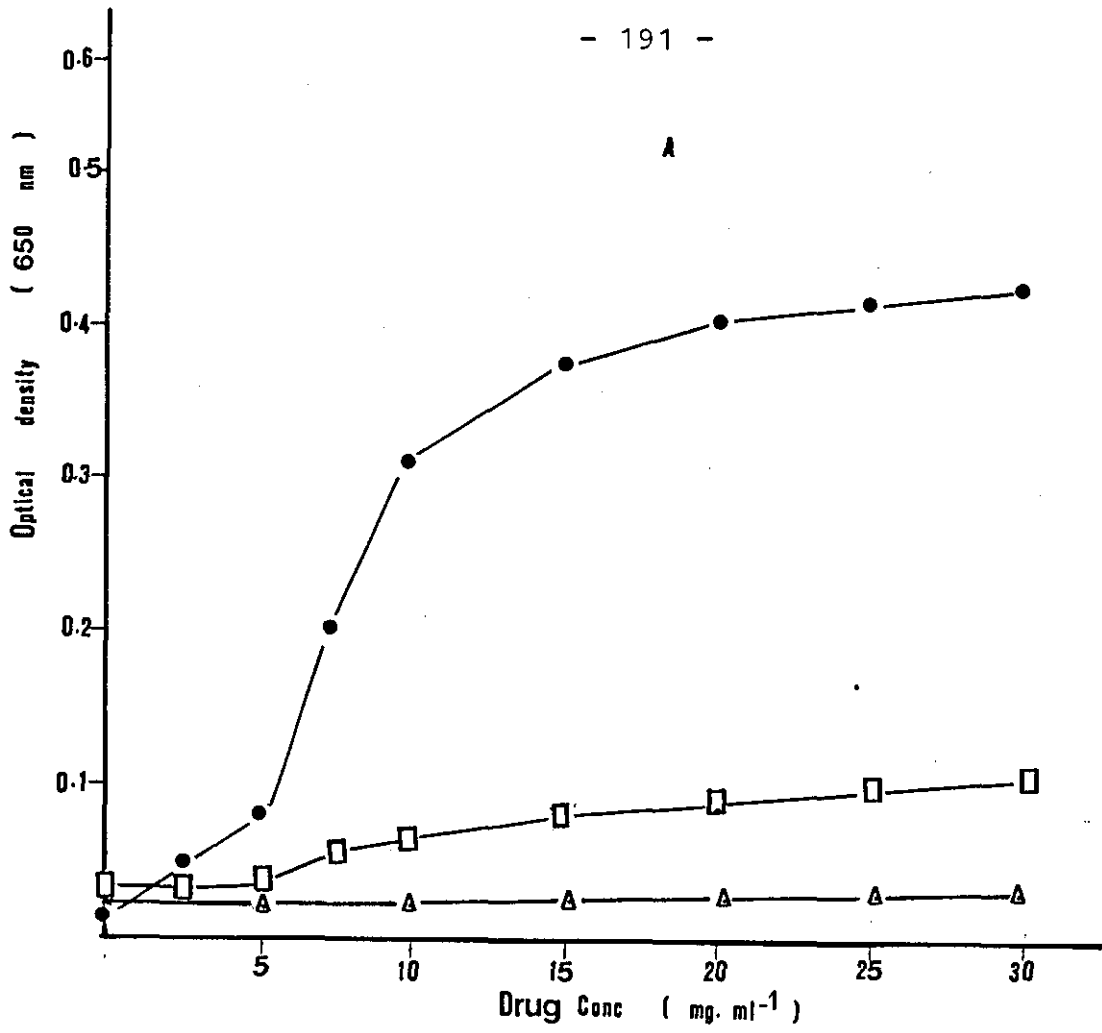


Fig. 3.100: The effect of Propranolol and Atenolol on the turbidity changes of lipid extract, and lipid dispersed preparations of (A) E.coli and (B) C.albicans suspended in CFM at 22°C. Contact time 10 min.

(A)

Cell concentration 4.2×10^9 cells.ml⁻¹

- x-x Lipid extract exposed to Propranolol
- Lipid dispersed preparations exposed to Propranolol.
- Lipid extract exposed to Atenolol
- +--+ Lipid dispersed preparations exposed to Atenolol.

(B)

Cell concentration 3.8×10^5 cells.ml⁻¹

- ⊗-⊗ Lipid extract exposed to Propranolol
- Lipid dispersed preparations exposed to Propranolol
- Lipid extract exposed to atenolol
- Δ-Δ Lipid dispersed preparations exposed to Atenolol.

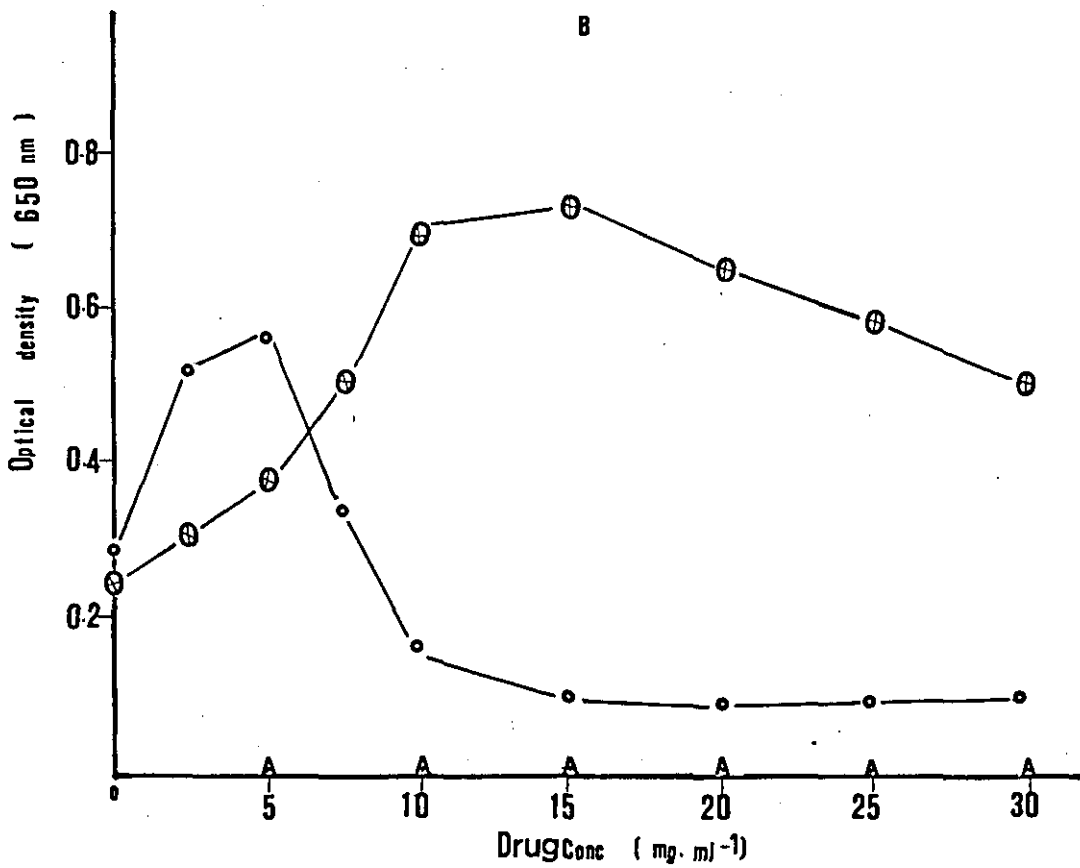
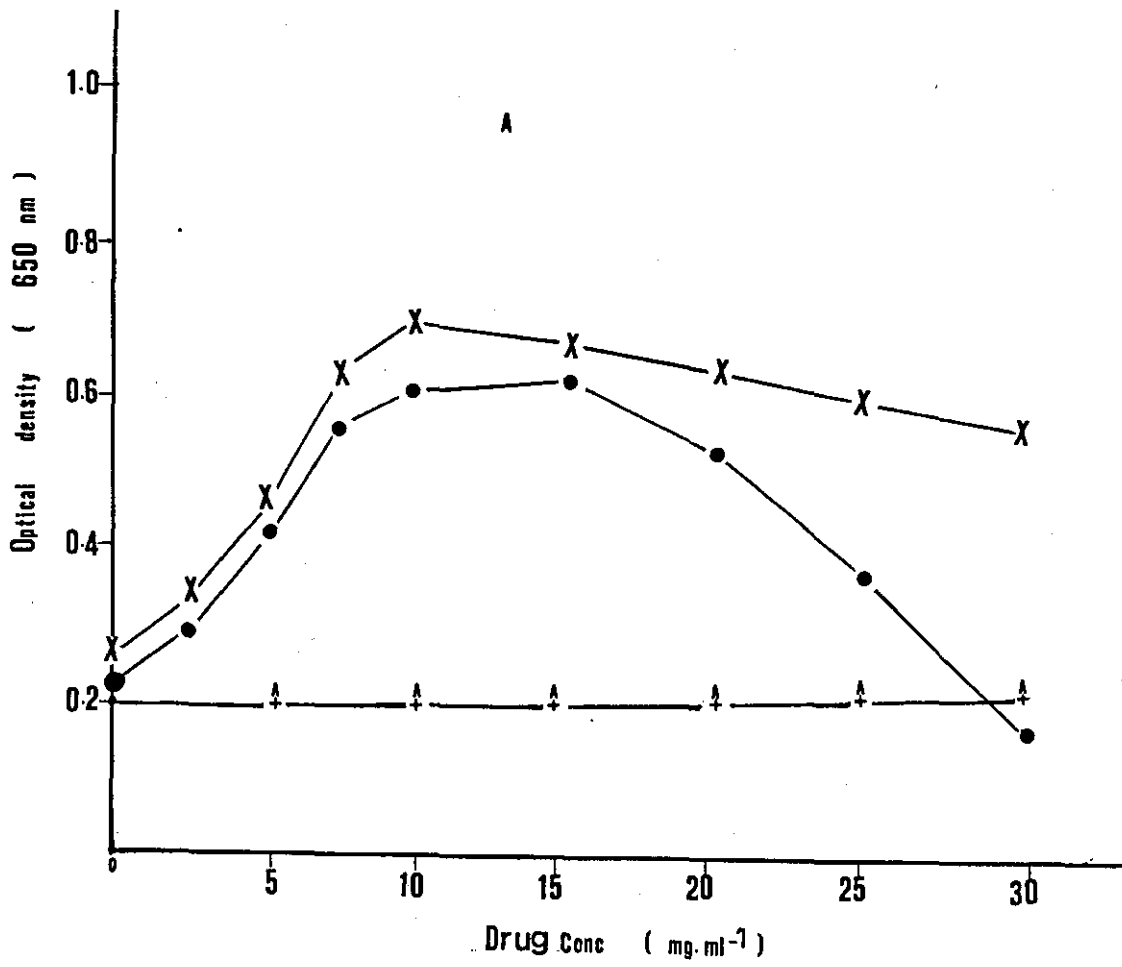


Fig. 3.101: The effect of (A) Propranolol and (B) Atenolol on the induced turbidity increases caused by different concentrations of HTAB on cells of E.coli suspended in CFM at 22°C. Contact time 10 min.

(A)

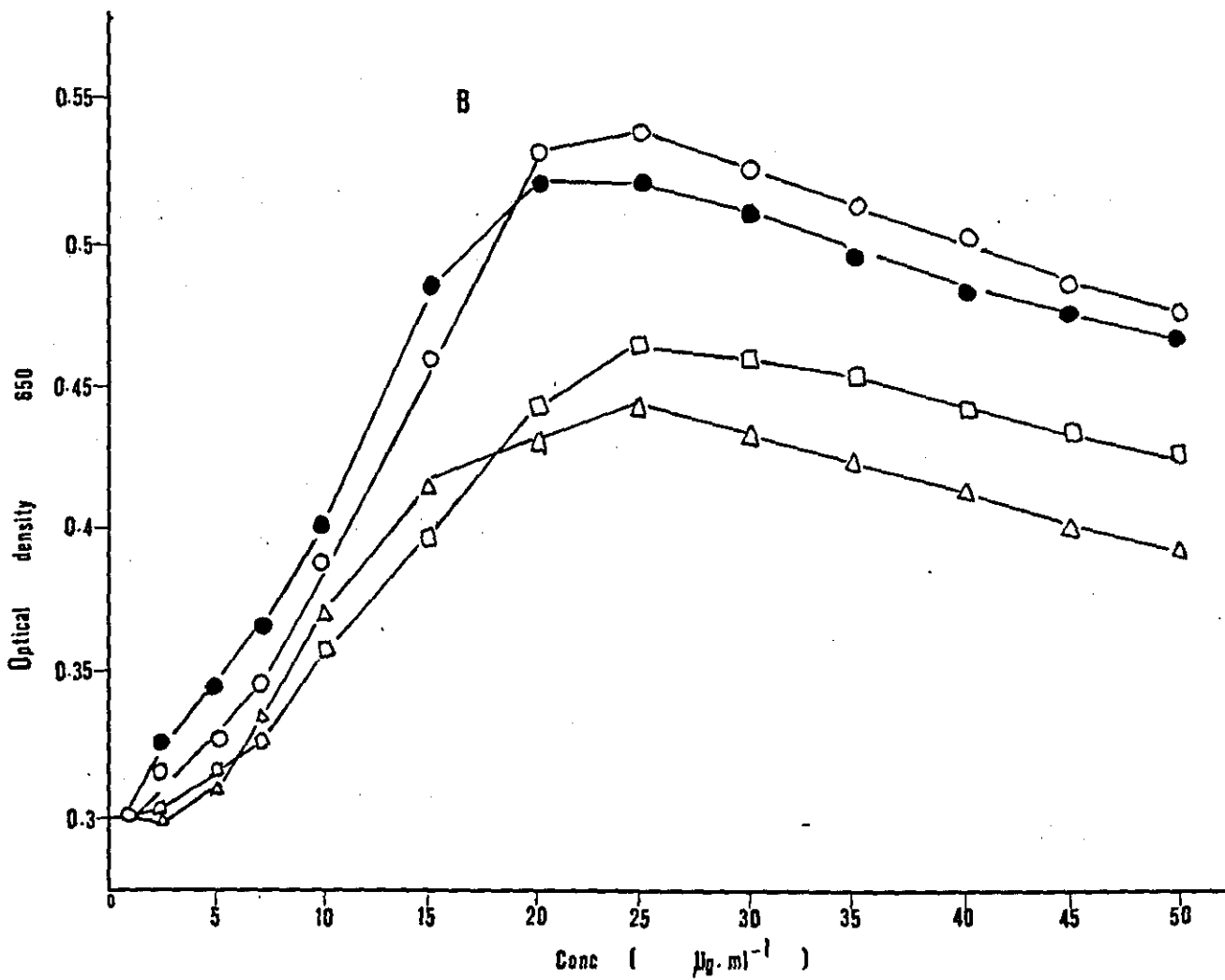
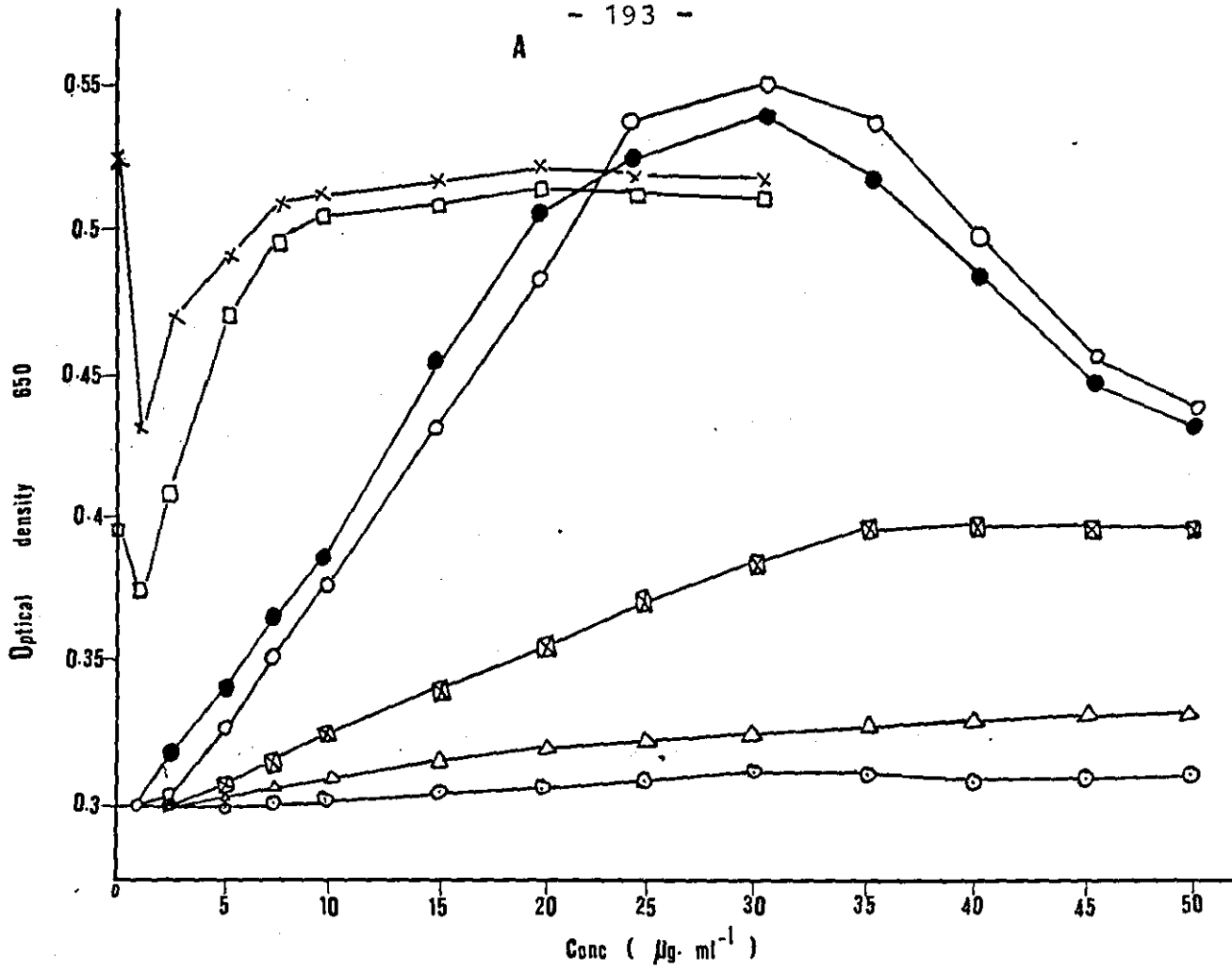
Cell concentration 4.2×10^9 cells.ml⁻¹

O-O HTAB
 ●-● HTAB + 0.005 mg.ml⁻¹ Propranolol
 ○-○ HTAB + 0.5 mg.ml⁻¹ Propranolol
 Δ-Δ HTAB + 1.0 mg.ml⁻¹ Propranolol
 ☒-☒ HTAB + 2.5 mg.ml⁻¹ Propranolol
 □-□ HTAB + 5.0 mg.ml⁻¹ Propranolol
 x-x HTAB + 20 mg.ml⁻¹ Propranolol

(B)

Cell concentration 4.2×10^9 cells.ml⁻¹

●-● HTAB
 O-O HTAB + 1.0 mg.ml⁻¹ Atenolol
 □-□ HTAB + 5.0 mg.ml⁻¹ Atenolol
 Δ-Δ HTAB + 10.0 mg.ml⁻¹ Atenolol



A. Fig. 3.102: The extent of protection by 0.25 mg.ml^{-1} Propranolol against the turbidity increase caused by different concentrations of HTAB on the cells of E.coli suspended in CFM at 22°C . Contact time 10 min. Cell concentration $4.2 \times 10^9 \text{ cells.ml}^{-1}$

O-O HTAB

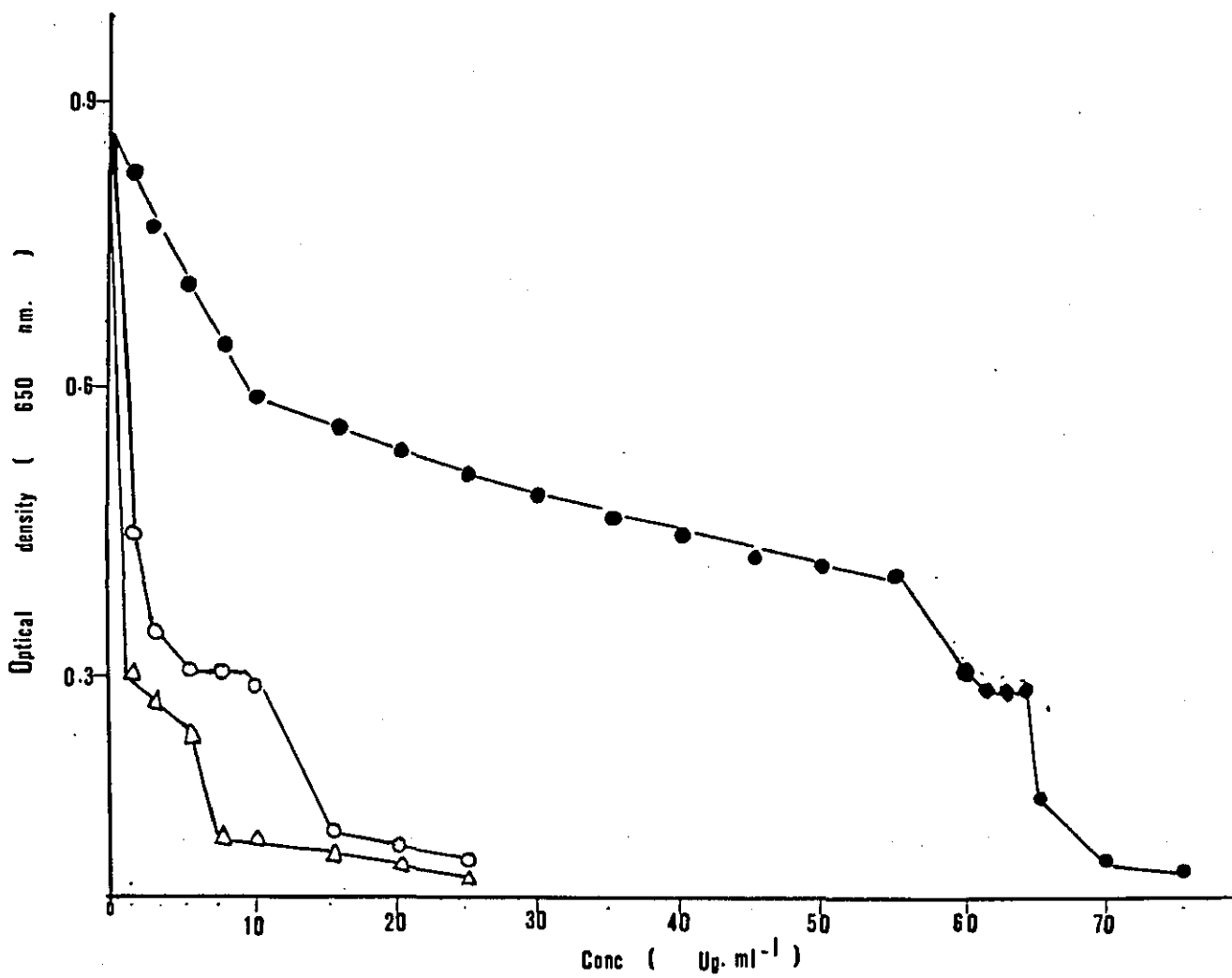
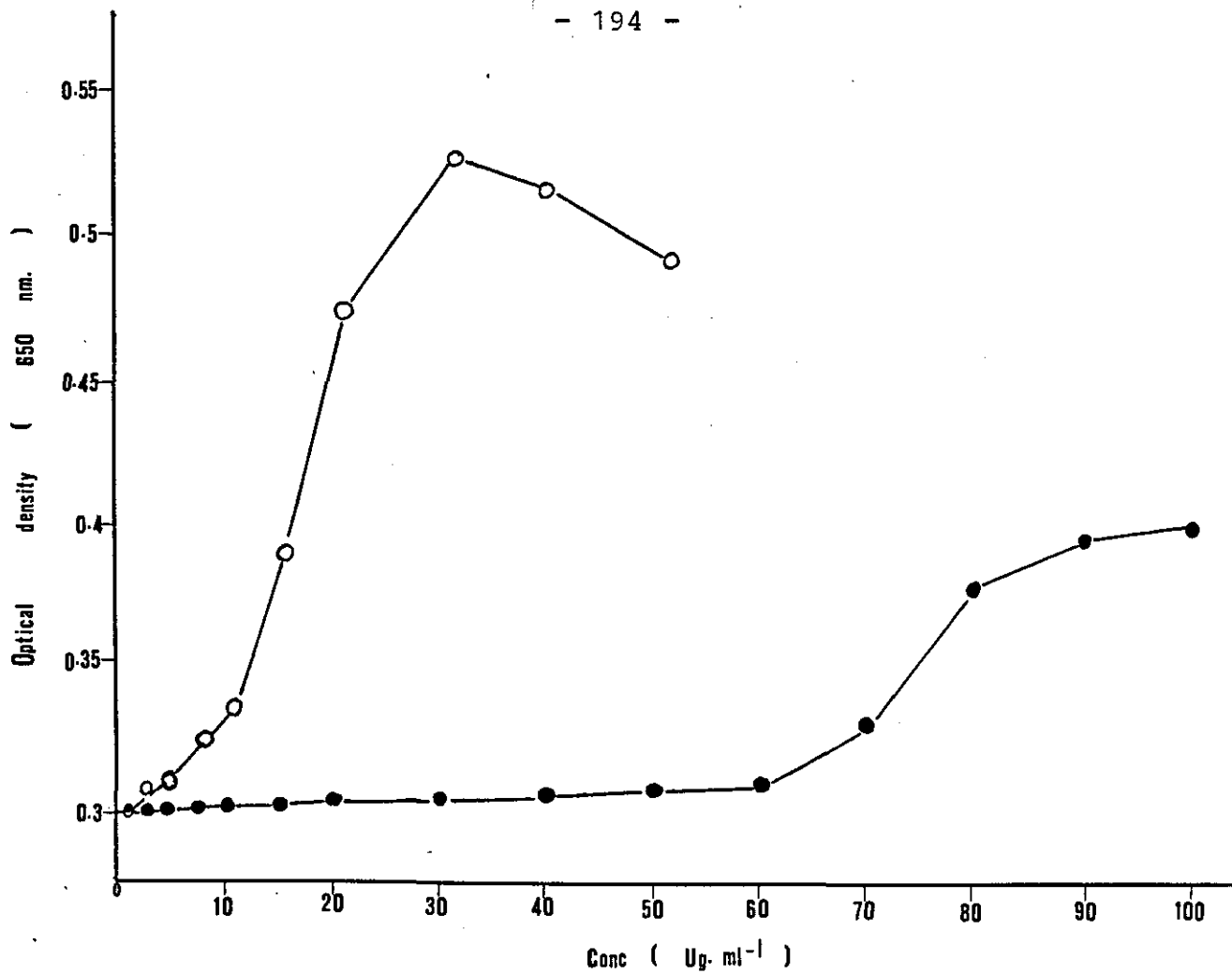
●-● HTAB + 0.25 mg.ml^{-1} Propranolol

B. Fig. 3.103: The effect of HTAB on the optical density of culture of E.coli in minimal growth media at 37°C containing Propranolol or Atenolol. Incubation time 18 hr. Initial cell concentration $4.2 \times 10^9 \text{ cells.ml}^{-1}$.

O-O HTAB

●-● HTAB + 0.2 mg.ml^{-1} Propranolol

Δ-Δ HTAB + 5.0 mg.ml^{-1} Atenolol



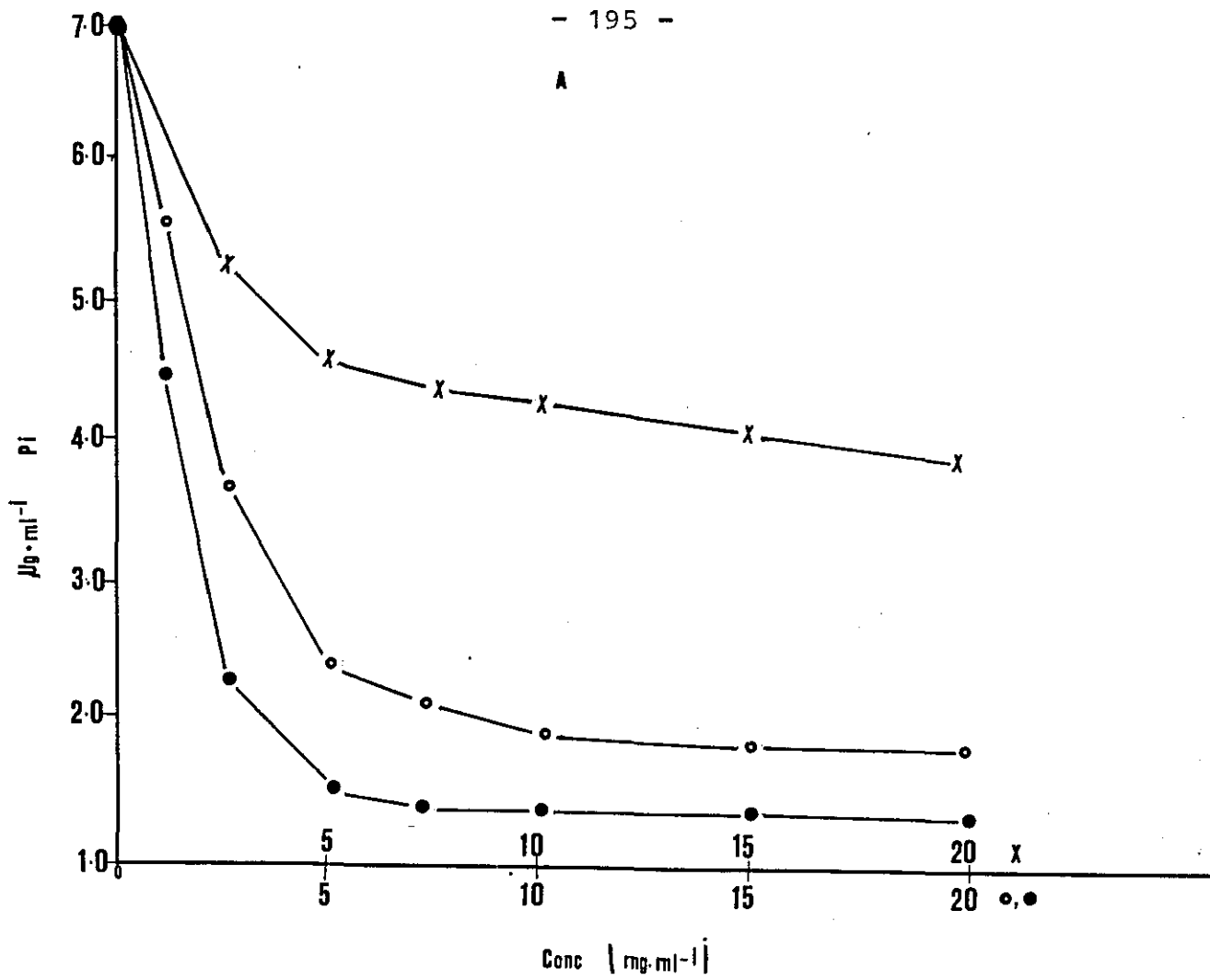
A. Fig. 3.104: The effect of β -blockers and HTAB on the activity of partially purified Mg^{2+} -ATPase from B.cereus. Temperature $37^{\circ}C$. Contact time 30 min.

Drugs: x-x Practolol) $mg.ml^{-1}$
o-o Propranolol)
●-● HTAB $\mu g.ml^{-1}$

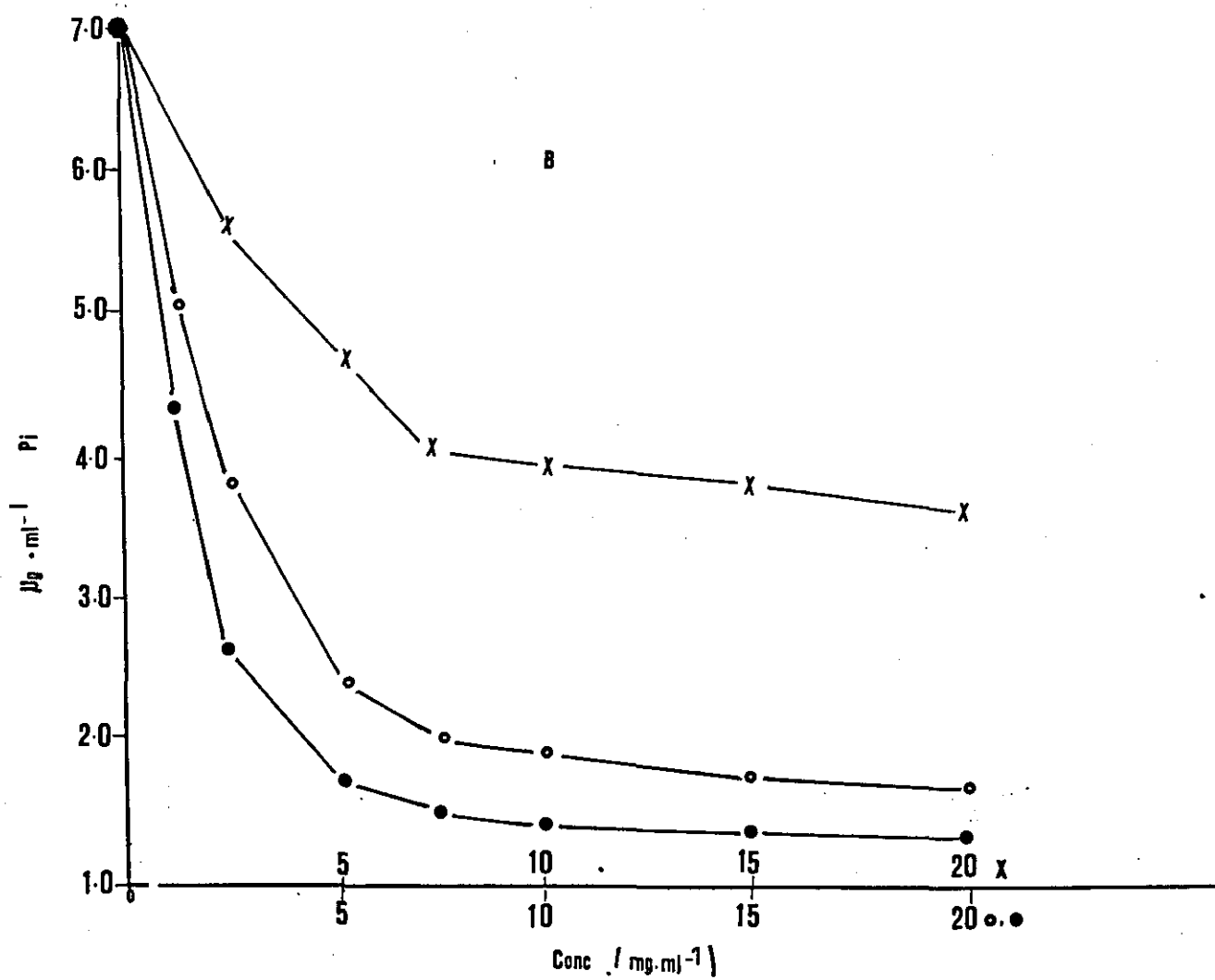
B. Fig. 3.105: The effect of β -blockers and HTAB on the activity of partially purified Mg^{2+} -ATPase from C.albicans. Temperature $37^{\circ}C$. Contact time 30 min.

Drugs: x-x Practolol) $mg.ml^{-1}$
o-o Propranolol)
●-● HTAB $\mu g.ml^{-1}$

A



B



A.

Fig. 3.106: Time Course for the activity of Mg^{2+} -ATPase from B.cereus treated with β -blockers.

Temperature $37^{\circ}C$.

β -blockers concentration o-o, Practolol (3.5 mg.ml^{-1}), ●-●, Propranolol (1.0 mg.ml^{-1}).

B.

Fig. 3.107: Time course for the activity of Mg^{2+} -ATPase from C.albicans treated with β -blockers.

Temperature $37^{\circ}C$.

β -blockers concentration o-o, Practolol (3.5 mg.ml^{-1}), ●-● Propranolol (1.0 mg.ml^{-1}).

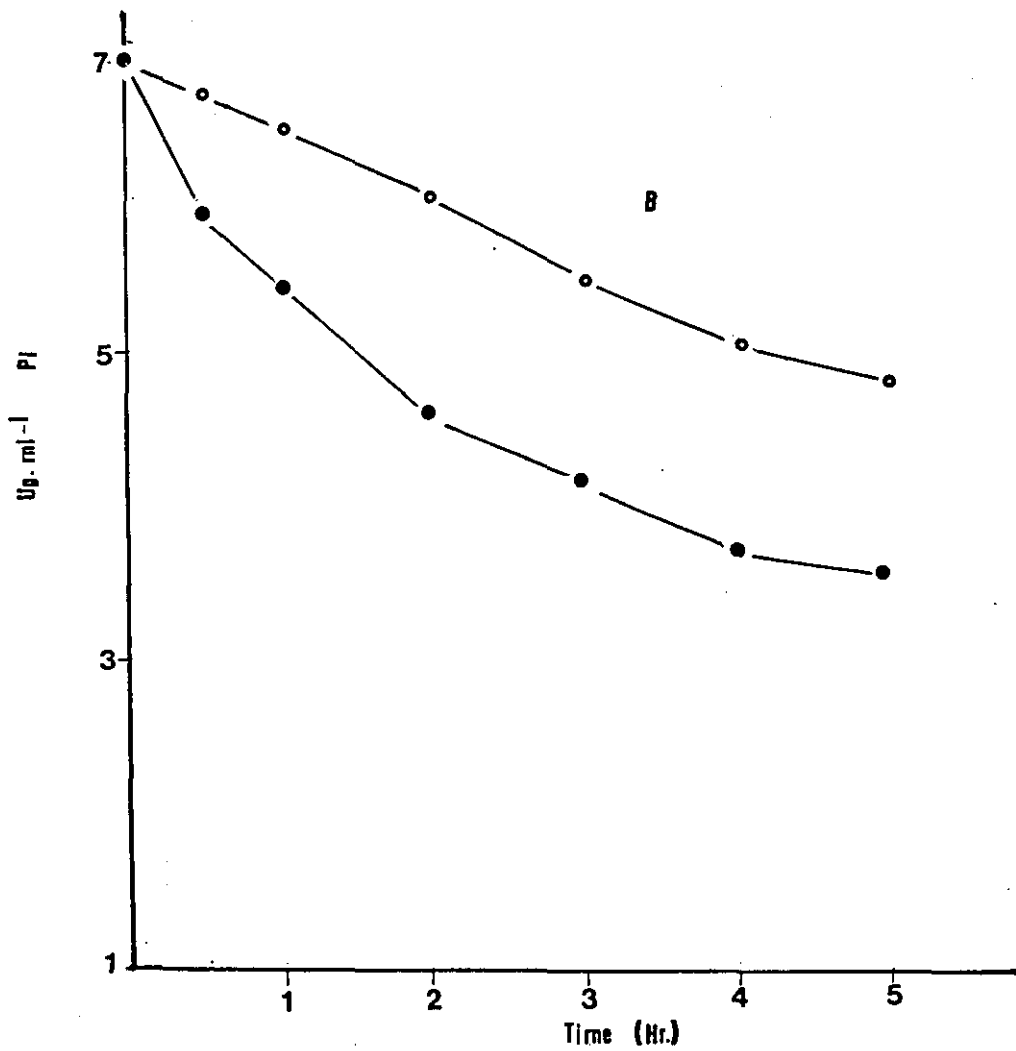
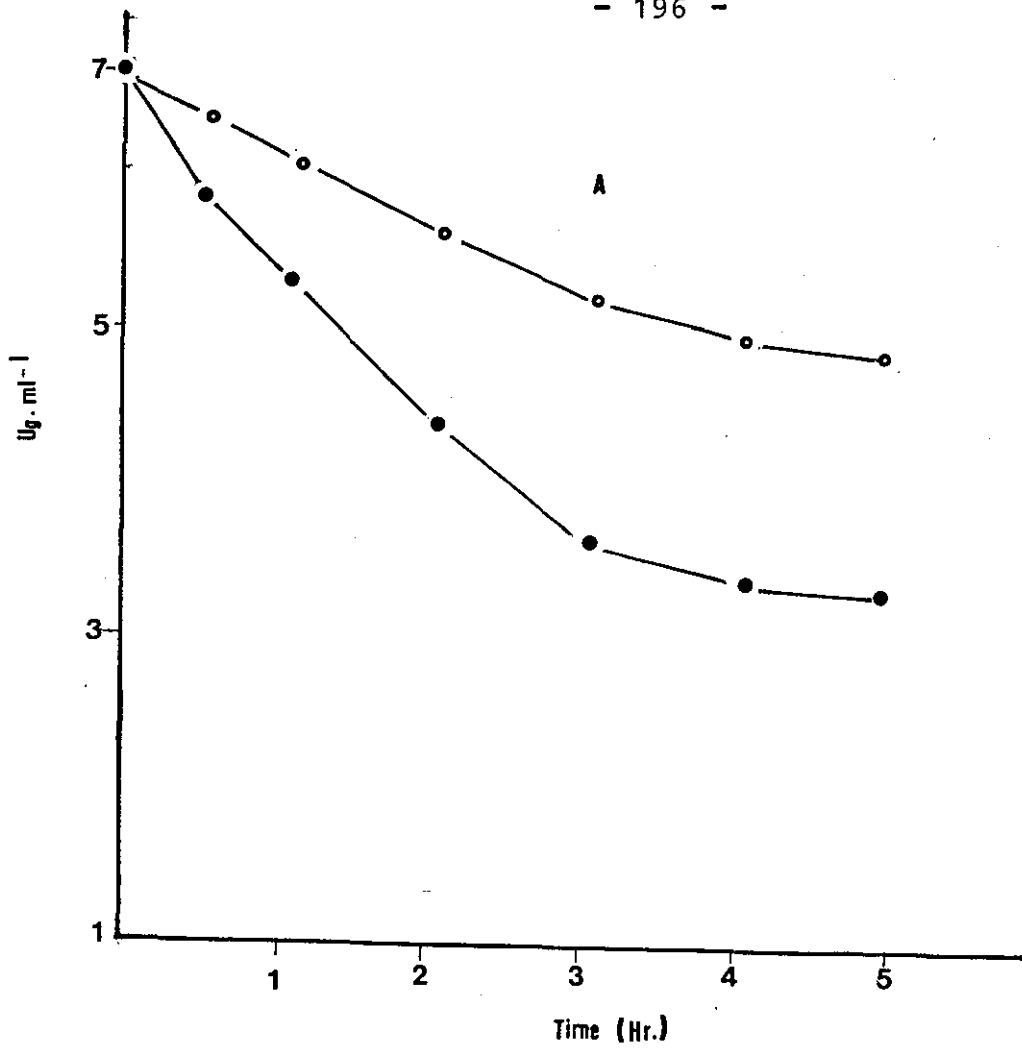
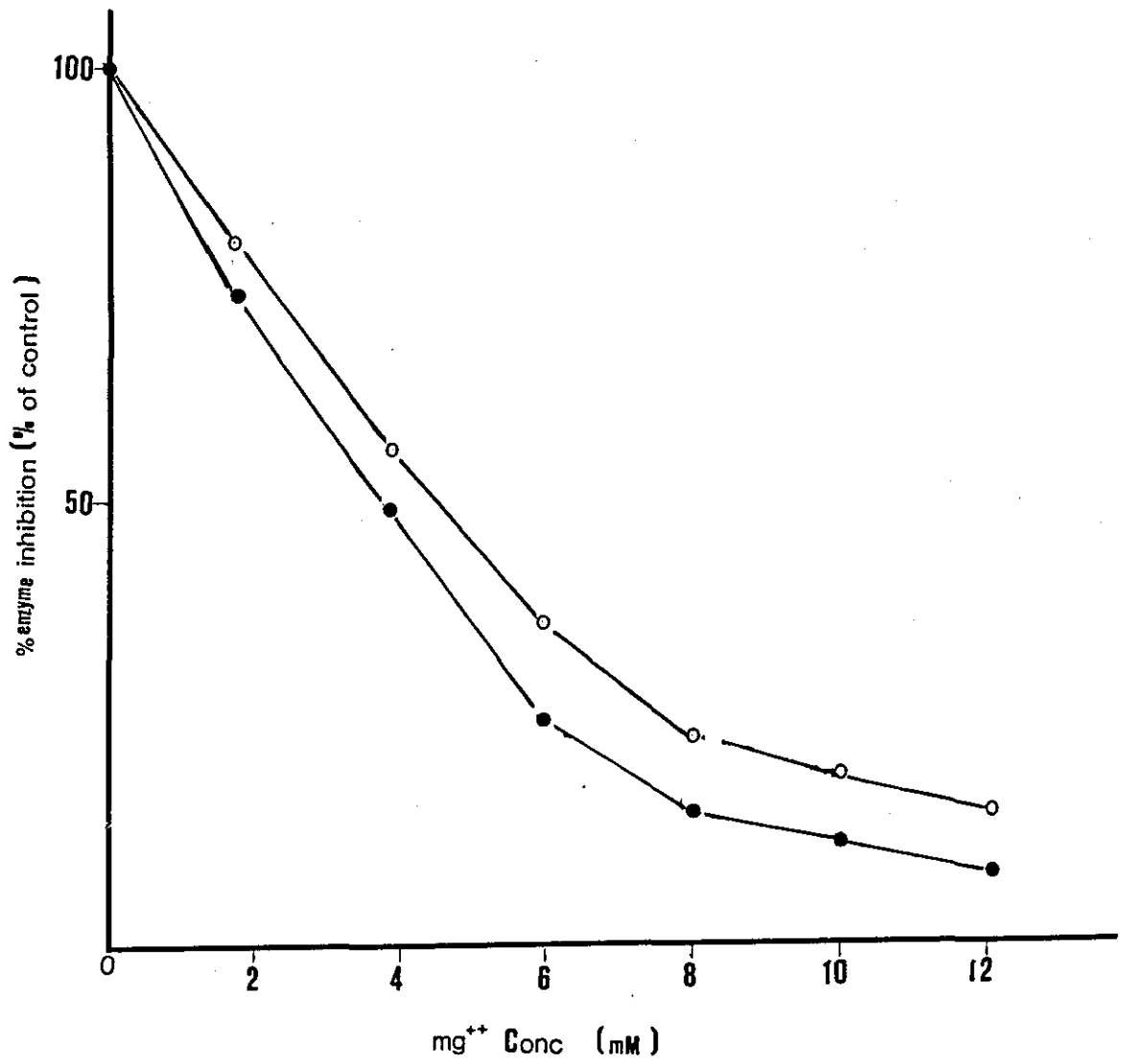
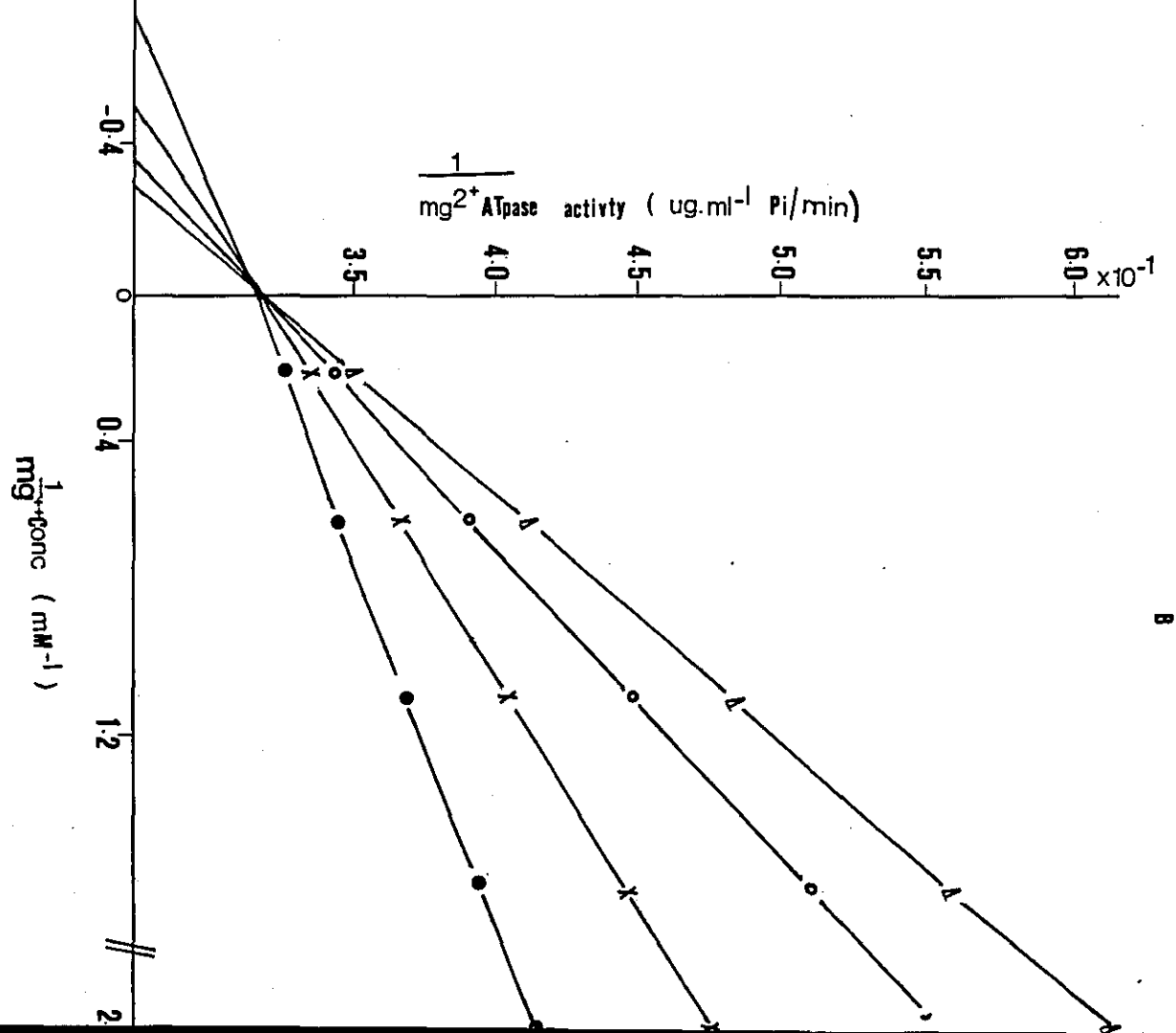
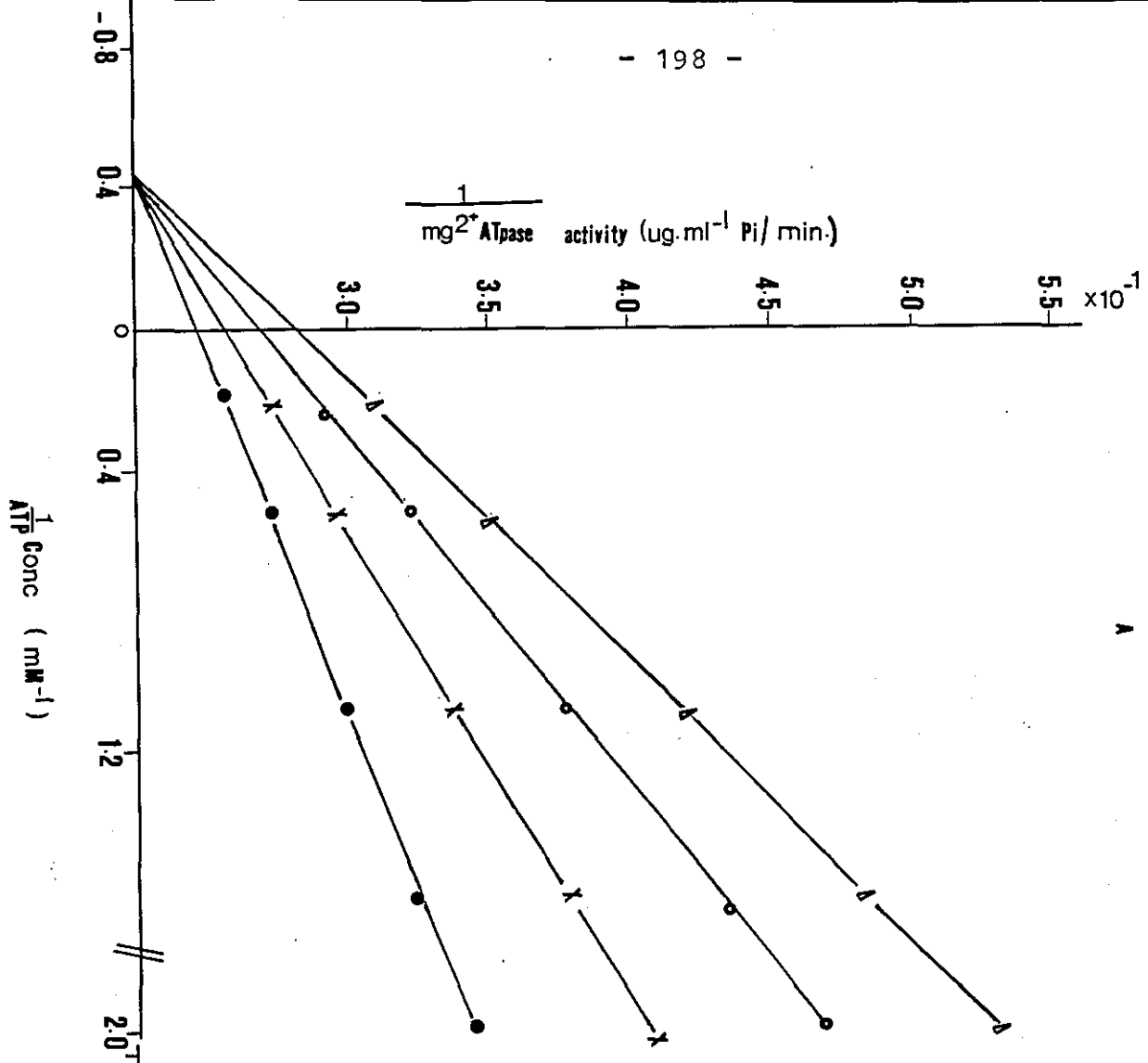


Fig. 3.108 : Reversal by Mg^{2+} ion of the inhibition caused
by 1.0 mg.ml^{-1} Propranolol ●-● and 5.0
 mg.ml^{-1} Atenolol O-O on Mg^{2+} -ATPase
C.albicans at 30°C . Contact time 30 min.



(A) Fig. 3.109: Double reciprocal plot of the kinetics of inhibition of C.albicans Mg^{2+} ATPase enzyme by various β -blockers and HTAB. The steady states were measured at varied ATP concentration in the absence of drug, ●-●; and in the presence of 5 mg.ml⁻¹ Practolol, x-x; 0.5 mg.ml⁻¹ Propranolol, O-O; and 0.5 μ g.ml⁻¹ HTAB, Δ - Δ . Temperature 30°C. Contact time 30 min. Cell concentration 3.8×10^8 cells.ml⁻¹.

(B) Fig. 3.110: Double reciprocal plot of the kinetics of inhibition of C.albicans Mg^{2+} ATPase enzyme by various β -blockers and HTAB. The steady states were measured at varied Mg^{2+} concentrations in the absence of drug ●-●; and in the presence of 5 mg.ml⁻¹ Practolol, x-x; 0.5 mg.ml⁻¹ Propranolol, O-O, and 0.5 μ g.ml⁻¹ HTAB, Δ - Δ . Temperature 30°C. Contact time 30 min. Cell concentration 3.8×10^8 cells.ml⁻¹.



(A) Fig 3.111: The effects of Propranolol and Atenolol on the activity of pure β -galactosidase enzyme. The final volume of assay medium, 4 ml containing 0.02M phosphate buffer, 3 mM ONPG, 2.0 units β -galactosidase enzyme and β -blockers at 30°C.
Drug, ●-●, Atenolol; O-O, Propranolol.

(B) Fig. 3.112: The effects of Propranolol and Atenolol on the activity of partially purified β -galactosidase enzyme from E.coli at 37°C.
Drug concentration (mg.ml⁻¹). O-O, 0.0; Practolol, x-x, 4.0; Propranolol, ●-●, 4.0.

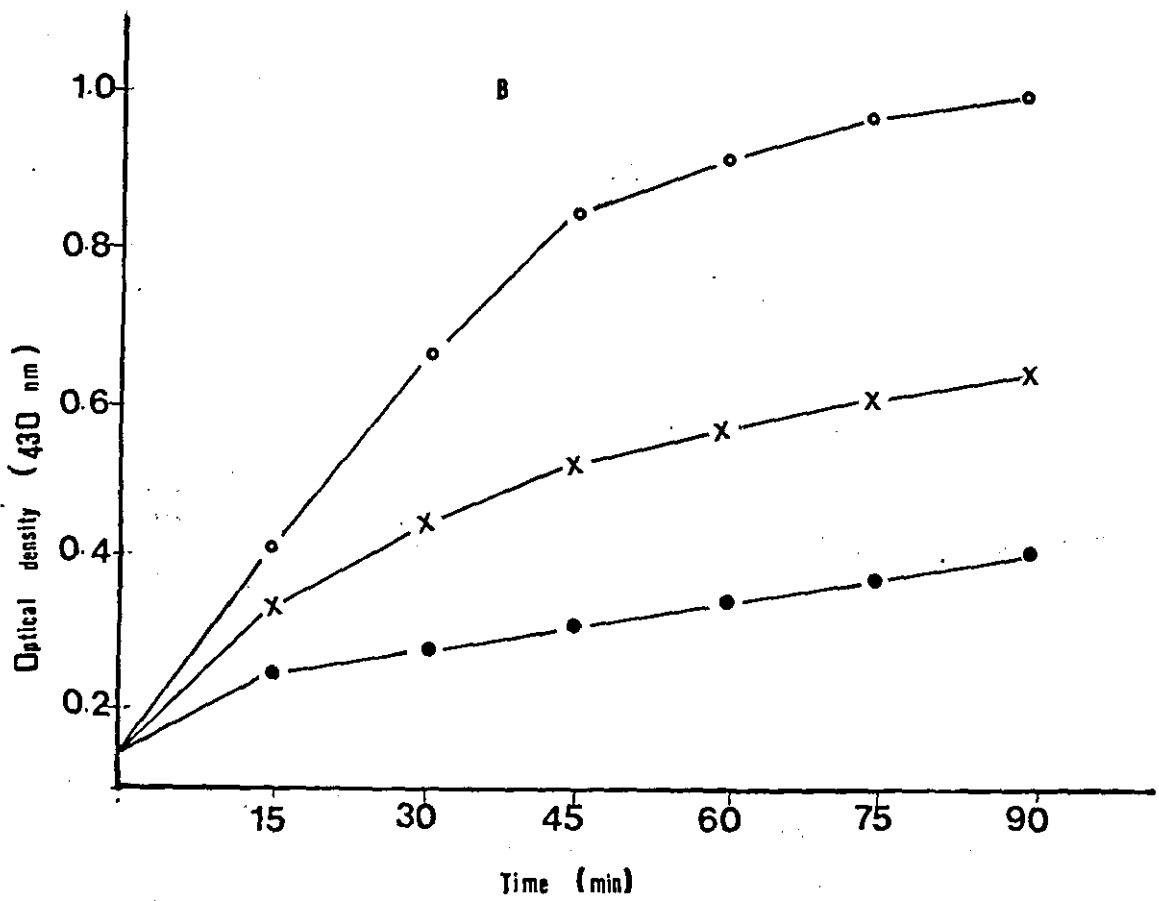
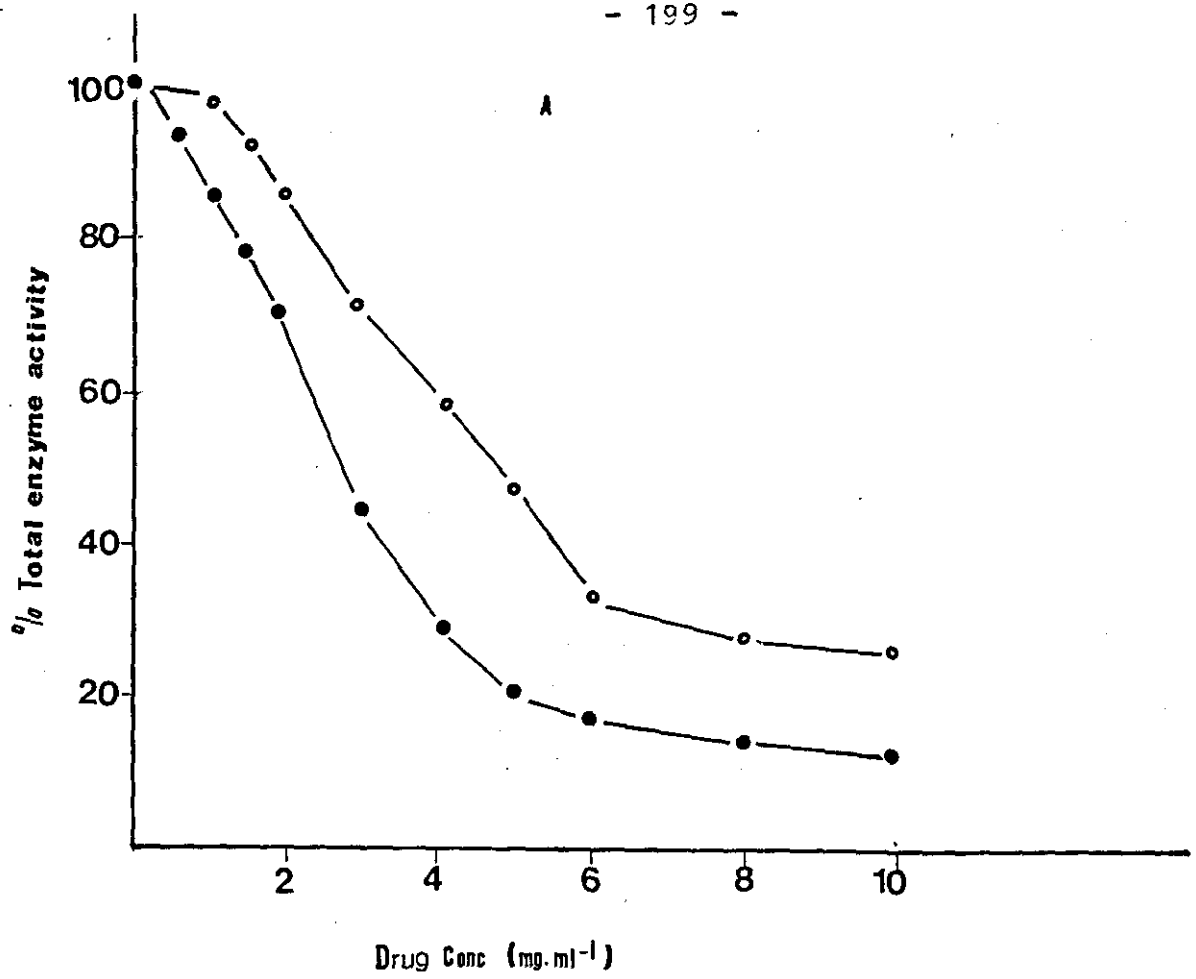


Fig. 3.113: Reversibility of Propranolol and Atenolol inhibition of partially purified enzyme of β -galactosidase from cells of E.coli by (A) Mg^{2+} ion and (B) Ca^{2+} ion at $37^{\circ}C$. Drug concentration (A) ($mg.ml^{-1}$).

o-o, 0.0; Practolol; x-x, 4.0,

Propranolol; ●-●, 4.0

Cation concentration 0.005M

Drug concentration (B) o-o 0.0;

Practolol, x-x, 4.0; Propranolol, ●-●, 4.0.

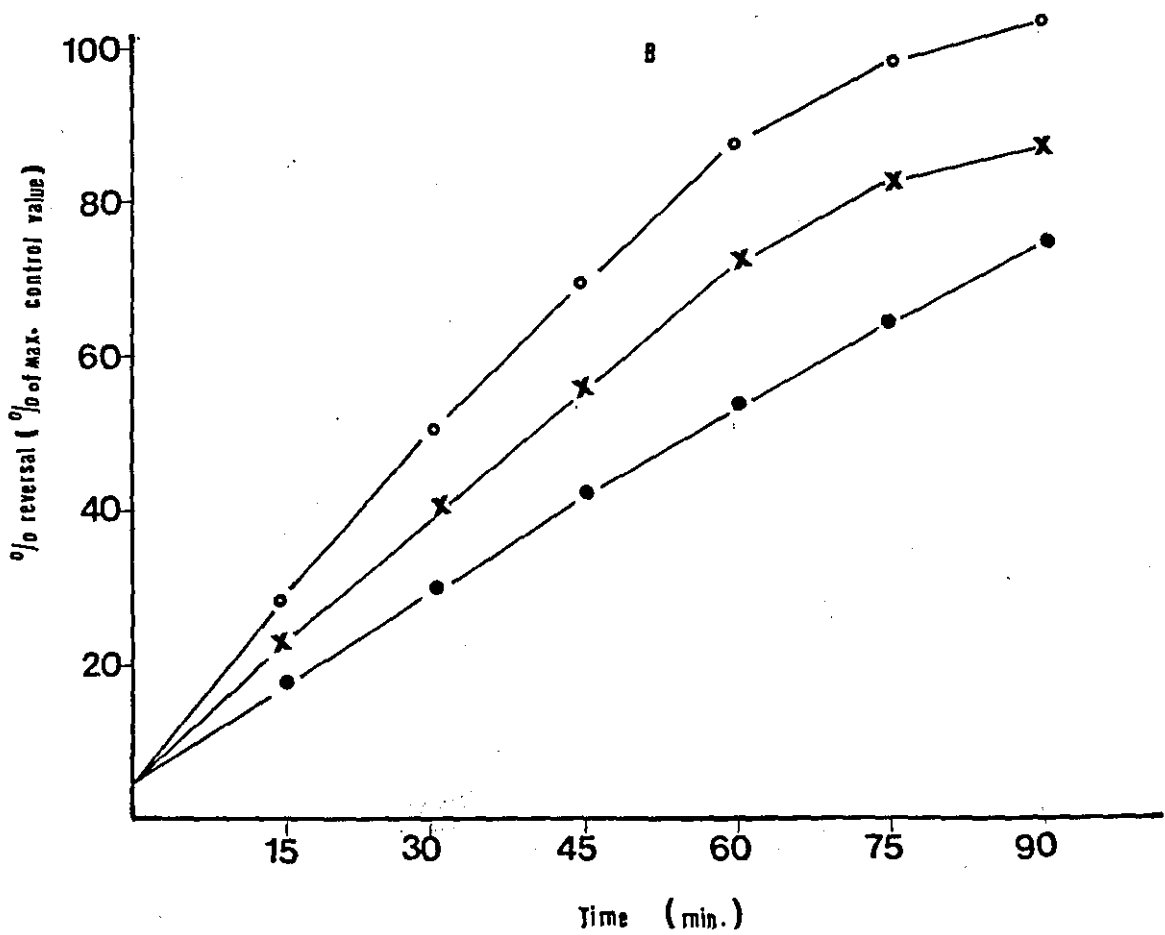
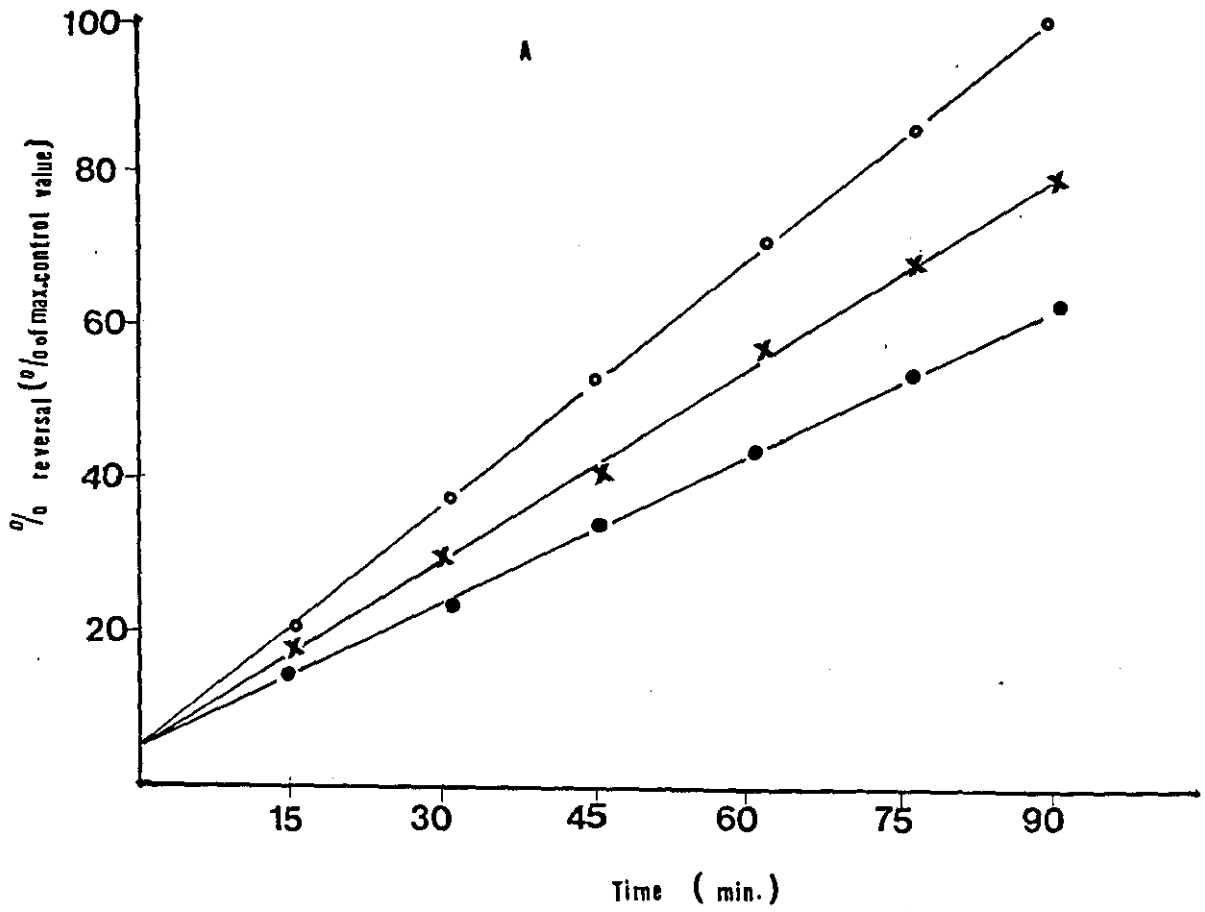


Fig . 3.114: Double reciprocal plot of the kinetics of inhibition of β -blockers. The steady state were measured at varied ONPG concentration in the absence of drug ●-●, and in the presence of 4 mg.ml⁻¹ Propranolol x-x and Atenolol O-O in the presence of 2.0 units β -galactosidase enzyme, 0.02M phosphate buffer. The reaction was stopped by the addition of 1.0M K₂CO₃ and the activity of the enzyme assayed at 430 (nm).

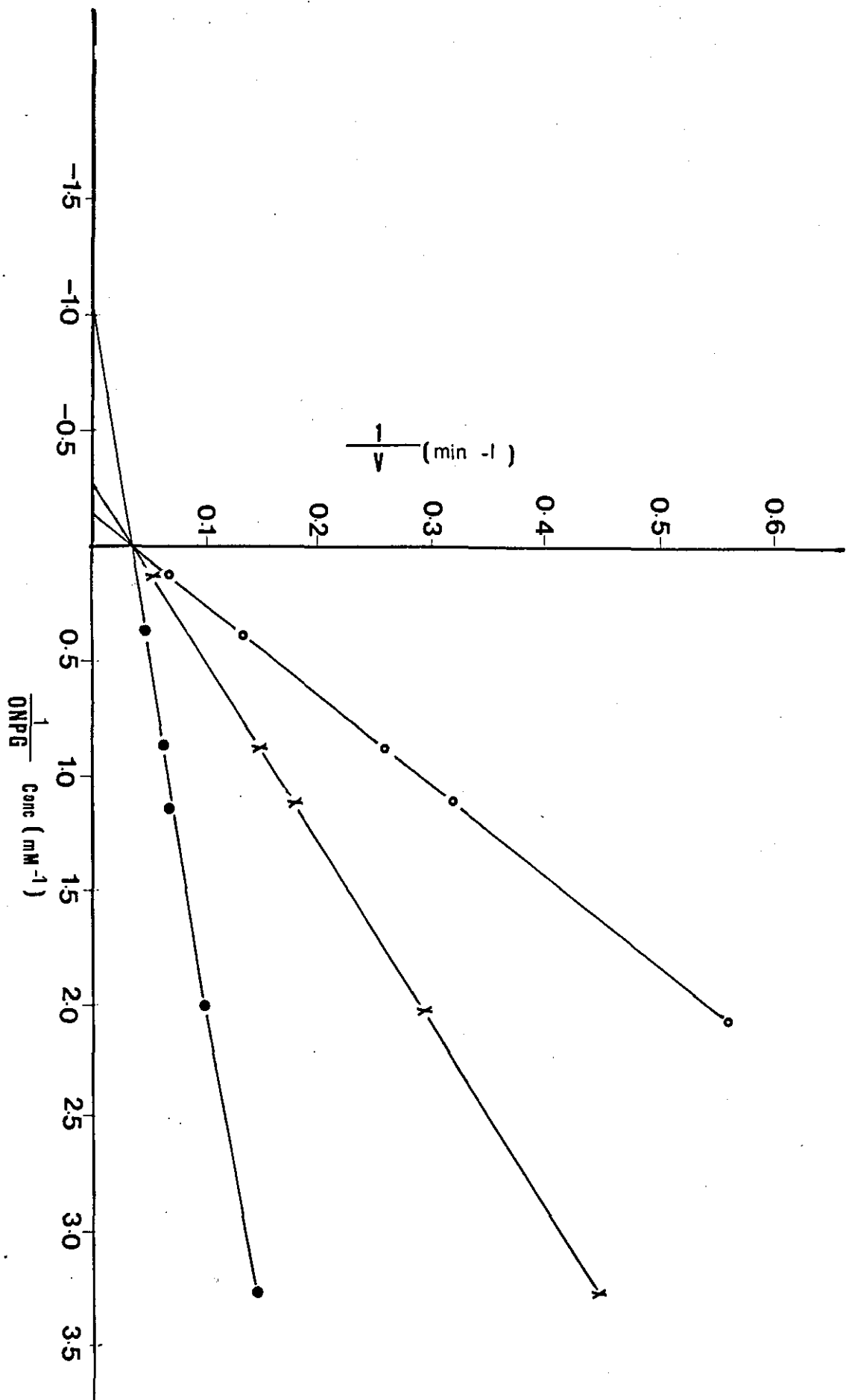


Fig. 3.115: The effect of varying concentrations
of β -blockers on the activity of
lysosomal enzymes of rat liver at 37°C
for 30 min.

Aryl sulfatase (A) Atenolol o-o
Propranolol. ●-●

β -glucuronidase (B) Atenolol o-o
Propranolol. ●-●

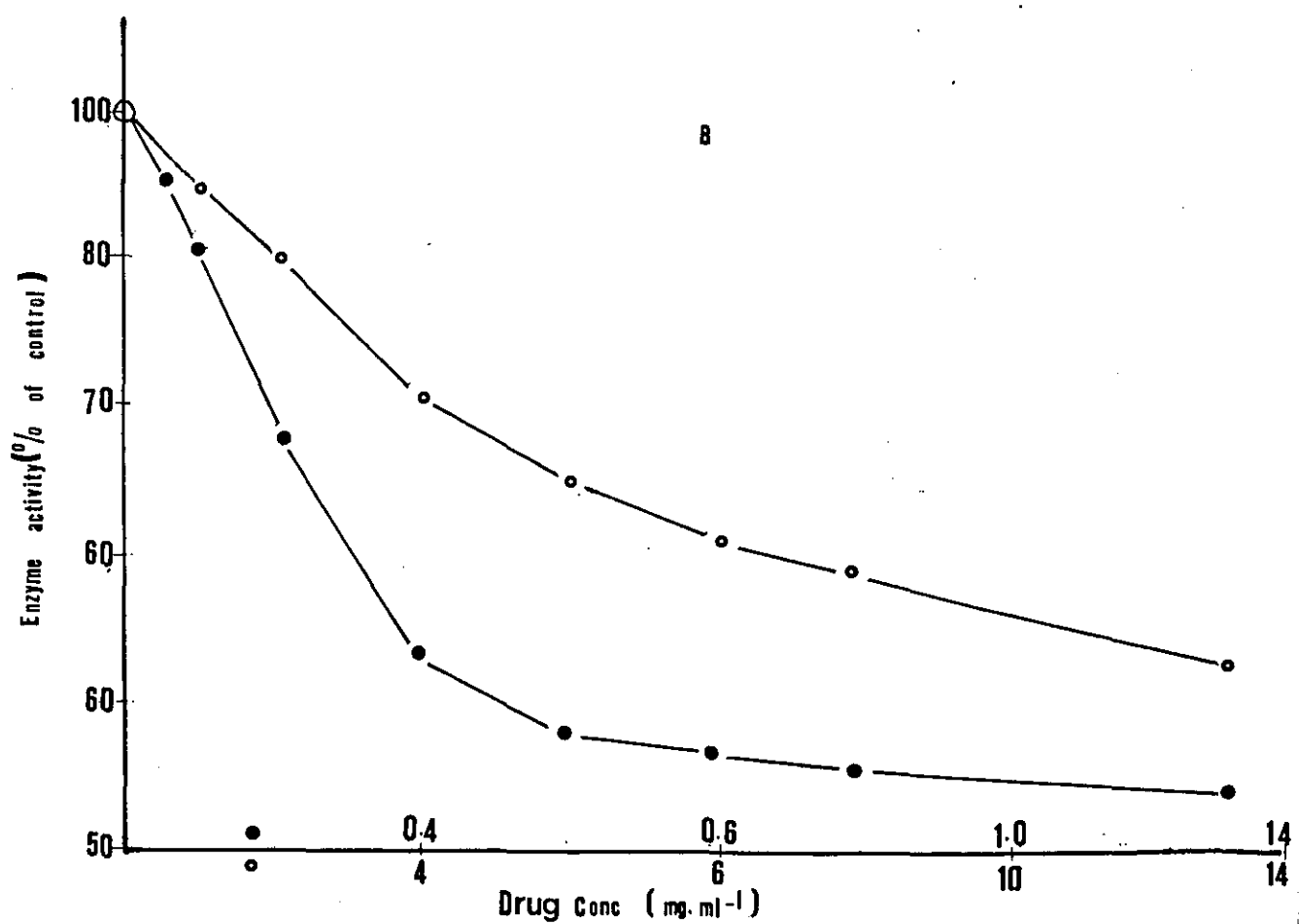
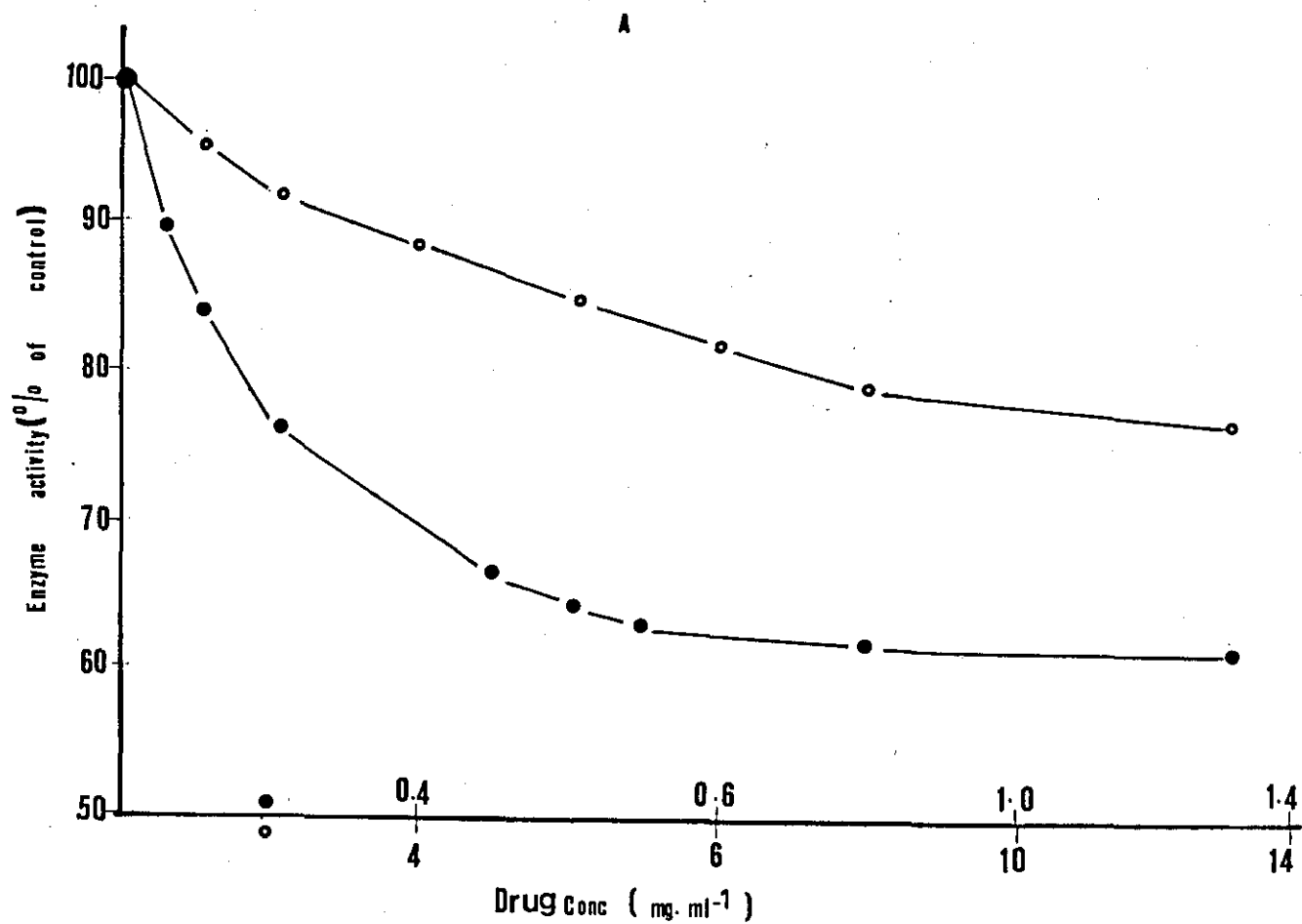
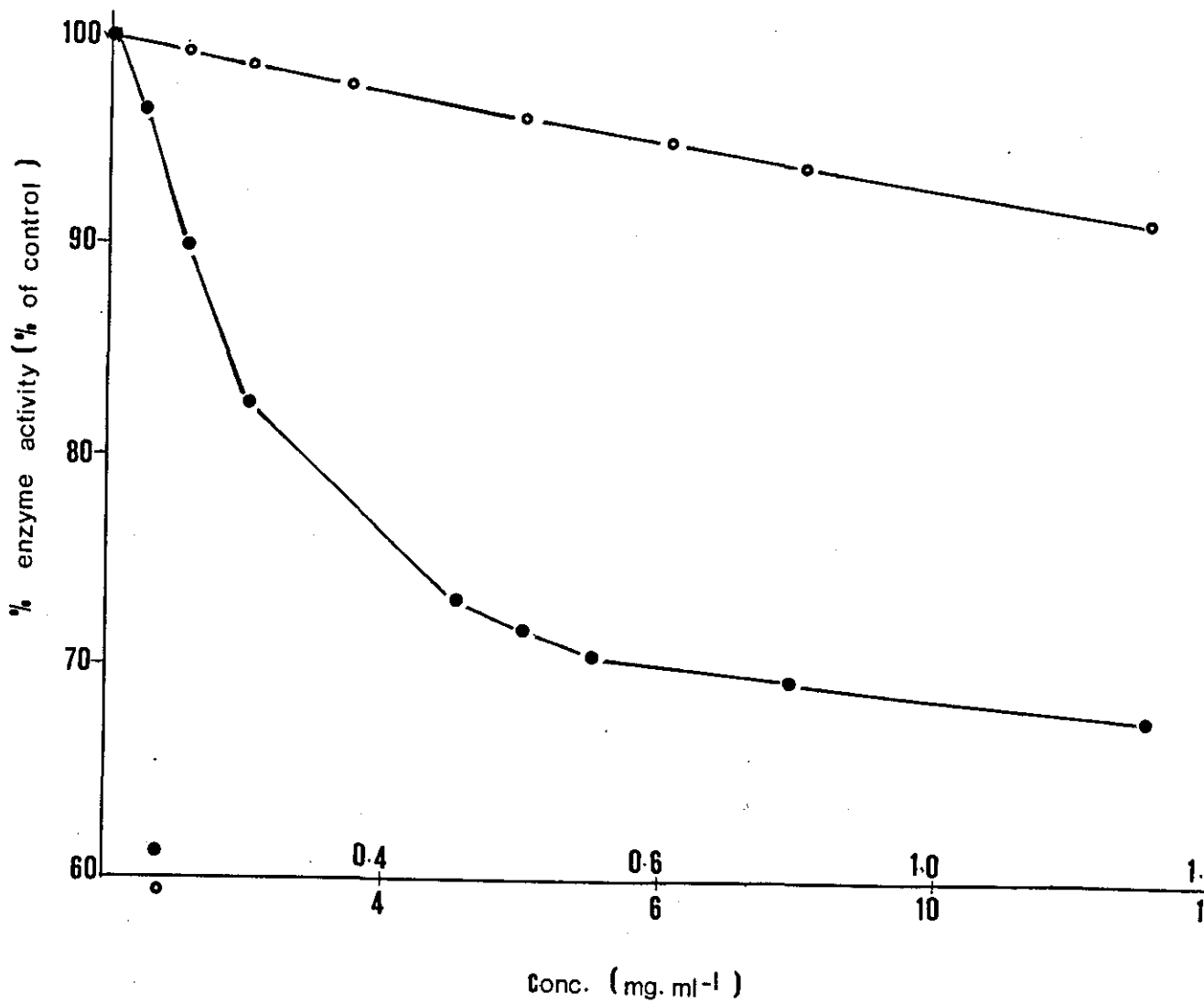


Fig. 3.116: The effect of varying concentrations of β -blockers on the activity of Lysosomal acid phosphatase of rat liver at 37°C for 30 min.

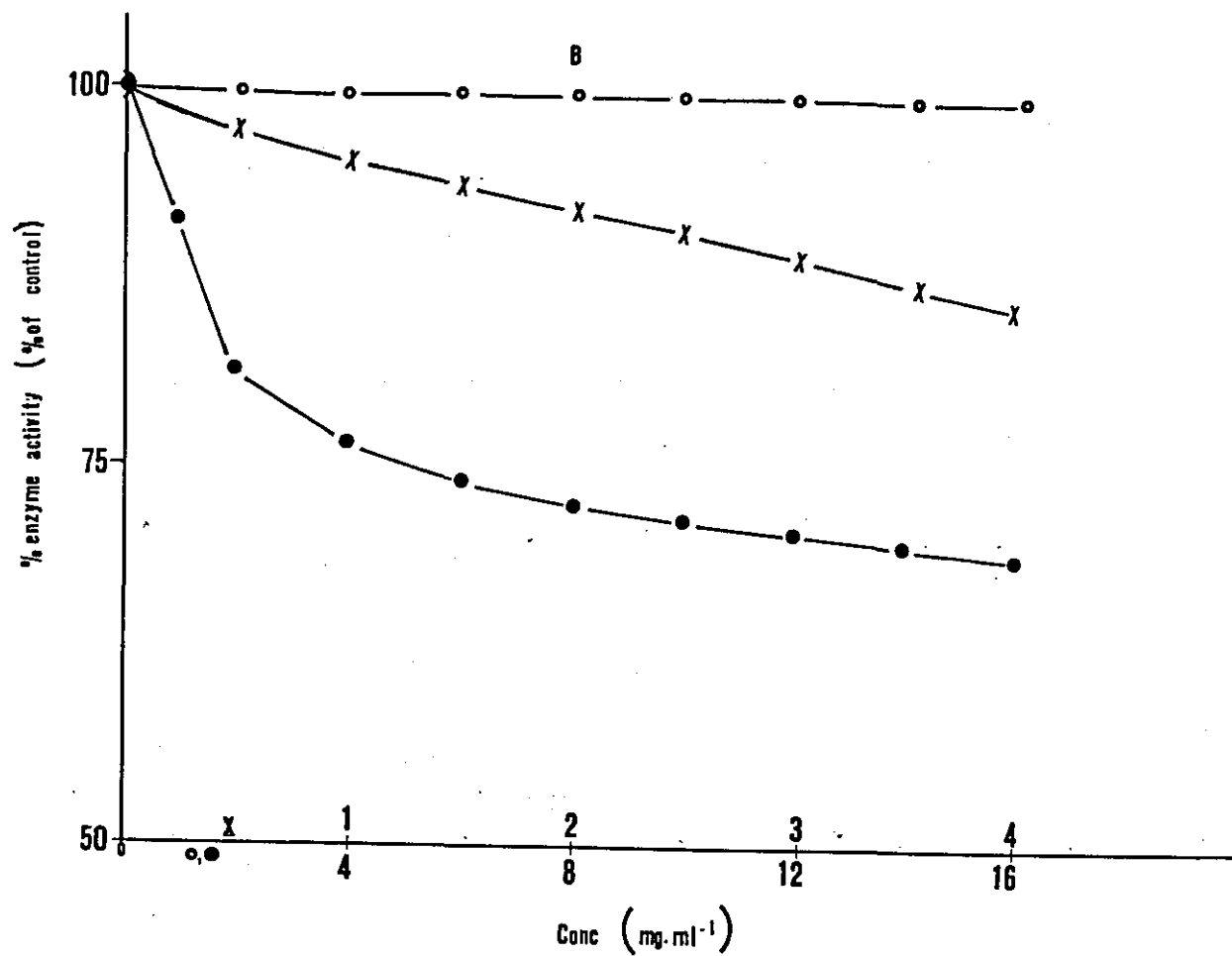
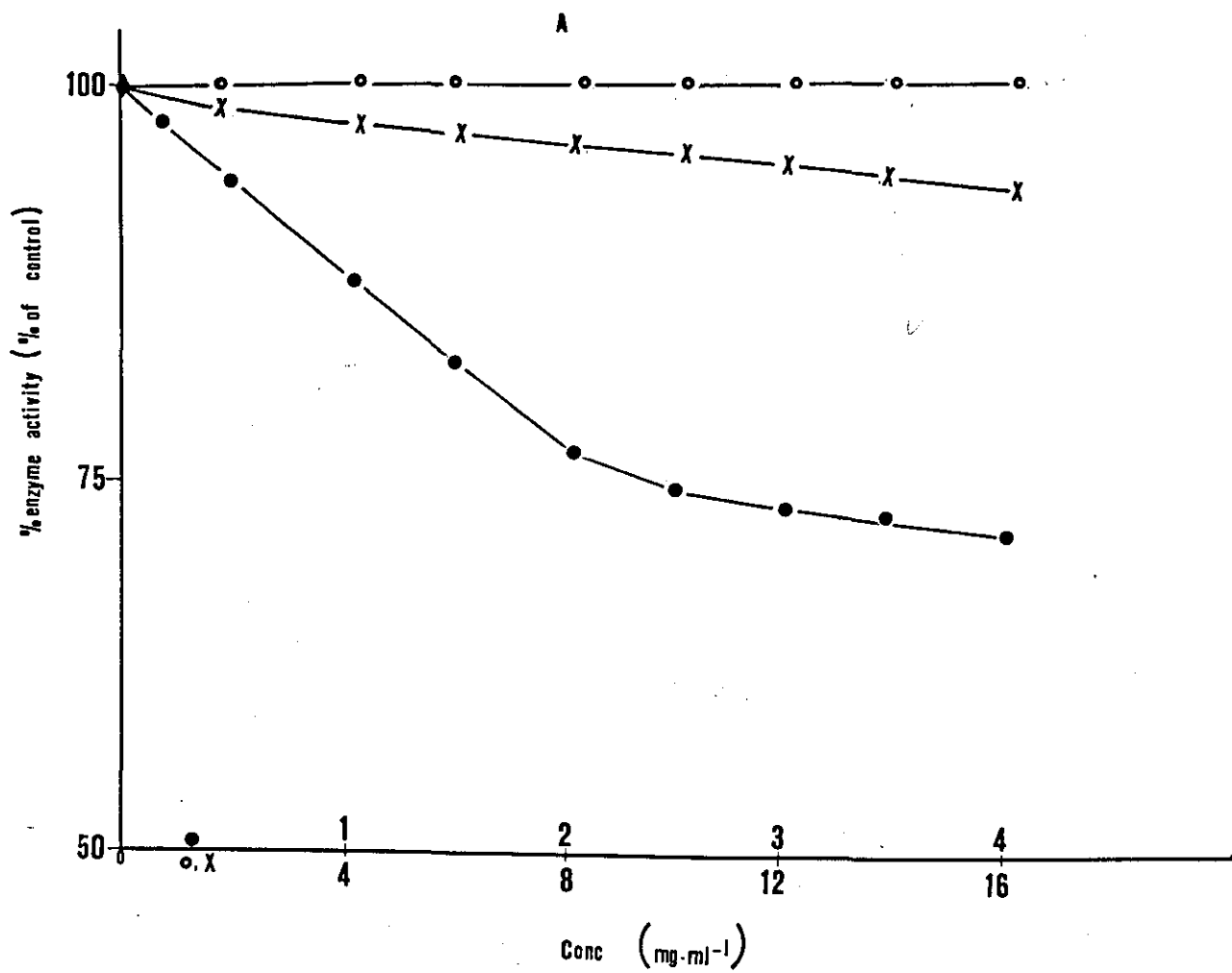
Atenolol, o-o; Propranolol ●-●.



(A) Fig. 3.117: The effect of varying concentrations of β -blockers on the activity of pure lysosomal enzymes at 37°C for 30 min.

(A) o-o, Atenolol on acid phosphatase
x-x, Atenolol on aryl sulfatase
●-●, Propranolol on aryl sulfatase.

(B) o-o, Propranolol on acid phosphate
x-x, Atenolol on β -glucuronidase
●-●, Propranolol on β -glucuronidase



(A) Fig. 3.118: Double reciprocal plot of the kinetics of inhibition of pure β -glucuronidase activity. The steady states were measured at varied concentrations of Phenolphthalein (PPT), glucuronic acid in the absence of drug, $\bullet-\bullet$; and in the presence of 4.0 mg.ml^{-1} Atenolol, $x-x$, and 4.0 mg.ml^{-1} Propranolol, $o-o$. Temperature 37°C . Contact time 30 min.

(B) Fig. 3.119: Double reciprocal plot of the kinetics of inhibition of pure aryl sulfatase activity. The steady states were measured at varied concentrations of P.nitrocatechol (NTC) sulphate in the absence of drug, $\bullet-\bullet$; and in the presence of 4.0 mg.ml^{-1} Atenolol, $x-x$, and 4.0 mg.ml^{-1} Propranolol, $o-o$. Temperature 37°C . Contact time 30 min.

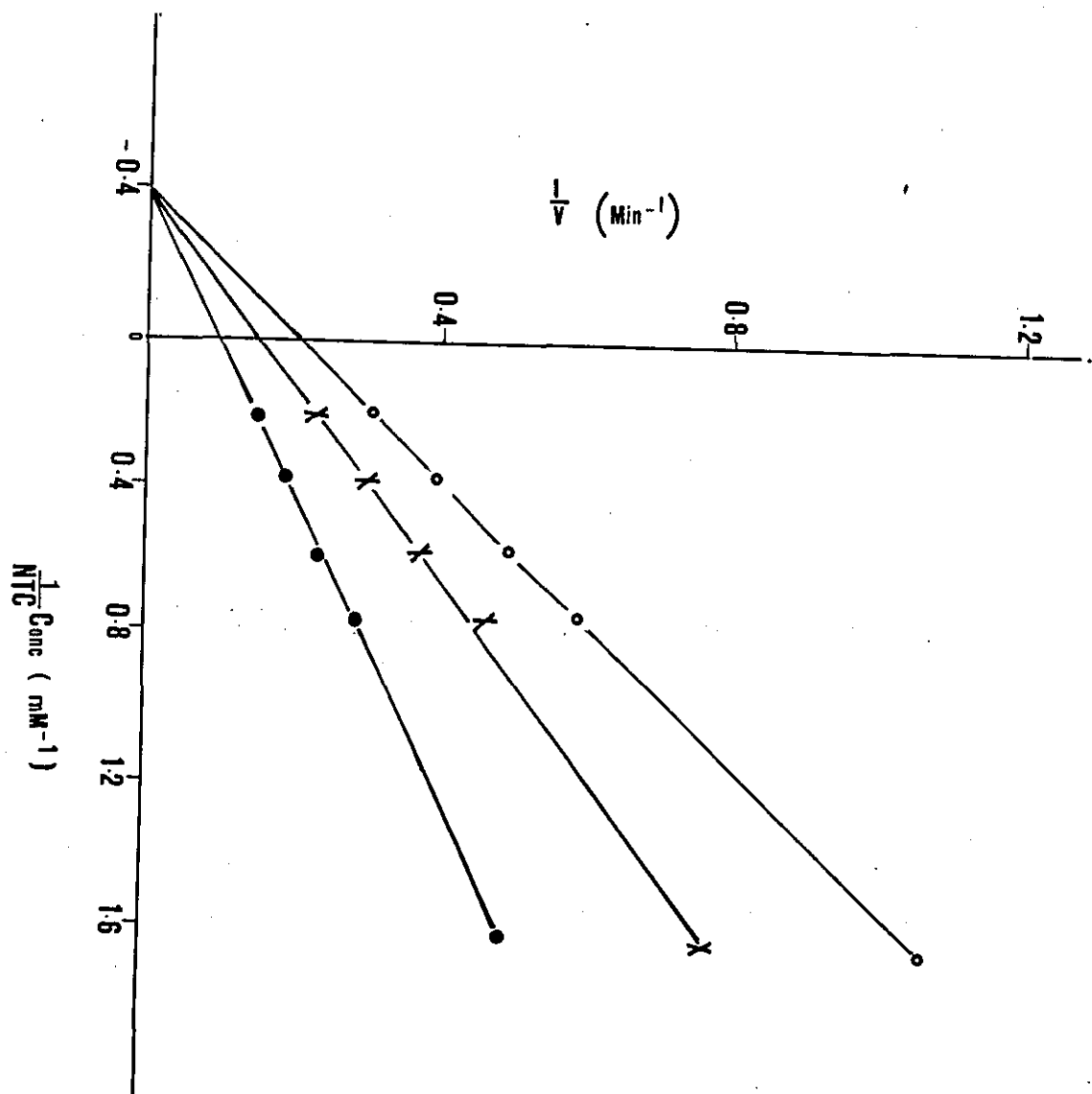
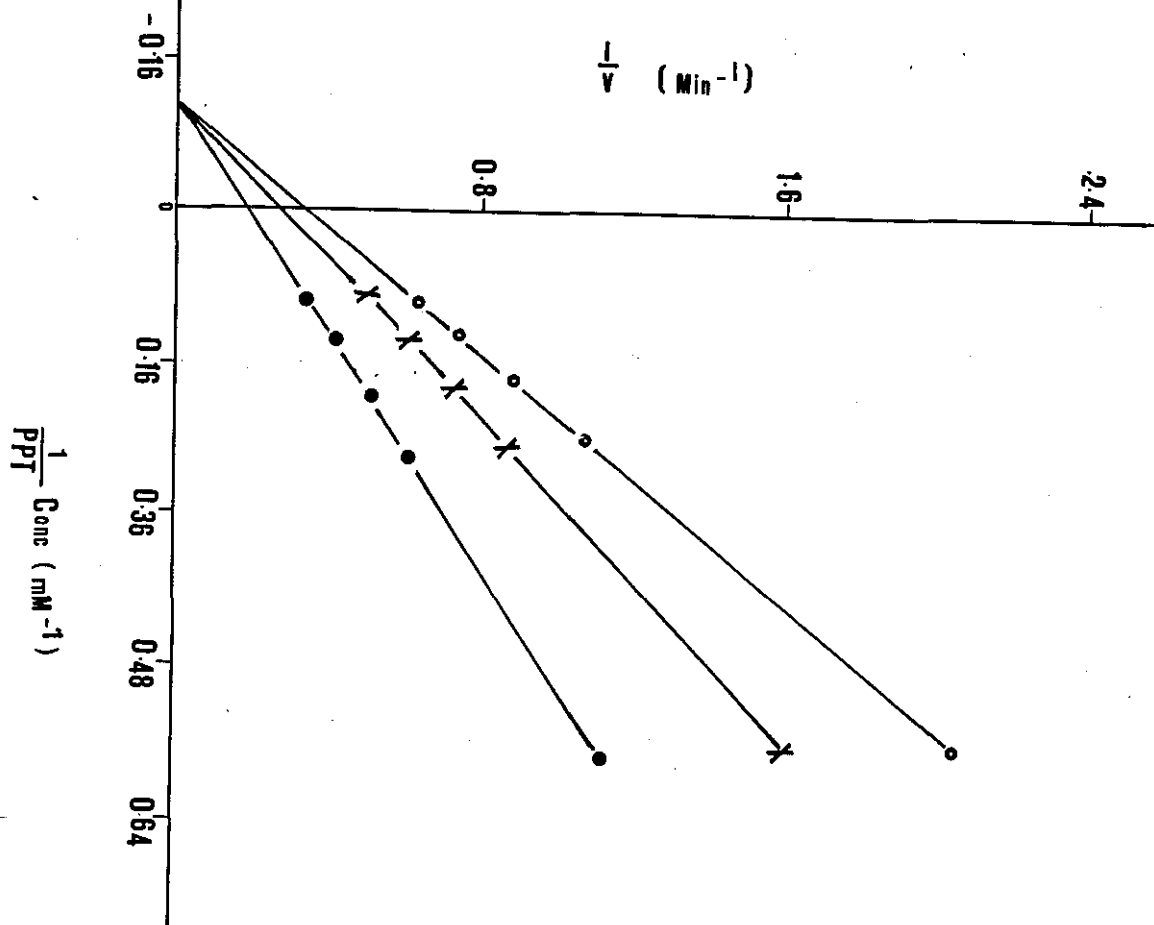
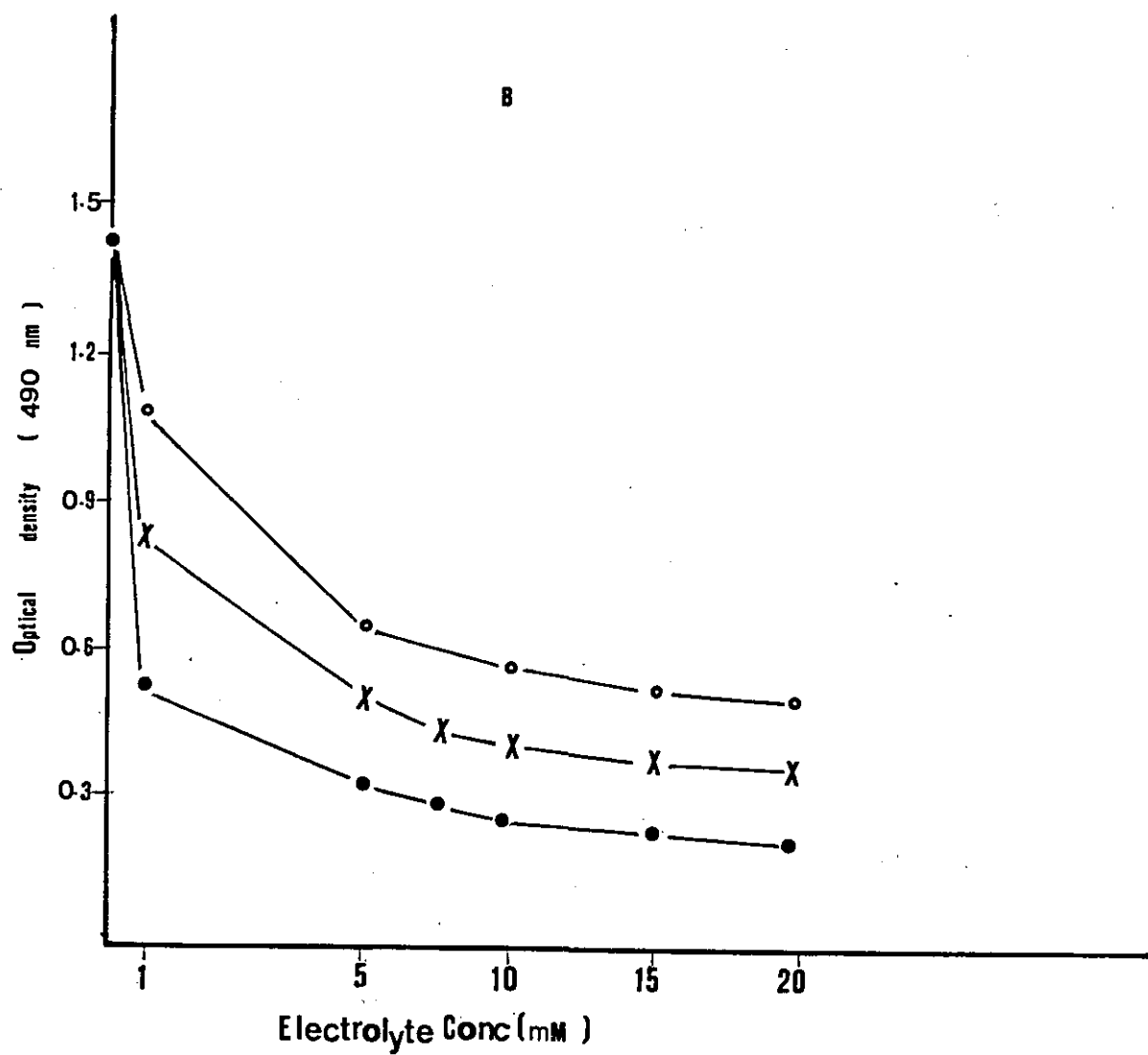
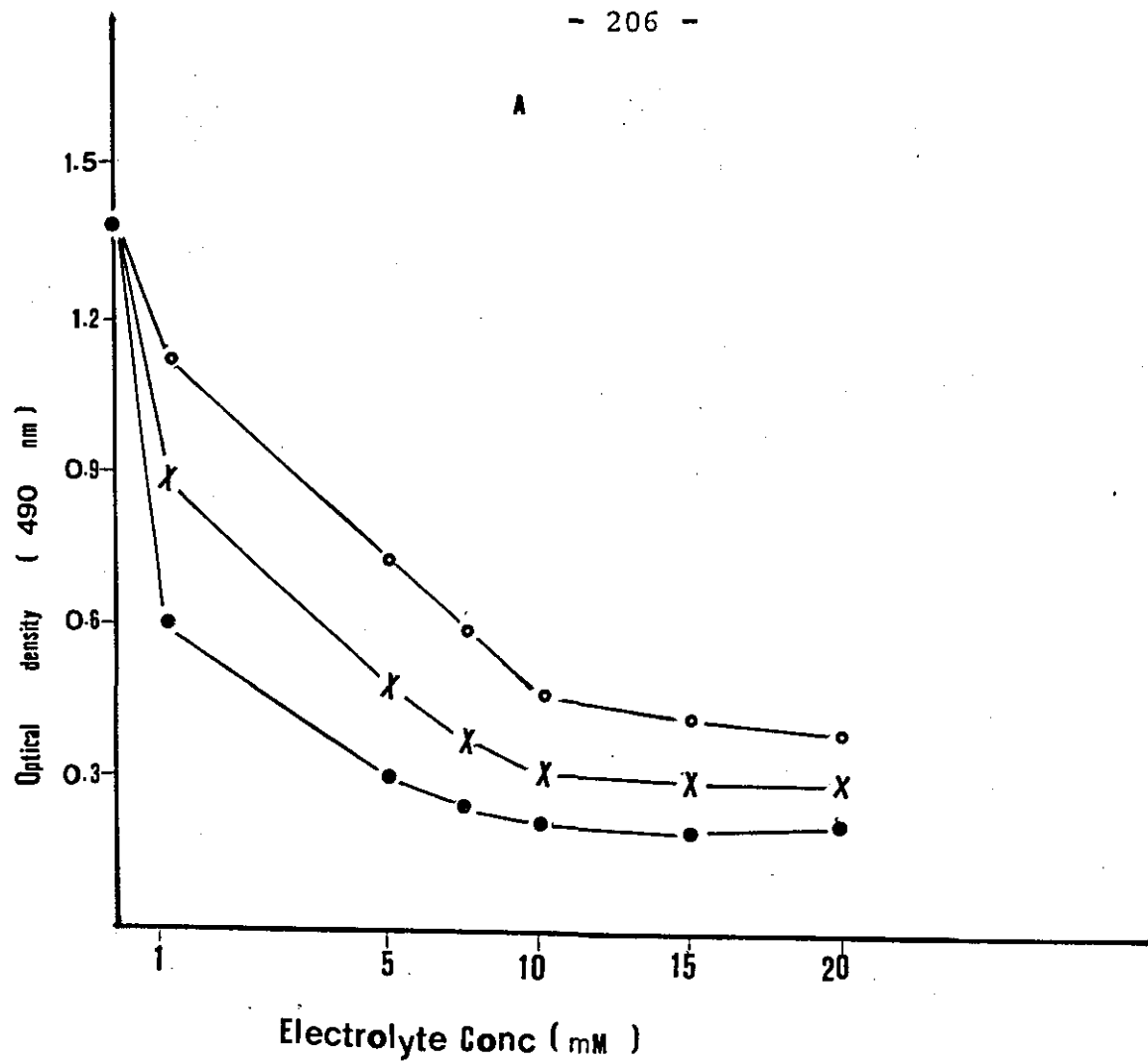


Fig. 3.120: Reversal by Mg^{2+} , Ca^{2+} and Zn^{2+} ions of the reduction of TTC from the cells of (A) E.coli (4.2×10^9 cells.ml⁻¹) and (B) C.albicans (3.8×10^5 cells.ml⁻¹) exposed to Propranolol at a concentration of 1.0 mg.ml⁻¹ at 37°C.

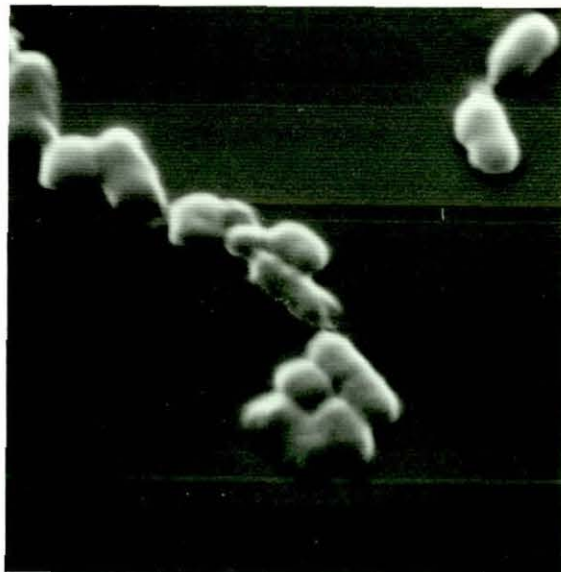
(A) Mg^{2+} ion, o-o; Ca^{2+} ion, x-x;
 Zn^{2+} ion ●-●.

(B) Mg^{2+} ion, o-o; Ca^{2+} ion, x-x;
 Zn^{2+} ion ●-●.



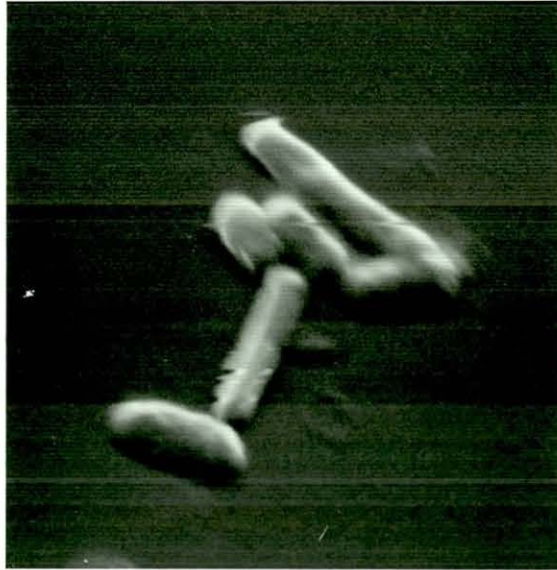
(A) Plate 3.1: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C for 15 min.

(B) Plate 3.2: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C for 15 min in the presence of 30.0 mg.ml⁻¹ atenolol (ca 2½ MIC).



(A) Plate 3.3: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C for 15 min in the presence of 0.5 mg.ml⁻¹ propranolol (Ca $\frac{3}{4}$ MIC), showing some filamentous forms without surface damages.

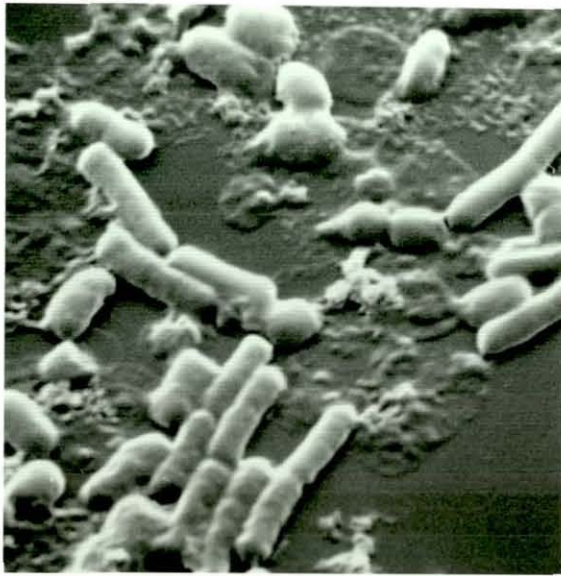
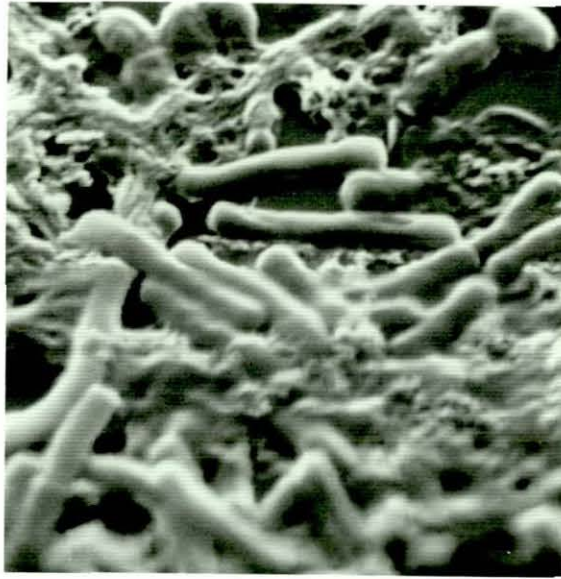
(B) Plate 3.4: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C for 15 min in the presence of 1.0 mg.ml⁻¹ propranolol (Ca $1\frac{1}{4}$ MIC). Some cells are similar to untreated ones, however some appear damaged.



(A) Plate 3.5: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C in the presence of 2.5 mg.ml⁻¹ propranolol. Some cells are damaged while some show surface damages.

(B) Plate 3.6: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C in the presence of 5.0 mg.ml⁻¹ propranolol. Some cells are damaged while some show surface damages.

(C) Plate 3.7: Scanning electron micrograph of E.coli cells suspended in growth medium maintained at 37°C in the presence of 7.5 mg.ml⁻¹ propranolol. Most of the cells are damaged, while some show surface damages.



(A) Plate 3.8: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min, showing yeast and filamentous forms.

(B) Plate 3.9: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min in the presence of 0.25 mg.ml⁻¹ (Ca ½ MIC) propranolol, showing yeast and filamentous forms without any surface damages.

(C) Plate 3.10: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min in the presence of 0.5 mg.ml⁻¹ propranolol (Ca MIC), showing yeast and filamentous forms without any surface damages.

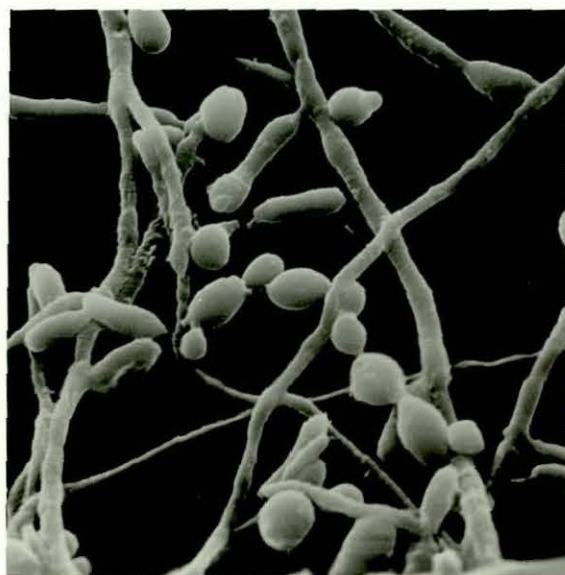
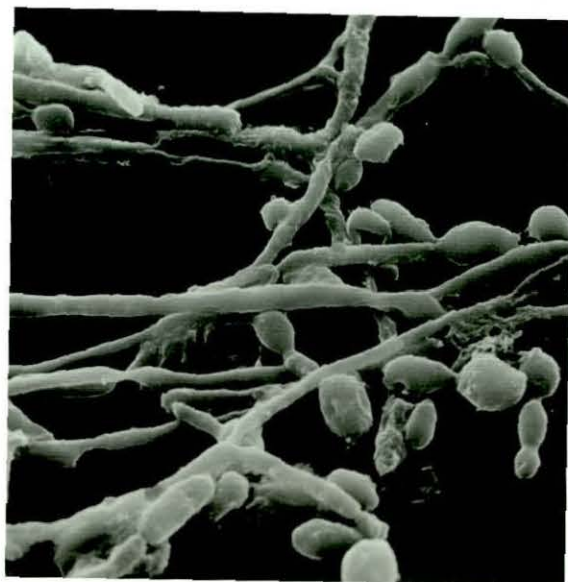


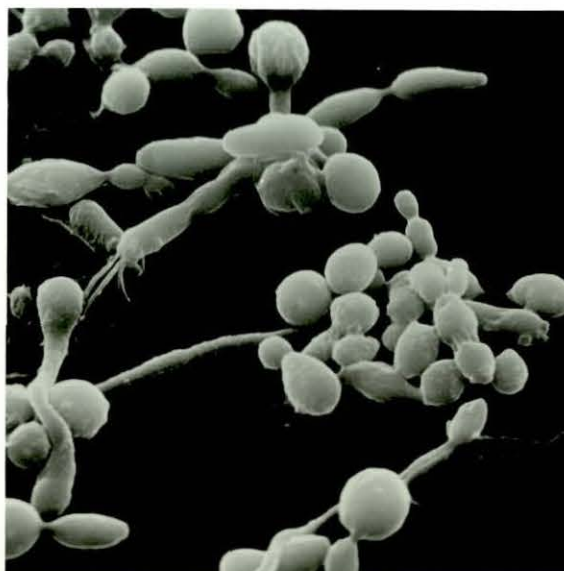
Plate 3.11: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min in the presence of 1.0 mg.ml⁻¹ propranolol (Ca twice MIC) showing some surface damages.

Plate 3.12: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min in the presence of 5.0 mg.ml⁻¹ propranolol showing surface damages.



Plate 3.13: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min in the presence of 10.0 mg.ml⁻¹ atenolol (Ca 1¼ MIC) showing yeast and filamentous forms without any surface damages.

Plate 14: Scanning electron micrograph of C.albicans suspended in growth medium maintained at 30°C for 15 min in the presence of 25.0 mg.ml⁻¹ propranolol showing yeast and filamentous forms without any surface damages.



CHAPTER FOUR

DISCUSSION AND CONCLUSION

CHAPTER FOUR

DISCUSSION

β -blockers affect microbial cells. Dependent on structure and concentration, they inhibit growth, membrane bound enzymes and respiration, and may cause cell lysis and loss of viability. Results obtained by studying such effects as well as other whole cell responses and those factors reducing these responses have been used to investigate the interactions of β -blockers and cell surfaces.

The MIC data (Table 3.1) show that microbial cultures are inhibited by both β -blockers and local anaesthetics. C.albicans and B.cereus show greater sensitivity to β -blockers and the structurally similar local anaesthetics compared to either E.coli or P.aeruginosa. In general, therefore, Gram-positive type organisms are more sensitive than Gram-negative types. This is in broad agreement with the findings of Fazly Bazaz and Salt, (1983a) who showed that local anaesthetics affect the growth of Gram-positive bacteria. Suggestions as to the reason for the greater susceptibility of Gram-positive bacteria or C.albicans compared to Gram-negative bacteria lie in the difference in the chemical composition of the surfaces of these organisms. The outer membrane of Gram-negative bacteria with its high lipid content, over the walls of Gram-positive bacteria and C.albicans, affords a greater protection to cells of Gram-negative bacteria from the influx of chemical agents. Indeed Lein and Hansch (1968) have shown that the greater content of lipids in Gram-negative bacteria is responsible in these organisms for slowing down the passage of highly lipophilic molecules. In addition to this, production of coating materials and the presence of a capsule may also contribute to an effective permeability barrier.

The sensitivity of the organisms however varies with different types of β -blocker and local anaesthetics. Thus propranolol, oxprenolol (β -blockers) and tetracaine (local anaesthetic) are effective antibacterial and

antifungal agents. β -blockers such as Metoprolol, Acebutolol, Timolol, Nadolol and Sotalol are intermediate acting, but Atenolol, Practolol, and Pindolol (β -blockers) as well as Procaine (local and procainamide anaesthetics) have little activity. These differences reflect structural differences between the individual β -blockers and local anaesthetics, the more lipophilic compounds having greater potency over less lipophilic compounds. Substitution of a benzene ring on to the aromatic ring of propranolol gives rise to a marked increase in the lipophilicity over others and therefore greater potency. On the other hand, the presence of electron-donating substituents in either atenolol or practolol decreases lipophilicity leading to reduced antimicrobial potency. Yu-Wing and Bhupat, (1977) have shown that structural differences in effective antimicrobial agents brought about an increase in lipophilicity, which give rise to enhanced permeability of cell surfaces. This is the probable cause of the greater effectiveness of highly lipophilic β -blocker (propranolol) and local anaesthetic (tetracaine) over atenolol and practolol or procaine and lignocaine. Similar results were obtained by Fazly Bazaz and Salt (1983 a).

Growth inhibition studies were also investigated by the shake culture method (Fig. 3.1 - 3.24). The reduction of growth in the presence of β -blocker and local anaesthetics is concentration dependent. At drug concentrations which approximate to minimum inhibitory concentration (tube dilution method), the growth rate approximates to zero. However, slight variations in the interpreted values of the MICs were obtained using either the tube dilution or the shaken culture methods which probably reflects the different incubation conditions and times. This was particularly notable with C.albicans with which pseudohyphae formation was influenced by shaking culture technique (reflecting defficient aeration). "Filament" formation is likely to bring about changes in overall cell size, consequently the number of available surface sites in the individual cells will vary. It has been suggested that different enzyme

systems control the rate of growth over different ranges of inhibitor concentration (Harris and Morrison, 1961). Thus, it is necessary that cell size and available surface remain unchanged if comparable results are to be obtained using either techniques. In addition to "filament" formation, production of clumps in cultures incubated by tube dilution techniques may result in variations of MIC values.

Growth occurs exponentially in the presence of lower concentrations of β -blockers and local anaesthetics for example, cells of E.coli treated with 0.25, 0.5 and 0.75 mg.ml^{-1} propranolol (Fig. 3.1) produced a growth pattern similar to that of untreated cultures, but with a decrease in the exponential growth rate. An exponential growth rate was not achieved with higher concentrations of either β -blockers or local anaesthetics. This is in general agreement with the findings of Lang and Rye (1972) and Fazly Bazaz and Salt, (1983 a) who showed similar characteristic growth for cells of E.coli treated with lower and higher concentrations of alcohols and local anaesthetics respectively. The reduction of the exponential growth rate in the presence of these agents, increases progressively at higher concentrations, and may, at concentrations above the MIC, cause a reduction in the culture optical density.

The inhibitory growth pattern shown in (Figs. 3.1 - 3.24) are similar to those produced, by Smith and Rosenkranz, (1970) and are different from those produced by some^{some} antibiotics (Harris and Morrison, 1961; Garrett and Miller, 1966). This growth pattern has been interpreted by Garret and Miller (1966) as indicative of inhibitors combining with receptor sites on the "biophase" following equilibration. Consequently, growth proceeds at a rate proportional to the fraction of uncombined receptor sites. Similarly, Harris and Morrison (1961) postulated that inhibitors combine with cellular enzymes competitively, the velocity of reaction occurring at a rate proportional to the concentration of uncombined enzyme. It thus follows that growth rate will decrease with increasing inhibitor

concentration, and at high concentration of the inhibitor, the growth rate is likely to fall to zero. This approach fits very closely with the obtained growth response curves (Figs. 3.1 - 3.24). The growth response curves in the presence of sulphonamides (Garrett and Wright, 1967), lincomycin (Mielck and Garrett, 1969), erythromycin (Garrett and Herman, 1970) and local anaesthetics (Fazly Bazaz and Salt, 1983a) are also in close approximation with this prediction. However, with antibiotics such as chloramphenicol and tetracycline (Garrett and Miller, 1966) and spectinomycin (Mielck and Garrett, 1970), a linear relation between growth rate and inhibitor concentration was observed, interpreted as suggesting that the inhibitors only need to occupy a small fraction of the receptor sites for total inhibition of bacterial growth. (Mielck and Garrett, 1970) attributed this inhibitory pattern as a consequence of multiple enzyme inhibition, where several enzymes are inhibited to differing extents after different threshold concentrations of inhibitor had been reached. With these agents, microbial cell growth is gradually inhibited, then followed by a new growth rate constant, linearly dependent on drug concentration.

To consider the reversibility of the effects of β -blockers and local anaesthetics (Figs. 3.25 - 3.36), cultures were treated with a range of concentrations of either β -blockers or local anaesthetics. Low concentrations (sub-MIC levels) of propranolol, oxprenolol, atenolol, practolol, tetracaine and procaine caused some growth inhibition, but on resuspension or dilution with fresh growth medium the cells grow at a rate similar to that of the untreated culture. Higher concentrations of these agents however showed, on resuspension or dilution, an initial lag phase after which growth proceeded at a rate lower than that of the control culture; the extent of this effect depended on drug concentration.

Reversibility of the actions of inhibitors on microbial cell growth has also been investigated by Lang and Rye (1972). They attributed this to an initial uniform

inhibition of the individual cells. Furthermore, the reversibility growth pattern shown in (Figs. 3.25 - 3.36) is very similar to that observed by Fazly Bazaz and Salt, (1983a). They attributed the initial lag phase to result from a need for the cell to recover or undergo repair before new growth commences, or that the cell may undergo "shock" following dilution. It follows therefore, that cell recovery from drug treatment can occur rapidly or slowly dependent on the concentration of the drug. Furthermore, it indicates that the actions of these drugs may be due to a non-specific, reversible attack on the cell, the extent of which depends on the concentration of the drug (at least up to the MIC value).

Considering the mechanism of interactions of β -blockers with the cell and the possible site of the actions, the effects of such compounds on the cell membrane and alterations of cell surface permeability were studied. In the first instance (action on the cell membrane) higher concentrations of β -blockers were used to observe cellular lysis and leakage of cellular materials. At higher concentrations, effective β -blockers (propranolol and oxprenolol) cause the leakage of cellular materials. This would result in cell death (loss of viability) depending on the concentration of the drug (see later).

The actions of propranolol on the release of K^+ , ^{32}P -labelled compounds, pentoses and 260 nm absorbing materials from cells of E.coli and C.albicans are shown in (Figs. 3.62 - 3.74). Atenolol and practolol did not cause any leakage of cellular material from microbial cells even after prolonged contact. Furthermore, SEM (plates 3.2, 3.13, and 3.14); . show no surface or cellular damage in the presence of atenolol; nor did SEM (Plates 3.3, 3.4, 3.9 and 3.14) of lower concentrations of propranolol even though at these levels both propranolol and atenolol caused growth inhibition.

Propranolol and oxprenolol caused the release of cellular materials from both E.coli and C.albicans. Low concentrations of propranolol and oxprenolol caused small leakage of cellular materials which increases as the concentration of these drugs is increased, reaching a

maximum at approximately $10 - 15 \text{ mg.ml}^{-1}$ and $15 - 20 \text{ mg.ml}^{-1}$ propranolol and oxprenolol respectively. Considering the actions on K^+ and ^{32}P -labelled compounds (Figs. 3.68 and 3.71), release occurred at a much lower concentration of propranolol and oxprenolol. For example, the threshold concentrations required to cause leakage of 260 nm and pentoses by propranolol (Figs. 3.62 and 3.70) was about 2.5 mg.ml^{-1} but was even lower (about 1.0 and 1.5 mg.ml^{-1}) for the release of K^+ and ^{32}P labelled materials. In addition, release of K^+ and ^{32}P -labelled materials occurred quickly. This is in agreement with the findings of Lambert (1979) who concluded that such rapid losses are more likely to be due to a direct interaction of the drugs with the cytoplasmic membrane.

Release of K^+ and ^{32}P -labelled compounds may include both those at the cell surfaces and those from the cytoplasm. Their release has been used as a model by other workers to assess direct membrane activity of effective compounds. The findings of Beggs (1983), clearly indicated that in contrast to miconazole, high levels of ketoconazole failed to cause any significant release of K^+ from C.albicans and C.parapsilosis, suggesting that miconazole can exert a direct membrane effect, whereas ketoconazole cannot.

Release of cellular materials from cells was complete within 15 or 20 minutes contact time (Figs. 3.73, 3.76 and 3.74) during which maximum release was obtained and after which leakage was either reduced or remained unchanged. In the same manner, the leakage - drug concentration profile show a maximum release at high drug concentrations as previously explained. Higher concentrations either brought about a slight change or reduced level of release.

Evidence in support of the release pattern with an essentially similar drug was provided by Leug and Bhupat (1977) for the action of tetracaine on P.aeruginosa, and Silva and Sousa, (1979) for chlorpromazine, nupercaine and tetracaine on B.cereus. Diphasic patterns of release have been observed by Rye and Wiseman 1964 and 1965) and by

Hugo and Longworth (1964); both suggested that they observed reduction in the amount of leaked material at high drug concentration resulted from the "sealing off" of cytoplasmic membrane by a surface film of chlorhexidine. This suggestion was not however supported by the electron micrographs of microbial cells treated by high concentrations of propranolol (Plates 3.6; 3.7 and 3.12). Similarly a sealing off effect was not observed by Fazly Bazaz and Salt (1983a) in electron micrographs of E.coli cells treated with high concentrations of tetracaine. Salton, (1951) implicated the re-adsorption of leaked material by the drug saturated cells as a cause of observed reductions of leakage of cellular materials at high concentration; leaked materials may have chemical groups likely to adsorb to complimentary sites on the exposed surfaces.

The change in the release profile at high drug concentration can partly be explained by the activity of autolytic enzymes. At 37°C the extent of the release of cellular materials was reduced (Fig. 3.67), but at 0°C, leakage of cellular material was apparent (Fig. 3.67). However, it is not certain, low temperatures usually stop the activities of autolytic enzymes. Further investigations are needed to substantiate this suggestion although Bläsi and Geisen, (1983) have demonstrated that autolytic enzymes do not cause lysis when the cells are grown under conditions of low pH (< 5.5) or low temperatures (below 1°C), and according to Lubitz and Halfman, 1984 and Höltze and Van Dium (1984) these enzymes are inactive at low temperatures. Thus it is possible these enzymes may have been denatured or inactivated at the low temperature used. In addition, released pentoses could be re-utilized by cells as a carbon source.

In the presence of β -blockers, the amount of ^{32}P released from E.coli (Fig. 3.72) is similar to the amount released with cold trichloroacetic acid (TCA), and the ^{32}P released from C.albicans cells in the presence of propranolol (Fig. 3.72A) is even greater than that released with cold TCA, which extracts the metabolic pool (Britten and McClure, 1962). This finding suggests that

leakage of cellular materials within the initial contact time, and even within 60 min exposure time (Fig. 3.64), longer exposure time shows that these β -blockers do cause the release of some cellular materials. Similar results were reported by Silvia and Sousa, (1979) on the release of K^+ from B.cereus with (100 mM) procaine, and Fazly Bazaz and Salt, (1983 a) on the release of pentoses from E.coli with 40 and 50 mg.m.⁻¹ of procaine and lignocaine respectively.

Cell lysis induced by lytic agents is the result of series of events leading to membrane disruption and breakdown with release of cellular materials. Lytic agents penetrate the organism by a series of specific uptake mechanisms that directly leads the compound to its target site (Salton, 1968; Brown, 1975; Braun and Frenz, 1980; Schaller and Dreher, 1981). The ability of lytic agents to penetrate the organism and to react with protein-protein as well as lipid and lipid-protein interactions (Gale, 1974; Lambert, 1978) and thus promote significant alteration in its permeability could contribute to lytic phenomena (Hancock and Raffle, 1981).

Observations that propranolol and oxprenolol at high concentrations led to cellular lysis and leakage of intracellular materials suggests that these drugs act by damaging the cell membrane. Furthermore, since neither atenolol nor practolol are effective lytic agents suggests an inability to effectively interact with the cell membrane in a manner likely to result in lost cellular materials.

Concentrations of drugs capable of inhibiting growth (e.g. lower concentrations of propranolol) do not cause loss of cell viability (Fig. 3.37 and 3.42). For example 0.25 and 0.5 mg.ml⁻¹ propranolol do not cause a reduction in cell viability in cells of E.coli and although 1.0 mg.ml⁻¹ propranolol did cause some loss of cell viability, the effect was small. In addition, 1.0 mg.ml⁻¹ propranolol, although not causing any release of 260 nm absorbing material and pentoses did initiate some loss of K^+ and ³²P-labelled materials. Similarly, 0.1, 0.25 and 0.5 mg.ml⁻¹

propranolol induces sufficient membrane damage to cause the leakage of metabolic pool from microbial cells. Release of the ^{32}P -metabolic pool can also occur within a period of 10 min exposure of cells in the presence of propranolol at a concentration of 2.5 mg.ml^{-1} (Fig. 3.71a). On the other hand the amount of 260 nm absorbing material, pentoses, and K^+ release by both propranolol and oxprenolol is lower than the amount released when the cells were boiled for 5 min.

Another chemical class which structurally alters the cell membrane are the local anaesthetics (Seaman, 1972; Fazly Bazaz and Salt, (1983a). Tetracaine has been shown to cause the release of metabolic pool phosphate, while 260 nm absorbing materials and pentoses are still not detected. Despite this, the leakage of 260 nm materials appears to be related to the viability of the organisms. Included in the material lost are metabolic pool aminoacids, the loss of these materials may result in the inhibition of m-RNA and nucleic acid biosynthesis (Rosenkranz and Carr, 1964; Lang and Rye, 1972) with consequent impairment of cellular processes such as protein synthesis. In addition aminoacids have been shown to be important in osmoregulation (Tempest and Meers, 1970). Their loss following drug treatment will undoubtedly lead to instability of the cellular osmotic barrier, consequently enhancing further lysis of the cell.

The leakage of other cellular materials (K^+ , ^{32}P -labelled materials and pentoses) may also be fatal. K^+ is needed for osmoregulation as well as forming part of metalloenzymes as in $\text{Na}^+ \text{K}^+ \text{-ATPase}$. Phosphates in addition to forming part of the cellular structure are also actively involved in certain energy transfer reactions in the cell and pentoses, apart from being important as a potential energy source, may also be required as building blocks for the synthesis of other important biological macromolecules, including the cell wall. Consequently, the leakage of this material will lead to cessation of microbial growth, but gross total leakage will undoubtedly lead to cell death.

Although atenolol or practolol are unable to cause

propranolol neither caused any loss in cell viability in cells of C.albicans nor leakage of cellular materials. Above threshold concentrations of 1.0 mg.ml^{-1} and 0.75 mg.ml^{-1} propranolol for E.coli and C.albicans respectively, cell viability was reduced by amounts related to drug concentrations. Concentrations of propranolol causing significant losses in cell viability did so within a short period of time (10 min), the overall extent of reduction being related to the drug concentration. Furthermore, the results show that increasing the contact time of microbial cells with β -blockers reduces cell viability significantly.

Indeed increasing the contact time of atenolol with both cells of E.coli and C.albicans (Fig. 3.40 and 3.42) causes loss of cell viability by an amount related to the concentrations of atenolol; such effects may not be detected with short contact times. This result is in agreement with the observations of Schmidt and Rosenkranz, (1970) who observed a reduction of viability of cells of E.coli treated with procaine and lidocaine after 1 hour, and suggested that lidocaine and procaine are bacterocidal when concentrations of 1% are used.

The bacteriostatic actions of β -blockers may be explained on the basis of known actions on cell membranes. According to Seeman, (1972), the membrane activity of many drugs is dependent on their interaction with phospholipid of biomembranes. Such interaction would ultimately lead to penetration of drug molecule into the membrane bilayer and accommodation in its hydrophobic-interior (Feinstein and Fernandez, 1975).

In the studies of cell viability, cells may be damaged during dilution and therefore are not able to recover and grow. This can be misleading and misinterpreted as cell death. To overcome this media containing 1% Tween 80 was employed but no change in the previously determined results was obtained.

The ineffective actions of atenolol as a bacteriostatic agent may lie in its inability to penetrate the cell. The difficulty in the penetration of the cell must

reside in the permeability barrier presented by the complex cell envelope of E.coli and the thick, rigid outer surface of C.albicans. Thus it is suggested that the microbial cell surface still retains most of its biological functions at the membrane level in the presence of atenolol.

Propranolol on the other hand may be lethal, perhaps due to interaction with membrane components with a consequent disruption of the permeability barrier, causing the release of periplasmic and/or cellular materials (Fig. 3.62 - 3.74). This is substantiated by the fact that the SEM (Plates 3.5; 3.6; 3.7 and 3.12) of cells treated with propranolol show alteration of the morphology of the cell surfaces. By contrast SEM (Plates 3.2, 3.13 and 3.4) of cells treated with atenolol did not show any surface damage even at high concentrations. It does not mean that some atenolol does not penetrate the cell and interact with the biological membrane. However, effective concentration may not be obtained, and it is likely that interaction of atenolol with components of the membrane is such that it is not necessarily lethal to the cells at least in the short term (Fig. 3.40 and 3.42). Thus the bacteriostatic actions of propranolol could result from the alterations of the cell structure following interactions with the components of biological membrane. Inhibition of biochemical reactions would then occur as a consequence of membrane alterations. The alteration of the permeability of the cytoplasmic membrane in terms of TTC reduction, inhibition of oxygen consumption and sugar uptake within the microbial cells have been observed with subinhibitory concentrations of β -blockers. These mechanism of actions have also been employed by other workers as an index for assessing the interactions of drugs with bacterial cell surfaces and membranes (Leung and Bhupat, 1977; Lambert, 1978).

The results for the effects of β -blockers on respiratory enzymes (Figs. 3.43 - 3.50) and (Figs. 3.51 - 3.59) show that β -blockers are capable of interfering with the activity of dehydrogenase enzymes as demonstrated by TTC reduction and by cellular oxygen consumption.

The inhibition of TTC reduction by β -blockers is

Dependent on concentration and upon the type of β -blockers as well as the type of the substrate used. Thus, the reduction of TTC in the presence of β -blockers is more influenced when lactate and malate are used as substrates (Figs. 3.45, 3.46, 3.49 and 3.50). The order of effectiveness in reduction of TTC by β -blockers is propranolol > oxprenolol > atenolol = practolol. Propranolol and oxprenolol inhibited endogenous as well as exogenous respiration while atenolol and practolol affected only exogenous respiration. Similar results were obtained with Candida albicans treated with ketoconazole by Shegematsu Jun Uno and Arai (1982), and they suggested that inhibition of endogenous respiration results from interference with oxidative metabolism of microbial cells.

Very low concentrations of β -blockers were required to inhibit dehydrogenase activity. Sub-MIC concentrations of propranolol (0.25 mg.ml^{-1}), oxprenolol (0.5 mg.ml^{-1}), atenolol and practolol (4.0 mg.ml^{-1}) inhibited the activities of this enzyme. The inhibition of dehydrogenase activity with sub-MIC levels of ketoconazole treated against C.albicans and local anaesthetic treated against E.coli have also been observed by Shegematsu Jun Uno and Arai (1983), Fazly Bazaz and Salt (1983 a). This level of β -blocker concentrations also inhibited microbial growth (Fig. 3.1 - 3.24), but did not cause any loss in cell viability (Fig. 3.37 and 3.42) suggesting that alterations of cell surfaces may not be solely responsible for the inhibitory activities of these enzymes, but may involve a more direct interaction between β -blockers and enzyme.

In addition to inhibiting respiration by interfering with the activities of dehydrogenase enzymes, β -blockers also affect respiration as detected by oxygen consumption (Fig. 3.51 - 3.59). Very low concentrations were also required to inhibit oxygen consumption as was the case for TTC reduction.

The uncoupling agent, 2,4-dinitrophenol (DNP) stimulates oxygen consumption (Figs. 3.54 and 3.58). None of the β -blockers used in this study behaved similarly as stimulation of oxygen in excess of 100 per cent was not

observed with either E.coli or C.albicans or with any of the substrates evaluated, suggesting that β -blockers may not be acting as uncoupling agents, Wei and Teng, (1985) have also shown that propranolol did not behave as an energy uncoupler but rather as an inhibitor of mitochondrial respiration through inhibition of Mg^{2+} -ATPase. Inhibition of Mg^{2+} -ATPase was also observed in the present studies (Figs. 3.104 - 3.108). The inhibition of Mg^{2+} -ATPase by β -blockers would lead to cascade effects, one of which is the energy-dependent system in microbial cells, and the effect on respiration can be rationalized with the chemiosmotic theory (Mitchell, 1979). The build up of electrochemical gradient following active pumping of protons across microbial membranes is utilized by the Mg^{2+} -ATPase complex to make ATP. In the presence of β -blockers Mg^{2+} -ATPase activity may be inhibited and thus the proton-gradient generated by respiration cannot be utilized and will then feed-back inhibit the active respiration. From this it is possible to suggest that the effect of β -blockers on respiration may partly or wholly be explained by inhibition of Mg^{2+} -ATPase.

However, Hugo and Bloomfield (1971) suggested that changes in membrane permeability may be responsible for a comparative increase in oxygen consumption in which case permeability of substrate within cells where the enzymes are localized is enhanced (Hugo and Street, 1952). This might explain the different responses of substrates to respiratory activities in the presence of β -blockers (i.e. the increase in the inhibition of malate and lactate dehydrogenases activity compared to the activation of glucose and succinate dehydrogenases). Malate and lactate dehydrogenases may be located at or near the cell surface where they are more accessible to the substrates compared to glucose and succinate dehydrogenases. However, since increase in oxygen consumption in excess of 100% was not detected, it is comprehensible to suggest that the different responses of substrates following changes in cell permeability in the presence of β -blockers is likely

through inhibition of Mg^{2+} -ATPase.

Propranolol caused greater inhibition of oxygen consumption compared to either metoprolol or practolol (Figs. 3.51 - 3.55). Exposure of microbial cells to propranolol for longer periods could possibly lead to impairment of oxygen consumption. This means that the energy (ATP) derived from respiratory activity, or from substrate level oxidation normally utilized for the active transport of sugars and aminoacids, and the production of ATP would be impaired, therefore growth of microbial cells would cease.

β -blockers inhibited the uptake of ^{14}C -glucose and mannose (Figs. 3.60 - 3.61). The overall profile is in a manner similar to growth inhibition studies observed in (Figs. 3.1 - 3.24), occurring at sub-MIC levels of propranolol and practolol. These results are similar to the findings of Van Den Bossche, (1974) on the effect of miconazole on the uptake of glucose by C.albicans. He suggested that the fall in the optical density of cultured microbial cells suspended in growth medium containing, for example, glucose as a carbon source is due to the unavailability of the carbon source needed for growth. The result of inhibition of uptake of sugars in the cell may be reduced growth. However, whether reduced growth rate reduces sugar uptake is not clear, as when treated cultures were allowed to continue to grow they did eventually take up the radiolabelled sugars in amounts equal to that of the untreated cultures (Fig. 3.60 - 3.61). Furthermore, growth was monitored by measuring the dry weight of cells at time intervals (Table 3.6a - 3.6b), and indicates that reduced growth in cultures of microbial cells treated with propranolol and practolol may grow to the same extent as the control if cultivation is continued until T_2 , which could be expected if treated cells grow at a rate slower than the untreated cultures.

The mechanism likely to prevent uptake of ^{14}C carbon sources may be due to changes in permeability properties of biological membranes (Abram, 1960). Since the outer membrane of Gram-negative bacteria or the candidal

cell wall do not carry out active transport mechanism (Costerton and Ingram, 1974), it is reasonable to suggest that inhibition of active transport is not the probable cause of inhibition of uptake of sugars. However, many substances penetrate through the cell envelope by either passive or facilitated diffusion. Facilitated diffusion involves catalyzed permeability of substances by a carrier with a binding site for sugars. It is suggested that either binding of sugar to this site is altered, or drug-cell surface interaction may be possibly leading to altered diffusion into cell.

Considering the amount of radioactivity recovered from various fractions of E.coli and C.albicans cells it was observed that the amount of radioactivity from various fractions treated with either propranolol or practolol was low compared to the untreated cultures (Table 3.4 - 3.5). This is in agreement to the findings of Van de Bossche (1974) on the study of uptake of ^{14}C glucose by C.albicans in the presence of miconazole and Hamilton (1975) on the uptake of ^{14}C glucose by S.aureus in the presence of alcohols. From these observations they suggested that a decreased uptake of glucose in the presence of drugs by microbial cells can affect growth and in addition they suggested that a wide range of effective drugs appear to act primarily on the microbial cell membrane inducing selective permeability changes. Furthermore, the amount of radioactivity recovered in cell envelope is small in untreated culture. In contrast the amount of radioactivity recovered in cell envelope is smaller than that recovered in cytoplasm in treated culture suggesting that uptake of ^{14}C sugar by microbial cells proceed at a rate slower than that of the untreated culture in support of altered permeability.

In addition to affecting uptake of sugars, β -blockers may also impair the utilization of the sugars in the cell. The utilization of glucose or mannose is usually a two-step process: the active uptake of the sugars, and the formation of phosphosylated sugars. Furthermore, the phosphoenol pyruvate phosphoenoltransferase system in the

uptake of sugars by S.aureus employs an enzyme which is extremely sensitive to a variety of chemical agents which may explain the inhibition of both glucose uptake and utilization (Hamilton, 1975; Postman and Roseman, 1976).

Harris and Harrison (1961), found a link between the uptake of adenine and glucose metabolism in yeast which may have been due to a combination of the purine with a dissimilation product to form nucleotides. The fact that propranolol and atenolol inhibited glucose uptake make it reasonable to suggest that any inhibition of glucose incorporations to purines would result in reduced utilization of glucose. The decreased incorporation of glucose into purines may impair such processes as biosynthesis of wall, as the first step in the route to the end product of Murien is the synthesis of UDP-muramic acid (Anwar and Vlaovic, 1979). Furthermore, since mannan is one of the basic constituents of wall of C.albicans (Rogers, 1963; Hunter and Rose, 1971; Rose, 1976), it is reasonable to suggest that reduced growth following inhibition of mannose uptake might be as a consequence of impairment of incorporation of mannan to the wall of this organism.

The effect of β -blockers on the uptake of glucose and mannose must be non-selective. This can be concluded from the different carrier system with a binding site specific for particular types of sugar (Lehninger, 1975). It means that the "carrier" protein for glucose and mannose are different and are sensitive to β -blockers. Considering the effect of β -blockers on β -galactosidase (Fig. 3.111 - 3.112), it was also found that these agents inhibited the activities of this enzyme, perhaps through the β -galactoside permease system resulting in the impairment of transport of lactose into the cell. These findings support the predictions in growth inhibition, that direct interaction of β -blockers with cell surfaces would lead to wide spread malfunction of the biochemical cell reactions.

The information so far presented indicates that relatively low concentrations of β -blockers rapidly inhibit several cellular functions. Inhibition of cellular

respiration and uptake of ^{14}C -sugars fit with this suggestion and are probably, therefore, the primary site(s) of action. As demonstrated by growth inhibition studies, this first stage can be reversed by resuspension in fresh growth media or by dilution, Lambert (1978) and Lang and Rye (1972) showed that phenylethyl alcohol (PEA) altered the cellular permeability barrier and concluded that its

effects resulted from a non-specific reversible attack on the cell membrane; the adsorption (of PEA) by the membrane altering several biochemical processes. It follows therefore, that such drugs form a loose association with the cell surfaces and it is likely that adsorption of antibacterial drugs by a cell is itself not a fatal event. However, secondary process leading to inhibition of the reproductive and metabolic process (bacteriostatic effect) may follow. Bacteriostatic effects would result if the permeability barrier is altered. This will result in free diffusion of small molecule, in which variations in the concentrations of metabolites will occur due to break down of transport processes. This could lead to an inhibition of various cellular processes indirectly by allowing leakage of small molecules from the cells or directly if the process is coupled to the membrane.

Various factors influence the distribution of drugs throughout the various possible locations within or beyond the biological membranes where they would affect specific or diverse biochemical functions. Thus the following parameters: uptake of β -blockers, lipid solubility, micelle formation and surface activity were investigated.

The uptake isotherms (Fig. 3.75 - 3.76) demonstrate adsorption to microbial surfaces. More propranolol is taken up than atenolol indicating that the difference in the chemical nature of both compounds is such that the affinity of propranolol to microbial membranes is higher than that of atenolol. Hancock and Raffle (1981) found a correlation between uptake and antibiotic sensitivity for whole cells and walls of species. On the basis of uptake data

for cetyltrimethylammonium bromide (CTAB or HTAB) by E.coli, Salt and Wiseman, (1970) suggested that individual cells first took up CTAB at or within cell surface layers. This surface adsorption was reversible. High concentrations resulted in further uptake and the penetration of the cytoplasmic membrane and subsequent membrane damage. Thus the antimicrobial activity of β -blockers can be explained by interaction between β -blockers and components of the plasma membrane in such a manner that sufficient β -blocker (such as propranolol) is taken up to saturate ionized sites on the outer surface and still leave enough for reaction with and disorganisation of the membrane consequently leading to leakage of low molecular weight metabolites.

The uptake isotherm (Fig. 3.7) is similar to that described by Fazly Bazaz and Salt (1983a) for the uptake of local anaesthetics by cells of E.coli. Propranolol shows a linear isotherm over much of the concentration range before saturating out, but atenolol shows a straight line isotherm (Fig. 3. 75). Seeman and Roth (1971) determined the membrane concentration and confidence limits at a particular anaesthetizing alcohol concentration, as well as the free energy of the interaction and suggested that membranes may contain either a finite number of independent sites (finite site model) or the membrane contains an infinite number of binding sites (Partition model). The finite-site model is characterized by an equilibrium constant, K , and a total number of binding sites, n . The partition model is characterized primarily by a partition coefficient, P . The finite site corresponds to "C" curve and the Partition model to "L" uptake isotherms (Giles and Smith, 1974). On the basis of this, propranolol and atenolol differ from each other, though "C" and "L" isotherms are not easily differentiated (Giles and MacEwan, 1960). Lang and Rye (1972), suggested that alcohols in simple solution in the aqueous biophase of the cell would rapidly be taken up at the cell membranes and produce a linear uptake. This would mean a reflection of total concentration of drug within the cell and not just drug bound at the cell membrane. Relating this to the type of

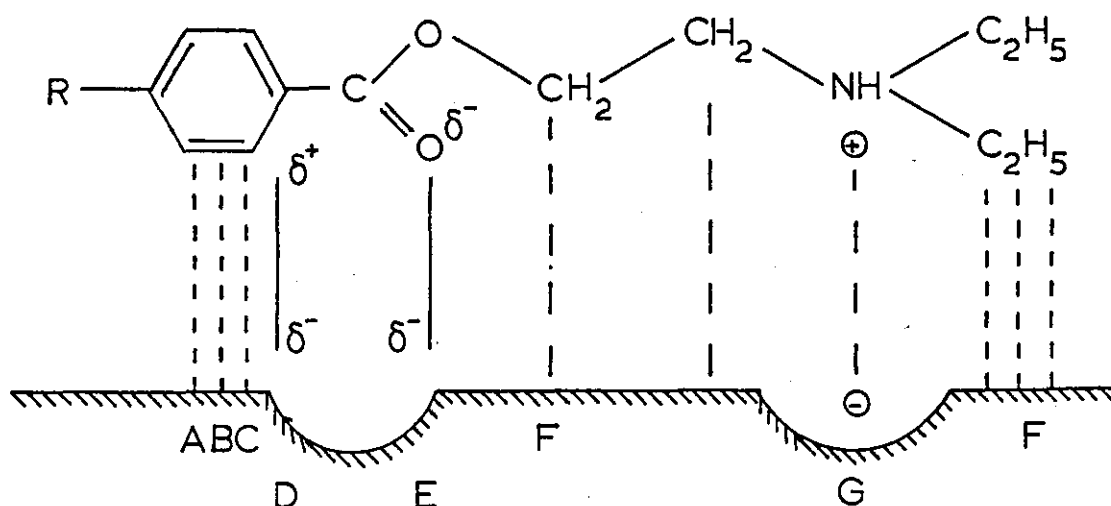
curve obtained for propranolol, propranolol concentration within the straight line region reflects total propranolol concentration inside the cell and this range of propranolol concentration covers those used in the investigation of this project. Fuller and Denyer (1985) reported that the uptake by E.coli of six phenolic agents approximately followed the "C" curve with initial concentration range. It is evident therefore that propranolol should be more effective than atenolol in inhibiting growth since within the cell the proportion of propranolol would be greater than that of atenolol.

Seeman and Roth (1971) found that the negative free energy of $-\text{CH}_2-$ (methylene group) in alcohol is about the same as the free energy of transfer per mole of $-\text{CH}_2-$ groups from an aqueous phase to a completely non-polar phase. Schneider, (1968) also arrived at the same conclusion and on the basis of this, suggested that the $-\text{CH}_2-$ groups of an alcohol may reside in some hydrophobic region of the membrane. This region consists of the non-polar portions of lipid molecule and non-polar interfaces between lipid and protein molecules or the hydrophobic regions of the protein molecules. Schneider, (1968) also found that the free energy of proteins increased for proteins which may undergo conformational changes. Thus, the site is probably the non-polar portion of lipid molecules or involves a protein which may undergo a conformational change on binding to a drug.

The molecular structure of β -blockers (Table 1.1) suggest cellular interaction similar to the alcohols or local anaesthetics as described above (Seeman and Roth, (1971; Schneider, 1968). According to Lang and Rye (1972) adsorbed molecules of this type would then interact with membranes by orientating perpendicularly to the plane of the membrane. This would allow co-operative adsorption; each membrane bound molecule facilitating the uptake of further molecules. Furthermore, the adsorption of the molecules on the membrane would also interfere with membrane bound enzymes by inducing a conformational change

in the membrane structure, or directly by interacting with the enzyme molecule.

Büchi and Perlia (1972) illustrated the various types of bonds which are formed when local anaesthetics are bound to the nerve membrane. This is illustrated in (Fig. 4.1). Since β -blockers are structurally similar to local anaesthetics it is likely that they could form similar types of bonds with biological membranes. An increase in the attachment of β -blockers (Fig. 4.2) to the receptor will depend on an increase of electrons at hydroxyl oxygen and on the $-OCH_2$ which in turn will depend on the substituent R on the benzene ring.

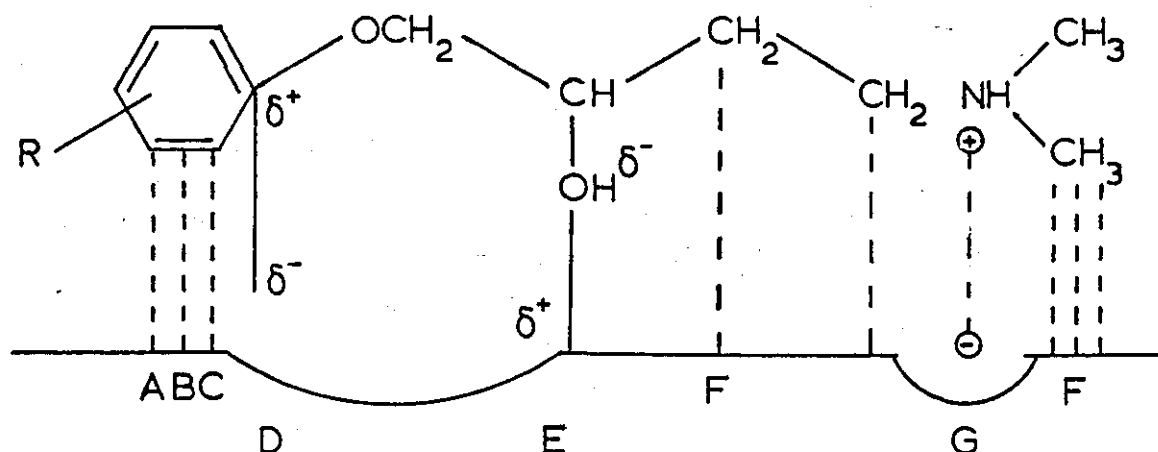


- ABC - Electron donor-acceptor binding
- D - Dipole attraction
- E - Hydrogen bond
- F - Van der Waals binding
- G - Electrostatic binding.

Fig. 4.1: Binding of local anaesthetics to the site of action (After Büchi and Perlia, 1972).

Electron density can be increased by electron donating substituents on the benzene ring. Therefore, propranolol having extra hydrophobic bonding site instead of electron donating group as in practolol or atenolol will have more capacity to attach to the receptor site, and will be more effective than either practolol or atenolol.

Although the derivative method for determination of



- ABC - Electron donor-acceptor binding
- D - Dipole attraction
- E - Hydrogen bond
- F - Van der Waals binding
- G - Electrostatic binding

Fig. 4.2: Illustrating the similarity between β -blockers and local anaesthetics binding on biological membrane

uptake (Fig. 3.75) of β -blockers used in this study is very sensitive, it does not account for the effect of the molecule of dansyl chloride attached to the β -blocker molecule. This will increase the bulk of β -blocker molecule, and may have been responsible for the "L" uptake isotherm profile for propranolol (Fig. 3.75). Such substitutions would certainly affect biological responses. Although the uptake isotherm for dansyl atenolol gave a

"C" type curve, such substitution would also affect the biological response of atenolol. It is therefore suggested that improvement in this technique requires, for example, determining the MICs of derivatised molecule or determining the lipid solubility characteristics of derivatised molecule and comparing this with underivatised β -blockers and corrections for the differences could then be taken into account. Furthermore, the concentration shown by the uptake isotherm represent the concentration of propranolol dansyl chloride or altenolol dansyl chloride. Therefore, the technique is limited by the difficulty associated with the determination of the actual β -blocker concentration. However, the technique is sensitive and fast though worthy of improvement.

Radiolabelled propranolol (Fig. 3.76b) gave a "C" curve for cellular uptake (Giles and MacEwan, 1960). The time course profile (Fig. 3.76, a) shows that uptake is rapid and with a high affinity suggesting the binding of propranolol to specific surfaces, the second phase of the curve thus reflecting complete saturation of available sites by propranolol.

In order to characterize the non-specific property of β -blockers as well as their potency and the site(s) of membrane perturbation their lipophilicity was assessed. The results are tabulated in (Table 3.10) and show that partition coefficients K^O/w increases with the pH of the medium in agreement to the findings of Hellenbrecht and Lemmer (1973). It also shows that K^O/w is greater with propranolol having greater lipophilic substituents over atenolol or practolol, oxprenolol being an intermediate. This finding supports Levy's (1968b) postulation that the physicochemical properties of β -blockers are determinants of their membrane affinity and therefore of their non-specific pharmacological effects.

Hellenbrecht and Lemmer (1973), measured the lipophilicity of a series of β -blockers and found that most apparent differences in lipophilicity of the β -blockers could be related to the ring-substituents of the compounds.

From these he observed that the order of increasing values of log P is: Propranolol > Oxprenolol > Atenolol ~ Practolol. This finding correlates well with the partition coefficients found experimentally in this investigation (Table 3.70). Thus the most lipophilic drug, propranolol, probably penetrates in the fatty acid side chains or reacts with hydrophobic proteins (Singer and Nicholson, 1972) in the membrane bilayer. The more hydrophilic drugs such as practolol or atenolol would probably bind superficially with the membrane. It also seems probable that the major factor affecting drug penetration is the lipophilic nature of the biological membrane, perhaps due to the affinity of drugs for these lipids and it is likely that lipids and proteins are involved in the "sites" which take up the drug. Schneider (1968) observed that the 'site' is probably the non-polar portion of lipid molecules or involves a protein which may undergo a conformational change on binding to the drug. Since β -blockers are amphipathic it is likely that they can interact with biological membrane, with the lipophilic part associating with the non-polar portions of lipid molecules, and the polar regions associating with themselves in the biophase. This type of interaction would normally result in membrane perturbation, and changes in membrane fluidity (Lenaz and Curatola, 1978).

In numerous other investigations the local anaesthetic properties of β -blockers have been assessed by their lipophilicity (Levy, 1968b; Engelhardt and Traunecker, 1969 and Davies, 1970). Again, Levy (1968b) has pointed out a close relationship between surface activity and local anaesthetic potency, measured on the rabbit cornea and supported by the experimental results obtained by Hellenbrecht and Lemmer (1972) with a series of β -blockers tested on the isolated frog sciatic nerve. They concluded that the unexpected low local anaesthetic property of practolol and atenolol and high local anaesthetic property of propranolol may be explained by the fact that the range solubility of practolol and atenolol is limited, while that

of propranolol is broad.

Although partition coefficients $K(O/w)$ correlate well with pharmacological and non-specific membrane activities it is by no means conclusive evidence and some other factors may also be involved. Löfgren, (1948) tested the Meyer-Overton hypothesis by trying to see whether there was an exact correlation between the partition coefficient and the effective blocking concentration on frog sciatic nerve for twenty eight local anaesthetics. Although eighteen of the compounds fell between two parallel lines, three compounds, one of which was procaine were appreciable out of line. Löfgren concluded that, although the partition coefficient was very important, there had to be at least one more determining factor in anaesthetic potency. One possible factor is surface activity which has been shown to be in close parallelism to lipid solubility, degree of ionization, and diffusion coefficient (Levy, 1968b). Consequently, in the present study the surface activity of β -blockers was assessed.

The results of the surface tension of aqueous solutions of propranolol and atenolol are represented in (Fig. 3.78). Propranolol was found to be several orders of magnitude more effective than atenolol in lowering surface tension. For a compound to be able to lower surface tension, it must accumulate at hydrophilic-lipophilic interfaces and migrate into the lipophilic medium (Hellenbrecht and Lemmer, 1972). Therefore, the surface activity is directly related to the respective partition coefficient. Such correlation has also been shown for four local anaesthetics (Büchi and Perlia, 1967). However, it is a good index of the partition coefficient and is still very important in determining the biological potency of a series of compounds. Propranolol would therefore be expected to be a more effective anaesthetic than either atenolol or practolol and to have stronger lipophilic binding with the components of membrane and perhaps therefore to exert a greater cytotoxic activity.

A Critical Micelle concentration (CMC) for both β -blockers and local anaesthetics (Fig. 3.77a - 3.77b) show

that whereas propranolol and tetracaine solubilize oil soluble dyes, practolol, atenolol and procaine did not. Similarly, Fazly Bazaz and Salt (1983a) found that while tetracaine and cinchocaine also solubilized Sudan B. Black, procaine and lignocaine did not. This agrees well with the previous findings of Farhadieh and Hall (1967) and of Salt and Traynor, (1979).

Formation of micelles cause changes in the physical properties of a surface active agent if the number of aggregation is fairly large (Farhadieh and Hall, 1967). The CMC for both propranolol and tetracaine reflects such changes and accounts for the formation of coalesced monomers. The CMC value for propranolol and tetracaine is 5.0 mg.ml^{-1} , and was not markedly changed in the presence and absence of cells (Fig. 3.77 a,b).

It is possible that propranolol and tetracaine are effective in a micellar state during which the uptake of these drugs saturate ionized sites on the cell surfaces as well as on membrane, and the CMC value could possible reflect the concentration at which changes such as the formation of monolayers at cell membrane occur. The more lipophilic is the compound, the higher the uptake of the compound from aqueous solution and its distribution within the lipid region of the cell. Atenolol and practolol have no detectable value of CMC. Since CMC value is related to the lipophilic nature of compounds the lower this value the higher is the potential antimicrobial activity of these types of compound. Formation of micelles is also the property of cationic antibacterial agents such as HTAB (Hexadecyl trimethyl ammonium bromide). These compounds have a marked surface activity and form colloidal aggregates (Salt and Wiseman, 1970).

It can be suggested from the data of physicochemical properties, that there is a close correlation between the non-specific membrane activity and antimicrobial effects of β -blockers. Further supportive evidence of membrane effects of β -blockers have been shown on their ability to solubilize erythrocyte and lysosomal membranes as well as changes in the turbidity of non-growing microbial cells.

Guttman (1940), first observed stabilization of spider crab nerve membranes by alkaline earths, as shown in resting potential measurements. Since then stabilization has been used to indicate a certain membrane alteration by active compounds. The local anaesthetic and phenothiazines ability to stabilize biological membranes was observed by Shigwald (1946) as well as observing the ability of diethazine to give an anaesthesia similar to that of procaine.

The biphasic action of β -blockers on erythrocyte membrane stabilization and lysis is shown in (Figs. 3.79 - 3.80). HTAB and local anaesthetics were used as comparison and representative of membrane active compounds. At very low concentrations of propranolol, tetracaine and HTAB, inhibition of release of haemoglobin occurred, but at higher concentrations, these compounds lyse the membrane directly. Atenolol did not protect the erythrocyte from the release of haemoglobin, but rather lysed the membrane at higher concentrations. Propranolol also caused a similar biphasic pattern of membrane stabilization and lysis with lysosomal membrane (Fig. 3.86 - 3.87).

The advantage of selecting the erythrocyte as a model for studying the mechanism of membrane activity is that it is relatively free of intracellular material and organelles. Any effect of a drug on osmotic haemolysis therefore might be interpreted as an effect on the membrane. The fact that propranolol stabilizes erythrocyte and lysosomal membrane suggests its ability to interact with biological membrane within certain concentrations ranges. Wiethhold and Lemmer, (1972) showed that the conformational changes brought about by propranolol were well correlated with the protective effect of this drug in human erythrocytes against hypotonic haemolysis. Mullin's, (1954) suggested that the mechanism of stabilization or anaesthesia is a physical occupation of space within the cell membrane; the bulkier the molecule then the more effective the stabilization, provided the molecule can reach the membrane and provided that its lipid solubility is high. This property fits very closely with the chemical

nature of propranolol and it is not surprising that atenolol does not stabilize biological membranes. On the other hand procaine with approximately an equipotent inhibitory effect on bacterial and candidal cells as atenolol, stabilizes the erythrocyte membrane (Fig. 3.80) suggesting that other factors as well as membrane stabilization are responsible for the membrane activity of compounds. It was observed that the concentration range that stabilizes either erythrocyte or lysosomal membranes, also impairs cellular functions such as respiration, enzyme activity, and inhibition of sugar uptake. Smith (1982) also observed that specific membrane activity includes protection against lysis, local anaesthetic activity, other receptor mediated effects, and inhibition of membrane-bound enzymes such as phospholipase A. It follows that the many membrane effects of widely different classes of drugs cannot be determined precisely. Atenolol which does not stabilize either erythrocyte or lysosomal membranes did inhibit the activity of cellular functions, suggesting different membrane interactions sufficient to inhibit cellular functions, but insufficient to cause stabilization of biological membrane.

The mechanism of lysis of erythrocyte or lysosomal membrane is probably similar to that discussed for microbial cells. From the lipoprotein nature of bacterial membrane and its response to surface active agents, it could be suggested that cells showing a similar lytic behaviour would possess a surface membrane of the same broad characteristics. Thus it is possible that the erythrocyte membrane surface could be regarded as possessing a critical collapse pressure and haemolysis could be achieved where the concentration of effective compounds produce surface pressure, sufficient enough to irreversibly collapse the membrane structure.

The relationship between stabilization-lysis and the release of K^+ from erythrocyte membrane (Fig. 3.81) show that the effect of propranolol is not on the intracellular matrix material. For example it may be that propranolol has some direct effect on the haemoglobin of the

erythrocytes without affecting the cell membrane. This possibility is unlikely, however, since not only is the haemoglobin prevented from escaping into the hypotonic test solution, but the K^+ release is also inhibited.

Seeman, (1966) found that the release from K^+ from red cells in hypotonic solution for 20 minutes was also prevented by low concentrations of Vitamin A.

That stabilization-lysis is a membrane phenomena is also supported by the studies on the effect of propranolol on HTAB induced stabilization-lysis of erythrocyte membrane³ (Fig. 3.85). Propranolol protected the erythrocyte membrane from the lytic effects of HTAB as well as potentiating the stabilizing effects of HTAB at low concentration, in a similar manner to divalent cations (Figs. 3.82, 3.83, 3.84). It is suggested that the extent of protection from the lytic effects of HTAB is referred to as the "stabilization factor" and is approximately 7.0 for propranolol. Thus, propranolol can produce membrane effects that resemble those of divalent ion resulting in membrane stabilization with decrease in membrane permeability.

Considering the biphasic pattern with that of lysosomal membrane treated with β -blockers (Figs. 3.85 - 3.87) the release of arylsulphatase and β -glucuronidase is not significant, likely to be due to subsequent inhibition of these enzymes following release by either propranolol or atenolol. De Duve, (1968) suggested that a compound is likely to affect lysosomes by either changing the permeability of the membranes or by inhibiting or activating their enzymes. Many anti-inflammatory drugs also exert their effects by both stabilizing lysosomal membrane and inhibiting the activity of some of these enzymes. Smith and Sabin, (1976) showed that steroids such as cortisone, dexamethasone and paramethasone not only stabilized lysosomal membrane and prevented enzyme release, but also inhibited arylsulphatase, while acid phosphatase and β -glucuronidase were not affected.

The optical density of non-growing cell suspensions of E.coli, P.aeruginosa, B.cereus and C.albicans were

influenced by propranolol and oxprenolol but not by atenolol, practolol and procaine over the concentration range studied (Figs. 3.88 - 3.93). Low concentrations of propranolol, tetracaine and oxprenolol did not cause any significant changes in turbidity, while higher concentrations caused a rapid and significant increase in turbidity. The result is in agreement with that of Salt, (1982) on the turbidity increases in non-growing cell suspensions of E.coli caused by propranolol and dibucaine, and that of Fazly Bazaz and Salt, (1983b) on the local anaesthetic induced turbidity increases of bacterial cell suspensions.

Suggestions as to the reason for such changes have been provided as the result of interactions of a variety of different antibacterial agents and bacterial cells. Not only do β -blockers and local anaesthetics induce turbidity increases; other compounds including cationic surfactants (Hugo and Longworth, 1964; Hugo and Frier, 1969; Salt and Wisemen, 1970; Salt, 1976), aldehydes (Munton and Russell, 1976) and phenolics (Beckett, Patki and Robinson, 1959; Lanikanra and Allwood, 1977) have also been reported to induce such effects.

Increases in turbidity of bacterial or candidal cell suspensions can be accounted for by either an increase in the refractive index of cell or by an increase in the reflecting surface area of the cell, or both (Hugo and Longworth, 1964). It follows that the size and shape of the cell may be affected following interactions of drugs with the cell surfaces. Further information in support of change in the surface area and refractive index of cell suspension following increase in turbidity increases is brought about by the observations of Beckett, Patki and Robinson (1959). They observed that increase in turbidity of E.coli in the presence of hexylresorcinol is due to changes in the refractive index and surface area of the cell. Munton and Russel, (1970); Lamikanra and Allwood, (1977) suggested that increase in the optical density is due to the effects of drugs on the cell wall. Venables and Russell, (1972) on the other hand attributed the increase in optical

density of suspensions of S.cerevisiae in the presence of nystatin as due to a reduction in cell volumes. In contrast, Hammond and Klinger, (1975) suggested that increase in the optical density of C.albicans in the presence of Candicidin as due to acidification of the cytoplasm resulting in the precipitation of cellular proteins.

The uptake profile (Fig. 3.75 - 3.76) suggests that changes in the reflecting surface of the cells might be due to the presence of the adsorbed drug which is responsible for the physical alteration of the cell surface, and therefore resulting to an increase in turbidity changes of non-growing cells of microbial suspensions, likely to satisfy the predictions of Munton and Russell, (1970) and Lamikanra and Allwood, (1977). Also SEM (Plates 3.7 and 3.12) show that 5 and 7.5 mg.ml⁻¹ propranolol caused some surface alterations of microbial cells. Furthermore, the fall in the optical density of microbial cells with time in the presence of 7.5 and 2.7 mg.ml⁻¹ propranolol (Fig.3.94) could possibly result to reduction of cell size following damage caused to the cell. In addition leakage caused by this level of propranolol concentration could possibly result in reduction in cell volume, or complete precipitation of cell constituents since nearly no change in turbidity was observed with higher β -blocker concentrations. In another explanation, changes in the surface properties of cells is as a consequence of cell death, have been implicated. Salt (1976) described these post mortem changes as due to penetration of cell membrane by effective compounds. This would satisfy the data on micellar formation at higher concentrations of propranolol or tetracaine (Fig. 3.77a,b). These concentrations not only resulted in the leakage of intracellular materials, but also caused significant reduction in culture viability (Fig. 3.37 and 3.38).

In addition, effective drugs (propranolol, oxprenolol and tetracaine) only induce turbidity increases beyond a particular threshold concentration. Below these concentrations, there may not be sufficient drug to penetrate the

cytoplasmic membrane, hence giving rise to the initial phase of the curve profile described earlier. This is supported by the fact that induced turbidity increases caused by propranolol in the presence of water and in buffer containing low phosphate (Figs. 3.95 a,b)

have a curve profile without the initial phase, suggesting the importance of ions in stabilizing biological membrane. In the absence of these ions, the threshold concentrations decreases, and therefore the cytoplasmic membrane is penetrated at a much lower concentration. Furthermore, the effect of time on the turbidity changes in cells of E.coli and C.albicans (Fig. 3.94) show similarity with those of leakage of cellular materials and uptake of ^3H - propranolol. Induced turbidity increases. leakage of cellular constituents, and uptake of propranolol are complete within a short period of time. Longer exposure times brought no change corresponding to the slower release at higher β -blocker concentrations.

Further evidence that induced turbidity increase is a consequence of changes at the cell membrane comes from the studies of turbidity changes of isolated cell envelope, intracellular materials and extracted and dispersed lipids of the cell. Only minor changes in optical density of bacterial and candidal cells occurred with the cell envelope treated with propranolol, whereas samples containing isolated cytoplasmic constituents showed marked increases in turbidity (Fig. 3.99b). This is in agreement to findings of Fazly Bazaz and Salt, (1983b), on the effect of local anaesthetics on the turbidity of isolated cell envelope and intracellular materials of cells of E.coli. This observation suggests that higher concentrations of propranolol must have penetrated through the cytoplasmic membrane into the cytoplasm, possible interacting with cellular constituents, and perhaps producing a change in the refractive index of the cell surface through accumulation of leaked materials on the surface of the membrane.

It was also observed that increases in turbidity occurred with lipid depleted cells and lipid dispersed

preparations (Fig.3.100). Increase in turbidity of lipid depleted cells by propranolol, suggest that lipids of membrane are not only the site at which propranolol interacts to cause turbidity increases, but that lipid need not be attached or associated with the whole cell for these changes to occur. Furthermore, since atenolol did not cause any increases in turbidity and was limited in toxicity, it is possible that even though some uptake was observed with this drug, insufficient was present to destructure the lipids in the lipophilic core of the cell membrane. Probably the loss of viability observed with atenolol after one hour contact time might result from the interactions of atenolol with the polar proteins of the membrane and that the difficulty in relating the ability of atenolol to cause loss of viability (after one hour exposure) with leakage of cellular materials might be due to sealing off or coagulation of these proteins at membrane surfaces. On the other hand, since propranolol is a lipophilic drug it is not surprising that it is able to solubilize or penetrate biological membranes. For propranolol the initial increase in turbidity of lipid dispersed preparation was followed by fall in optical density, this fall probably reflects the solubilization of the lipids in the lipophilic core of propranolol micelles.

Considering the effect of different concentrations of propranolol and atenolol on the induced turbidity increases of cells of E.coli caused by HTAB (Fig.3.101), it is evident that low concentrations of propranolol, 0.5, 1.0 and 2.5 mg.ml^{-1} affected the turbidity increases caused by HTAB. But 0.5 and 1.0 mg.ml^{-1} , propranolol neither solubilizes Sudan B. Black nor causes any significant leakage of 260 nm absorbing materials or pentoses. Although 2.5 mg.ml^{-1} propranolol caused the leakage of cellular materials from microbial cells as well as reducing the increase in turbidity caused by HTAB, the overall pattern remains unchanged, whereas the suppressive effects of 0.5 and 1.0 mg.ml^{-1} altered the overall pattern of the curve. The phases or regions characteristics of induced turbidity

profile are altered. It is suggested that these low concentrations stabilized or protected the microbial cell from changes associated with induced turbidity increases caused by HTAB in a similar manner in which propranolol protected erythrocyte membranes from the lytic effect of HTAB (Fig. 3.85). The extent of protection afforded to cells from the HTAB induced turbidity changes increases as the concentration of propranolol decreases, reaching a limit at 0.05 mg.ml^{-1} , at which propranolol is unable to stabilize or protect the cells from the effect of HTAB. At this concentration, the overall profile of the curve is similar to that caused by HTAB alone.

At higher concentrations of propranolol the effect of HTAB on culture turbidity is additive. Thus the threshold concentration for HTAB is significantly reduced, completely abolishing the initial phase of the curve, suggesting the non-specific actions of such β -blockers (e.g. propranolol), which are characterized by their lipophilic nature and are usually additive (Chien and Hansch, 1968). On the other hand, atenolol, at all levels of concentrations studied, did not produce any significant changes on the induced turbidity increases caused by HTAB. (Fig. 3.101b).

The extent to which 0.25 mg.ml^{-1} propranolol protected or stabilized cells of E.coli to HTAB induced increases in turbidity was investigated. The findings suggest an increase in the threshold concentration of HTAB (Fig. 3.102). As with the erythrocyte membrane, the extent of stabilization is referred to as the stabilization factor (SF) and is approximately 9 which is similar to that estimated for erythrocyte membrane protection. The result of this finding is related to the effect of 0.2 mg.ml^{-1} propranolol on the MIC of HTAB on E.coli. In the absence of propranolol, the MIC value for HTAB is in the range of $5 - 7.5 \text{ mg.ml}^{-1}$, whereas cells pre-incubated with 0.2 mg.ml^{-1} propranolol, the MIC value decreases to the range of $60 - 70 \text{ } \mu\text{g.ml}^{-1}$ from which the protective factor was estimated to be approximately 10. These values are close or fall within the range obtained for the induced turbidity changes (SF),

suggesting that the membrane is protected by propranolol from penetration by the HTAB.

The results of MIC of β -blockers on microbial cells supplemented with magnesium (Table 3.2) show that drug induced growth inhibition of microbial cells is reduced in the presence of Mg^{2+} ion. Furthermore, divalent ions reduce drug-induced inhibition of succinate dehydrogenase (Fig. 3.120), Mg^{2+} -ATPase (Fig. 3.103), β -galactosidase (Fig. 3.113) stabilization-lysis of erythrocyte membrane (Figs. 3.82 - 3.83) and drug induced turbidity changes (Figs. 3.97 - 3.98). Similar reduction by cations of drug induce inhibition of various cellular processes have been reported by several workers. In each case, a link between the interaction of divalent cations and of drugs on the cell membrane has been proposed.

The protective effects of divalent metal ions like Ca^{2+} , Mg^{2+} and Zn^{2+} were described by Cope (1980) as due to either an equilibration with intracellular divalent ions, thus preventing loss from the cell, or to competition between divalent metal ion and drug for negatively charged sites within the cell. The results of Kessels and Belde (1985) show that Ca^{2+} decreases not only the Cd^{2+} -induced K^+ release but also the toxic effects of Cd^{2+} on yeast growth and suggested that the protective effects of Ca^{2+} is due to the reduction of Ca^{2+} uptake by cells. Furthermore, Luijck and Sande (1986) showed that divalent cations such as Mg^{2+} and Ca^{2+} strongly inhibited the mitomycin C-induced protein-H dependent decline in culture optical density, possibly by stabilizing the outer membrane or preventing autolysis. Previously Leduce, (1982) demonstrated that strong induction of Cloacinogenic cells, resulting in large amounts of protein H, causes a strong induction of both phospholipase A activities and the formation of numerous permeability zones. This process leads to severe damage to the membranes and ultimately to cell lysis, possibly by induction of autolytic system of E.coli. Ca^{2+} and Mg^{2+} ions interfere with this autolytic process and thus inhibit the decline in culture optical density and cell lysis.

Measuring the proportions of divalent metal ions in the cell envelope as a measure of resistance has proved valuable in linking divalent metal ions and biological function, and thus to the association of these metal ions with membrane macromolecules. Studies with four strains of P.mirabilis (Norris and Rogers, 1985) show that whereas methicillin was inhibitory to two of the strains, the drug was ineffective against the other two. Furthermore, resistance of P.mirabilis to polymyxin, acriflavine, benzalkonium chloride, cetrимide, chlorhexidine, sodium lauryl sulphate and tetracycline was demonstrated in a comparative manner. Total lipid, phospholipid and protein occurred in higher amounts in the sensitive strains whereas the highest levels of cations (Ca^{2+} and Mg^{2+}) were found in the resistant strains. In the same manner, Ps.aeruginosa was found even more resistant than the resistant strains of P.mirabilis. The analysis of cations (Ca^{2+} and Mg^{2+}) composition clearly show that these ions are present in large amounts of Ps.aeruginosa compared to the two strains of P.mirabilis.

Thus the reduction of drug action by divalent metal ion has been used in this study to assess the membrane activities of β -blockers. Various effects of practolol and atenolol are also reversed by divalent metal ions, suggesting that these drugs possess some degree of membrane activities, possibly a different perturbation of membrane to that of the active compound propranolol, or the same type of interactions, but to a lesser extent.

As pointed out in (Table 1.3) enzymes such as Mg^{2+} -ATPase are metalloenzymes and have been shown to be activated by and bound to magnesium and calcium. Succinate dehydrogenase, isolated from different sources (including bacteria) was found to contain non-haem iron (Freedman, 1981). Malate and lactate dehydrogenases isolated from various sources have been shown to contain zinc as well as iron (Hughes, 1981). It is possible that competition or displacement of ions by β -blockers alters enzyme active conformation and causes

reduction in their activities. In the presence of exogenous divalent cations competition will also occur, but this time causing the displacement of β -blockers and therefore maintaining protein conformation. Consequently their activities would be maintained in the presence of β -blockers. This is substantiated by the fact that double reciprocal plot of kinetic inhibition of the activity of Mg^{2+} -ATPase in the presence of a fixed concentration of propranolol and practolol and varying concentrations of Mg^{2+} is competitive (Fig. 3.107b) suggesting that β -blockers and magnesium competed for the same active site.

Various responses to the actions of β -blockers on whole cell suspensions have so far been analysed. Specific responses such as the inhibition of membrane bound and associated enzymes have also been investigated within the present study in an attempt to substantiate the various responses associated with whole cell effects. (For example, inhibition of respiration and uptake of radiolabelled sugars). It was found that β -blockers caused a dose dependent inhibition of Mg^{2+} -ATPase (Figs. 3.104-3.105). HTAB, a membrane active compound (Hamilton, 1975) also inhibited the activity of this enzyme in a dose dependent manner. Sub-MIC concentrations of these drugs were required to inhibit the enzyme. Prolonged contact time between drug and enzyme preparation resulted in a decrease in enzyme activity. Enzymes from B.cereus and C.albicans were equally affected, and propranolol was a more potent inhibitor of Mg^{2+} -ATPase than practolol.

Inhibition of Mg^{2+} -ATPase by propranolol has been investigated in other membrane systems. Meltzer and Kassir (1983) showed that propranolol inhibited the calmodulin-activated Ca^{2+} -ATPase of human erythrocyte membranes. Wei and Teng (1985) observed the inhibition of mitochondrial Mg^{2+} -ATPase, and the depression of muscle myosin B by propranolol was thought by Honig (1968) to be a consequence of inhibition of Ca^{2+} -ATPase. The

physical and enzymatic properties of solubilized membrane ATPase(s) from bacterial and fungal systems as elucidated by Abrams (1960) are similar in a number of ways to those extracted from animal mitochondrial membranes in which acts as a coupling factor in oxidative phosphorylation. Thus the inhibition of Mg^{2+} -ATPase in cells of B.cereus and C.albicans might explain the effect of β -blockers on respiration. Furthermore, changes in membrane permeability may thus be explained by the inhibition of Mg^{2+} -ATPase since the physical state and the functioning of this enzyme controls membrane permeability and therefore transport of materials across biological membranes. Indeed, Hashimoto and Satoh, (1979), Katze and Repke, (1974) showed that propranolol and some other β -blockers inhibit the calcium uptake by impairing active transport. By use of a series of varying fixed concentrations of the substrate (ATP) in the presence of different drug concentrations, it was found that propranolol and practolol are competitive inhibitors of membrane Mg^{2+} -ATPase (Fig. 3.77a). Wei and Teng (1985) also show that the inhibition of Mg^{2+} -ATPase by propranolol is non-competitive towards ATP using mitochondrial membranes. This might be expected since β -blockers bear no structural similarity to ATP. Direct action on the enzyme will undoubtedly lead to changes in its tertiary structure and consequent denaturation. Alternatively, it could also cause a localised change in structure of the membrane bilayer, a perturbation likely to result in change in the structure of the membrane associated enzyme molecule.

Further evidence on the interaction of β -blockers on this enzyme molecule is provided from the results of the kinetic studies of inhibition of Mg^{2+} -ATPase by β -blockers in the presence of different concentrations of Mg^{2+} (Fig. 3.110b). Propranolol and practolol were shown to be non-competitive inhibitors of Mg^{2+} activated Mg^{2+} -ATPase. It is possible that these drugs either bind Mg^{2+} , thereby making Mg^{2+} unavailable to the enzyme or that these drugs occupy those sites on the membrane that would bind Mg^{2+} or would interact with bound Mg^{2+} . The K_m (Table 3.8b) is

probably too low to account for the association between the divalent ions and β -blockers compared to the binding of divalent ions to Mg^{2+} -ATPase. Thus, this low affinity would not be expected to prevent the association of Mg^{2+} with ATPase. Levin and Weiss (1977), Weiss and Prozialeck (1980) showed that the association between propranolol and divalent ions is low compared with the binding of calmodulin with ATPase. Furthermore, propranolol binds to biological membranes (Weiss and Prozialeck, 1980), and since propranolol binds to divalent ion activated Mg^{2+} -ATPase with too low an affinity to account for the inhibition, it is reasonable to conclude that competitive inhibition of divalent ion-activated Mg^{2+} -ATPase by β -blockers was due to occupancy of these sites on the membrane that would normally bind Mg^{2+} or Ca^{2+} .

Current studies of the effects of β -blockers on β -galactosidase, have shown that atenolol is a more effective inhibitor of the purified enzyme (Fig. 3.111) than propranolol. Kinetic data (Table 3.10) also show the greater potency of atenolol over propranolol. Both propranolol and atenolol are competitive inhibitors of the activity of the enzyme. In an attempt to establish whether the greater inhibitory activity of atenolol over propranolol can also be extended to the unpurified β -galactosidase from E.coli, the same amount of each drug was used. The result, represented in Figure 3.112 shows that under the conditions of these experiments, propranolol was a more potent inhibitor of β -galactosidase than atenolol.

The possible explanation for the greater potency of atenolol over propranolol on the purified enzyme could be that atenolol has greater affinity for sites on the enzyme normally occupied by ONPG compared to propranolol. On the other hand, the greater potency of propranolol over atenolol on the unpurified enzyme could be greater membrane activity of propranolol compared to atenolol. The production or the synthesis of β -galactosidase in the cytoplasm will depend on the transport of lactose inside the cytoplasm, β -galactosidase being an insoluble enzyme (Lehninger, 1975). The transport of lactose across biological membranes is

mediated by β -galactosidase permease a membrane bound enzyme. It is reasonable to suggest that the greater activity of propranolol could be a damage to the tertiary structure of β -galactosidase permease, either through membrane collapse or interaction with the enzyme.

Rosenkranz and Carr, (1965) reported that inhibition of enzyme induction resulted from altered cellular permeability and concluded that its effect resulted from reversible attack on the membrane.

β -blockers inhibit the activity of lysosomal enzymes. Arylsulphatase and β -glucuronidase were inhibited by atenolol and propranolol; acid phosphatase was slightly affected by propranolol, but not by atenolol (Figs. 3.115 - 3.116). Lysosomes are cytoplasmic organelles containing a variety of acid hydrolases (De Duve, 1959; Novikoff, 1961). This means that the investigation of the effect of β -blockers on the activity of lysosomal enzymes does not reflect the interactions of β -blockers with lysosomal membrane as its enzymes are not membrane bound, though they may be membrane associated. The results represented (Fig. 3.86 - 3.87) show that propranolol is able to interact with lysosomal membrane, causing stabilization at lower concentrations and lysis of lysosomal membrane at higher propranolol concentrations. This would lead to the release of acid hydrolases and subsequent inhibition by propranolol.

An additional experiment was carried out in an attempt to investigate the effect of propranolol and atenolol on the activity of commercial, purified lysosomal enzymes. Purified arylsulphatase and β -glucuronidase were inhibited by propranolol and atenolol, acid phosphatase was not affected. The exact mechanism by which the two enzymes are inhibited, and not acid phosphatase, remains uncertain but it is likely to involve a direct effect of the drug on the molecular structure of arylsulphatase and β -glucuronidase. In the same manner Smith and Sabin, (1976) observed that steroidal anti-inflammatory drugs (hydrocortisone, dexamethasone and paramethasone) inhibited the activity of aryl sulphatase in a dose dependent manner but had no effect

on the activity of β -glucuronidase. Further investigations led them to conclude that these steroidal anti-inflammatory drugs block enzyme activities directly while exacting no influence on membranes. In another experiment, investigating the kinetic studies of the inhibition of arylsulphatase and β -glucuronidase by propranolol and atenolol, it was found that propranolol and atenolol inhibited the activities of these enzymes in a non-competitive manner (Figs. 3.118 - 3.119, Table 3.11a - 3.11b), suggesting that these drugs bind to sites on the enzyme other than those normally occupied by their substrates, and fit very closely to the prediction of direct interaction of β -blockers and molecules of the enzyme.

The inhibition of the enzymes so far described is by no means the only enzymes that are likely to be affected by β -blockers. Other sensitive enzymes may be present in biological membranes. Their activities are likely to be affected by β -blockers either by specifically interacting with enzyme molecule as determined by kinetic experiments or by indirectly interacting with the membrane components, likely to result in changes which would then influence enzyme activities. In this way, β -blockers would affect a variety of biochemical processes, some of which such as respiration and uptake of sugars, have been illustrated. The impairment of these biochemical properties were shown to occur at lower growth inhibitory concentrations but probably this would make it impossible for the microbial cell and indeed animal cell to recover from the damage to the membrane caused by higher β -blocker concentrations.

CONCLUSIONS

β -blockers like local anaesthetics possess anti-microbial activity with similar mode of actions, possible connected with membrane damage through interactions with the components of microbial membrane. However, not all β -blockers or local anaesthetics are effective antimicrobial agents. Propranolol (a β -blocker) and tetracaine (a local anaesthetic) are more effective than either atenolol and practolol (β -blockers) or procaine (a local anaesthetic). Oxprenolol is also effective as antimicrobial agent but is less effective than propranolol.

Effective antimicrobial agents such as propranolol, oxprenolol or tetracaine caused a rapid release of cellular materials, an increase in turbidity of non-growing microbial cells, and propranolol caused the loss of cell viability. In contrast, other β -blockers (e.g. atenolol and practolol) and local anaesthetics (e.g. procaine) do not possess these properties, but like the effective agents, they inhibited cell growth, respiration, uptake of sugars, and membrane associated enzymes and in each case, in general, are less potent compared to the effective β -blockers or local anaesthetics. Physicochemical properties revealed that increase in antimicrobial activity is correlated with membrane affinity of the β -blockers. Thus the most lipophilic propranolol probably penetrates deeper and interacts with the membrane lipids and hydrophobic proteins, whereas the less lipophilic β -blockers (atenolol and practolol) bind superficially with surface proteins and phospholipids of the membrane. Other membrane-active agents, which include alcohols, anilides, phenols, chorhexidine and cationic detergents also have affinity for biological membranes and probably interacts in such a manner as to promote the leakage of cellular materials from the cytoplasm of the microbial cell.

The precise location of the site of action of β -blockers in the cytoplasmic membrane still remains to be identified. The similarity in terms of growth inhibition associated with atenolol or practolol and procaine, and their differences in erythrocyte membrane stabilization,

only associated with procaine, suggests that the mechanism is at least different in this respect, and can be determined in further investigations.

The biochemical effects of β -blockers on prokaryotic and eukaryotic cells are similar, and often unpleasant for therapeutical use. Inherent membrane activity (membrane-stabilization) has been attributed to be responsible for the toxicity of β -blockers (Smith, 1982; Cooper, 1986). Inhibition of phospholipase A induces swelling of mitochondria (Smith, 1982) and depression of myocardial contractility may induce hypotension (Cooper, 1986). Both are associated with membrane-stabilizing activity of drugs. The present study is consistent with the view that some of the in-vitro effects can be accurately predicted by the long term use of cellular dispersions of both prokaryotic and eukaryotic cells in the screening of potentially toxic drugs, by assessing their membrane-stabilizing activity which will likely indicate their ability to cause toxic effects in overdosage.

REFERENCES

- Abrams, A. (1965). The release of bound adenosine triphosphatase from isolated bacterial membranes and the properties of the solubilized enzymes. *J. Biol. Chem.* 245, 3676.
- Abrams, A., McNamara, P., and Johnson, F. (1960). Adenosine triphosphatase in isolated bacterial cell membrane. *J. Biol. Chem.* 235, 3659.
- Aguilera, M. A. and Williams, V. E. M. (1965). The effects on cardiac muscle of β -receptor antagonists in relation to their activity as local anaesthetics. *Br. J. Pharmacol. Chemother.* 24, 332.
- Akiyama, S. and Igisu, H. (1979). Effects of d,L-propranolol and other beta adrenergic receptor blocking agents on membrane fluidity of erythrocytes. *Jpn. J. Pharmacol.* 29, 144.
- Alhquist, R. P. (1948). Study of the adrenotropic receptors. *Am. J. Physiol.*, 153, 586.
- Ames, G. F. L., Spudis, E. N., and Nikaido, H. (1974). Protein composition of the outer membrane of *Salmonella typhimurium*: Effects of lipopolysaccharide mutations. *J. Bacteriol.* 117, 406.
- Angus, B. L., Carey, A. M., Caron, D. A., Kropinski, A.W.B. and Hancock, R. E. W. (1981). Outer membrane permeability in *Pseudomonas aeruginosa*: comparison of a wild-type with antibiotic supersusceptible mutant. *J. Antimicro. Agents. Chemother.* 21, 299.
- Angus, B. L. and Hancock, R. E. (1983). Outer membrane porin proteins F.P. and D1 of *Pseudomonas aeruginosa* and PhoE of *Escherichia coli*: Chemical crosslinking to reveal native oligomers. *J. Bacteriol.* 155, 1042.
- Anwar, R. A. and Vlaovic, M. (1979). Purification of UDP-N-acetylenolpynuroylglucosamine reductase from *Escherichia coli* by affinity chromatography, its subunit structure and absence of afflavin as the prosthetic group. *Can. J. Biochem.* 57, 186.
- Aronson, J. M. and Preston, R. D. (1960). An electron microscopic and x-ray analysis of the walls of selected low phycomycetes. *Proc. R. Soc. B.* 152, 346.

Baccino, F. M., Rita, G. A. and Zuretti, M. F. (1972). Studies on the structure of bound sedimentability of some rats liver lysosome hydrolases. *Biochem. J.* 122, 363.

Barrett, A. M. and Carter, J. (1970). Comparative Chronotropic activity of β -blockers antagonists. *Br. J. Pharmac.* 40, 373.

Bayer, E., Gom, E., Kallenegger, B. and Uhman, R. (1976). Separation of amino-acids by high power performance liquid chromatography. *Anal. Chem.* 48, 1106.

Beckett, A. H. and Patki, S. J. (1959). The interaction of phenolic compounds with bacteria. *J. Pharm. Pharmacol.* 11, 307.

Behr, M., Pugsley, A. and Schnartman, C. (1980). Regulation of the OmpA outer membrane protein of *Escherichia coli*. *J. Bacteriol.* 147, 972.

Beggs, W. H. (1983). Comparison of miconazole and ketoconazole induced release of K^+ from *Candida* species. *J. Antimicrobial. Chemother.* 11, 381.

DeBennard, L. (1958). Associations of molecules with membrane lipids: lecithin and cholesterol. *Bull. Soc. Chim., Biol.* 40, 161.

Bell, D. J. and Northcote, D. H. (1950). The structure of a cell-wall polysaccharide of bakers yeast. *J. Chem. Soc.* 1944.

Belliveau, J. W. and Lanyi, J. K. (1978). Calcium transport in *Halobacterium halobium* envelope vesicles. *Arch. Biochem. Biophys.*, 186, 98.

Bhupath, Yu Wing (1977). Mechanism of action of tetracaine hydrochloride against *Pseudomonas aeruginosa*. *J. Infect. Disease*, 135, 679.

Bhattacharyya, P. and Barnes, E. M. (1978). Proton-coupled sodium uptake by membrane vesicles from *Azotobacter vivelandii*.

Bishop, C. F., Blank, F. and Gardener, P. E. (1960). The cell wall polysaccharides of *Candida albicans*. Glucan, Mannan and Chitin. *Can. J. Chem.*, 38, 869.

Black, J. W., Duncan, W. A. M., and Shanks, R. G. (1960). Comparison of some properties of pronethalol and propranolol. Br. J. Pharmacol, 56, 119.

Bloch, K. E. (1983). Sterol structure and membrane function. Critical Reviews in Biochemistry, 14, 47.

Bläsi, V., Geisen, R., Lubitz, W., Henrich, B. and Plapp, R. (1983). In "The larger of penicillin" CR. Harkenbeck, J. V. Höltze and Labischinski, H. (eds.). pp. 205-210, de Gryter, Berlin - New York.

Bloomfield, S. F. (1974). The effect of the phenotic antimicrobial agent Fentichlor on energy coupling in Staphylococcus aureus. J. Appl. Bacteriol., 37, 117.

Van Den Bossche, H. (1974). Biochemical effects on the uptake and/or utilisation of purines, pyrimidines, nucleotides, amino acids and glucose by Candida albicans. Biochem. Pharmacol., 23, 887.

Braun, P. C. and Calderon, R. A. (1981). Chitin synthesis in Candida albicans. Comparison of yeast and hyphal forms. J. Bacteriol., 135, 1972.

Braun, V., Gnirke, H., Henning, U., and Rehn K. (1973). Model for the structure of the shape-maintaining layer of the Escherichia coli cell envelope. J. Bacteriol., 114, 1264.

Braun, V. and Rehn, K. (1969). Chemical characterization, spatial distribution and function of a lipoprotein (Murien-lypoprotein) of the Escherichia coli cell wall. The specific effects of trypsin in the membrane structure. Eur. J. Biochem. 10, 426.

Britten, R. J. and Mclure, F. T. (1962). The amino-acid pool in Escherichia coli. Bacteriol. Rev. 26, 292.

Brown, M. R. W. and Melling, J. (1975). In "Resistance of Pseudomonas aeruginosa" (eds. Brown, M. R. W., John Wiley & Sons Ltd, London) pp 71.

Büchi, J. and Perlia, X. (1972). In "Drug design". Vol. 3., Eds. Ariens, E. J., Academic Press: New York and London.

- Carbib, E., Blowers, B and Roberts, R. L. (1983). Vectorial synthesis of a polysaccharide by isolated plasma membrane. Proc. Natl. Acad. Sci., USA, 80, 3318.
- Cartwright, C., Juroszek, J. R., Beavan, M. J., Ruby, F. M. S., Demorais, S. M. F. and Rose, A. H. (1986). Ethanol dissipates the proton-motive force across the plasma-membrane of Saccharomyces cerevisiae. J. Gen. Microbiol. 132, 369.
- Casey, R. P. and Broger, C., Theben, M., and Azzi, A (1981). Studied on the molecular basis of H^+ translocating by cytochrome C. Oxidase. J. Bioenerg. Biomembr., 13, 219.
- Cebrian, J. (1972). N. M. R. evidence for the hydrophobic interactions of local anaesthetics. 290, 51.
- Chai, J. and Foulds, J. (1978). Two bacteriophages which utilizes a new Escherichia coli major outer membrane protein as part of their receptor. J. Bacteriol. 135, 164.
- Chattaway, E. W., Homes, M. R. and Barlow, A. J. E. (1968). Cell wall composition of the mycelial and blastopore forms of Candida albicans. J. Gen. Microbiol. 51, 367.
- Chen, P. S. Jr., Toribara, T. Y. and Warner, H. (1965). Microdetermination of phosphorus. Anal. Chem. 28, 1756.
- Chopra, I. and Shanes, S. (1981). Susceptibility of protein synthesis in Escherichia coli to tetracycline and minocycline. J. Gen. Microbiol. 124, 187.
- Citonelli, J. A. and Smith, F. (1955). The polysaccharide associated with yeast invertase. J. Am. Chem. Soc. 77, 5682.
- Commager, H. and Judis, J. J. (1965). Mechanism of action of phenolic disinfectants: Effects of glucose and succinate metabolism. J. Pharm. Sci. 54, 1436.
- Cooper, P. (1986). Membrane stabilizing activity and toxicity. The Pharmaceutical Journal (Quarterly reviews, August, 30) p. 251.
- Cope, J. E. (1980). Mode of action of miconazole on Candida albicans: Effect on growth, viability and K^+ release. J. Gen. Microbiol., 119, 245.

Costerton, J. W. and Ingram, J. W. (1971). Structure and Functions of the cell envelope of Gram-negative bacteria. *Bacteriol. Rev.*, 87, 110.

Cruickshank, J. M. and Neil-Dwyer, G. (1985). β -blocker brain concentrations in man. *Cardiol. Suppl.*, 1, 34.

Datta, D. B. and Arden, B. (1977). Major proteins of the Escherichia coli outer cell envelope membrane as bacteriophage receptors. *J. Bacteriol.* 131, 821.

Davies, R. H. (1979). Identification of biologically active conformation in flexible drug molecules. *Int. J. Quantum. Chem., Quantum, Biol. Symp.* No. 6, p.203.

Dawson, R. M. C. and Hemington, (1960). The phospholipids of erythrocyte ghosts of various species. *Biochem. J.* 77, 226.

Donohue-Rolf and Schaechter, M. (1980). Translocation of phospholipids from the inner to the outer membrane of Escherichia coli. *Proc. Natl. Acad. Sci. USA.* 77, 1867.

Duran, A. and Cabib, E. (1979). Chitin synthetase distribution on the yeast plasma membrane. *Science*, 203, 363.

De-Duve, C. (1959). In "Subcellular particles". (Eds. T. Hayaskei). p. 128. Ronald Press, New York.

Eliam, Y. and Lavi, H. (1985). Active extrusion of potassium in the yeast Saccharomyces cerevisiae induced by low concentrations of trifluoperazine. *J. Gen. Microbiol.*, 131, 2555.

Engelhardt, A. and Traunecker, W. (1969). The pharmacology of phenoxypropranolamine derivatives of β -adrenergic blocking agents. *Naunyn-Schmiedeberg's. Arch. Pharmak. exp. Path.*, 263, 203.

Epstein, S. E. and Borer, J. S. (1976). Protection of ischaemic myocardium by nitroglycerin: Experimental and clinical results. *Circulation*, 53 (Suppl. 1), 191.

Eze, M. O. and McElhaney, R. N. (1981). The effect of alterations in the fluidity and phase state of the membrane lipids on the passive permeation and facilitated diffusion of glycerol in Escherichia coli. J. Gen. Microbiol., 124, 299.

Farhadien, B. and Hall, W. A. (1967). Aggregation of certain medicinal amines on aqueous solution of their salt. J. Pharm. Sci., 56, 18.

Fazly Bazaz, B. S. and Salt, W. G. (1983a). Local anaesthetics as antimicrobial agents: Structure-action considerations. Microbios. 37, 45.

Fazly Bazaz, B. S. and Salt, W. G. (1983b). Local anaesthetic induced turbidity increases: Implications of interactions with intact bacterial cells and with subcellular fractions. Ibid, 36, 135.

Feinstein, M. B. and Fernandez, S. M. (1975). Fluidity of natural membranes and phosphatidyl serine and ganglioside dispersions. Effects of local anaesthetics, cholesterol and protein. Biochim. Biophys. Acta. 413, 354.

Fitzgerald, J. D. (1969). Perspectives in adrenergic beta-receptor blockade. Clin. Pharmacol. Ther., 10, 292.

Freedman, R. B. (1981). Membrane-bound enzymes. In Finean J. B., Mitchell, R. H. (eds.): membrane structure. Amsterdam. Elsevier/North-Holland Biomedical Press. pp. 161-215.

Fuller, S. I. and Denyer, S. P. (1985). The mode of action of membrane active compounds on Staphylococcus aureus. Lett. Appl. Microbiol., 1, 13.

Gale, E. F. and Johnson, A. M. (1974). The release of K^+ from Candida albicans in the presence of polyene antibiotics. J. Gen. Microbiol., 87, 20.

Garrett, E. R. and Herman, S. M. (1970). Kinetics and mechanisms of action of drugs on microorganisms XI: Effect of erythromycin and its supposed antagonism with lincomycin on the microbial growth of Escherichia coli. J. Pharm. Sci., 59, 1448.

Garrett, E. R. and Miller, G. H. (1966). Kinetics and mechanism of action of antibiotics on microorganism B: Choranphenicol and tetracycline affected Eschericha coli. Ibid. 55, 593.

Garrett, E. R. and Wright, Ol K. (1967). Kinetics and mechanisms of action of drugs on microorganisms Vii, Quantitative adherence of sulfonamide action on microbial growth to a receptor site model. Ibid. 56, 1576.

Ghuysen, J. M. (1968). Bacteriolytic enzymes in the determination of wall structure. Bacteriol. Rev. 32, 425.

Gibson, D. G. (1974). Pharmacodynamic properties of β -adrenergic drugs in man. Drugs, 7, 8.

Giles, C. H. and MacEwan, T. H. (1960). A system of classification of solution absorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface area of solids. J. Chem. Soc. 3973.

Giles, C. H. and Smith, D. (1974). A general treatment and classification of the solute adsorption isotherm: Theoretical consideration. J. Colloid. Interface, Sci. 47, 755.

Gill, E. N. and Vaughan-Williams, E. M. (1964). Local anaesehttic activity of the β -blocking drug pronethalol. Nature, 201, 199.

Gilpatrick, J. (1981). Resistance to ergosterol biosynthesis inhibiting fungicides in laboratory strains of yeast. J. Plant Pathol., 87, 240.

Gorin, P. A. J. and Perlin, A. S. (1956). A mannan produced by *saccharomyces roux*. Can. J. 34, 1796.

Gravito, R. M. and Jenkins, J. (1983). X-ray diffraction analysis of matrix porin, an integral membrane protein from Escharicha coli outer membrane. J. Mol. Biol, 164, 313.

Guttman, R. (1940). Stabilization of spider crab membranes by alkaline earths, as manifested in resting membrane potential measurements. Gen. Physiol., 23, 343.

Hamilton, W. A. (1975). Energy coupling in microbial transport. *Adv. Microbial. Physiol.*, 12, 1.

Hammond, S. M. and Kliger, B. W. (1975). Studies on the optical density changes in suspension of Candida albicans by the polyene antibiotic candicidin. *Microbios.*, 13, 15.

Hancock and Raffle (1981). Involvement of the outer-membrane in gentamicin and streptomycin uptake and killing in pseudomonas aeruginosa. *Anti microbial. Agents. et Chemoter*, 19, 777.

Harkins, W. D. and Jordans, H. F. (1930). A method for the determination of surface and interfacial tension from the maximum pull on a ring. *J. Am. Chem. Soc.* 52, 1751.

Harris, M. and Morrison, G. A. (1961). Multiple inhibitions of cell metabolism by a single substance. *Nature, Lond.*, 191, 1276.

Hassid, W. Z. and Joslyn, M. A. (1941). The molecular constitution of an insoluble polysaccharide from yeast, Saccharomyces cerevisiae. *J. Am. Chem. Soc.* 63, 295.

Hashimoto, K., and Sotoh, H. (1979). Effects of etafenone and antiarrhythmic drugs on Na^+ and Ca^{2+} channels of guinea pig arterial muscle. *J. Cardiovasc. Pharmac.*, 1, 561.

Hatefi, Y. and Hanstein, W. G. (1969). Solubilization of particulate proteins and non-electrolytes by chaotropic agents. *Proc. Natl. Acad. Sci. USA*, 62, 1129.

Hellenbrecht, D. and Lemmer, B (1973). Measurement of hydrophobicity, surface activity, local anaesthesia and mycodial conduction velocity as quantitative parameters of the non-specific membrane affinity of nine β -adrenergic blocking agents. *Naunyn Schmiedeberg. Arch. Pharmacol.*, 277, 211.

Holtze, J. V. and Van Dium, J. (1984). In "Cell Wall synthesis and autolysis" (Nombela eds.). *Elsievier Scientific, Amsterdam*, pp. 205.

Honig, C. R. and Reddy, Y. S. (1978). Effects of catecholamine analogs on control of actomyosin by calcium. The nature of the negative inotropic receptor. *J. Pharm. Ther.*, 184, 330.

Hooper, A. B. and Dispirito, A. A. (1985). In bacteria which grow on simple reductants. Generation of a proton gradient involves extra cytoplasmic oxidation of substrate. *Microbiol. Rev.*, 49, 140.

Hughes, (1981) see P 264

Howe, R. and Shanks, R. G. (1966). Optical isomers of propranolol. *Nature*, 210, 1336.

Hugo, W. B. (1954). The use of 2, 3.5-triphenyltetrazolium bromide in determining the dehydrogenase activity of BACT. Coli. *J. Appl. Bacteriol.*, 17, 31.

Hugo, W. B. (1956). The action of phenol and 2-phenoxyethanol on the oxidation of various substances by Escherichia coli and disrupted cell preparation of the organism. *J. Gen. Microbiol.*, 15, 315.

Hugo, W. B. (1978). Membrane-active antimicrobial drugs - A reappraisal of their mode of action in the light of the chemiosmotic theory. *Int. J. Pharm.*, 1, 127.

Hugo, W. B. and Bloomfield, S. F. (1971). Studies on the mode of action of the phenolic antibacterial agent fentichlor against Staphylococcus aureus and Escherichia coli: The effect of fentichlor on the metabolic activities of S.aereus and E.coli. *J. Appl. Bacteriol.*, 34, 509.

Hugo, W. B. and Freer, M. (1969). Mode of action of the antibacterial compound Dequalinium Acetate. *Ibid.* 17, 118.

Hugo, W. B. and Longworth, R. (1964). Some aspects of the mode of action of chlorhexidine. *J. Pharm. Pharmacol.*, 16, 655.

Hugo, W. B. and Street, H. E. (1952). The effect of phenol, 2-phenoxyethanol and cetyltrimethyl ammonium bromide on the oxidation of various substrates by Escherichia coli. *J. Gen. Microbiol.*, 6, 90.

Hunter, K. and Rose, A. H. (1971). In Yeasts (eds. Rose, A.H. and Harrison, J. S., London and New York Academic Press) p.211.

Hughes, N. N. (1981). In "The inorganic chemistry of Biological Processes" (2nd eds. John Wiley and Sons, Chichester, New York, Brisbane, Toronto). pp. 89.

Hunsley, D. and Burnett, J. H. (1970). The ultrastructural architecture of the walls of some hyphal fungi. J. gen. Microbiol., 62, 203.

Ichihara, S. and Hussain, M. (1981). Characterization of new membrane lipoproteins and their precursors of Escherichia Coli. J. Biol. Chem. 256, 3125.

Inouye, M. and Shaw, J. (1972). The assembly of a structural lipoprotein in the envelope of Escherichia coli. Ibid. 246, 8154.

Inouye, M. (1974). A three dimensional molecular assembly model of a lipoprotein from the Escherichia coli outer membrane. Proc. Natl. Acad. Sci. (Wash.), 71, 2396.

Ishikawa, S. and Lehninger, A. (1962). Reconstitution of oxidative phosphorylation in preparations from Micrococcus lysodieticus. J. Bio. Chem. 237, 2401.

Ito, K. and Bassford, P. J. (1981). Protein localization in Escherichia coli. Is there a common step in the secretion of periplasmic and outer membrane protein? Cell. 24, 707.

Johnig, F. (1979). Structural order of lipids and proteins in membranes. Evaluation of fluorescence anisotropy data. Proc. Natl. Acad. Sci. USA, 76, 636.

Jogun, K. H. and Stezowski, J. J. (1976). Chemical structural properties of tetracycline derivatives: coordination and conformational aspects of oxytetracycline metal ion complexation. J. Amer. Chem. Soc., 98, 6018.

Joklik, W. K., Willet, H. P. (1984). In "Zinsser Microbiology" (18th eds. Appleton-Century Crofts"-Nowalk, Connecticut) pp. 24.

Juliano, R. L. (1973). The proteins of the erythrocyte membrane. Biochim., Biophys. Acta. 300, 341.

Kates, M. and Allison, A. (1961). Phosphatides of human blood cells and the role in spherocytosis. Ibid. 48, 571.

Kessels, B.G.F., Belde, P.J.M. (1985). Protection of Saccharomyces cerevisiae against Cd^{2+} toxicity by Ca^{2+} .

J. Gen. Microbiol., 131, 2533.

- Kornberg, R. D. and McConnell, H. M. (1971). Lateral diffusion of phospholipids in a vesicle. *Proc. Natl. Acad. Sci. USA.* 68, 2564.
- Lambert, P. A. (1978). Membrane-active antimicrobial agents. *Prog. Med. Chem.* 15, 88.
- Lamikara, A. and Allwood, M. C. (1977). Effects of polyethoxyalkyl phenols on the optical density of Straphylococcus aureus. *J. Appl. Bacteriol.* 42, 387.
- Lang, M. and Rye, R. M. (1972). The uptake by Escherichia coli and growth inhibitory properties of benzylalcohol and phenylethyl alcohol. *J. Pharm. Pharmacol.* 24, 219.
- Leduce, M. and Kasra, R. (1982). Induction and control of the autolytic system of Escherichia coli. *J. Bacteriol.* 152, 26.
- Lee, A. G. (1977). The interaction between phospholipids and chlorpromazine, propranolol, and practolol. *Mol. Pharmacol.* 13, 474.
- Lehninger, A. L. (1975). In "Biochemistry". The molecular basis of cell structure and function (2nd eds. Worth Publishers) Inc. New York. pp.
- Lenard, J. and Rothman, J. C. (1976). Transbilayer distribution and movement of cholesterol and phospholipid in biological membrane. *Proc. Natl. Acad. Sci. USA.* 73, 391.
- Lenaz, G. and Parenti-Castelli, G. (1974). In "Membrane proteins in transport and phosphorylation". (eds. by Azzone, G. T., Klingenberg, M. E., Quagliariello, E. and Siliprandi, M.). North Holland, Amsterdam, 23-28.
- Lenaz, G. and Curatola, E. (1976). Protein interactions - Spin and Fluorescence probe studies on the effect of n-alkanols on phospholipid vesicles and mitochondrial membranes. *Arc. Biochem. Biophys.*, 172, 278.
- Lenaz, G. and Curatola, G. (1978). A conformational model for the action of general anaesthetics at the membrane. Theoretical consideration. *Arc. Biochem. Biophys.* 27, 378.
- Lessler, M. A. and Brierly, G. P. (1970). "Oxygen electrode measurements in biochemical analysis", In "Methods of Biochemical analysis". 17, 1.

Levin, P. M. and Weiss, B. (1977). Binding of tri-fluoperazine to the calcium-dependent activator of cyclic nucleotide phosphodiesterase. *Mol. Pharm.* 13, 690.

Levy, J. V. (1968a). Myocardial and local anaesthetic actions of β -adrenergic receptor blocking agents: Relationship to physicochemical properties. *Eur. J. Pharmacol.* 2, 250.

Levy, J. V. (1968b). Surface tension effects of β -adrenergic blocking drugs. *J. Pharm. Pharmacol.* 20, 813.

Leung, Y. A. and Bhupat, D. R. (1977). Mechanism of action of tetracaine hydrochloride against *Pseudomonas aeruginosa*. *J. Infect. Disease.* 156, 679.

Lien, E. J. and Hansh, C. (1968). Structure-activity correlations for antibacterial agents on Gram-positive and Gram-negative cells. *J. Med. Chem.* 11, 430.

Lindenschmidt, R. C. and Witschi, H. P. (1984). Propranolol induced elevation of pulmonary collagen. *J. Pharmacol. et. Exp. Ther.* 232, 346.

Löfgren, N. (1948). "Studies on local anaesthetics: Xylocaine a new synthetic drug", Hoeggströms, Stockholm.

Lucchesi, B. R. (1970). Beta adrenergic receptor blocking agents. Part 1. *Pharmacology Uni. Mich. M. Ctr. J.* 36, 127.

Lugtenberg, B. and Meijers. (1975). Electrophoretic resolution of the major outer membrane proteins of *Escherichia coli* K12 into four bands. *FEBS. Lett.* 58, 254.

Luria, M. H. and Anderson, E. J. (1966). Acute and chronic effects of adrenergic beta blocking agent (propranolol) in treatment of cardiac arrhythmias. *Circulation*

Lurrink, J. and Van Der Sandle, C. (1986). Effects of divalent cations and of phospholipase A activity on excretion cloacin Df 13 and lysis of host cells. *J. Gen. Microbiol.* 132, 825.

Luzzati, U. (1968). In "Biological Membranes". (D. Chapman, eds). Academic Press, New York.

Main, B. G. and Tucker, H. (1985). In "Medicinal Chemistry". Academic Press Inc. (London) Ltd. pp. 69.

Mejbaum, W. (1939). Estimation of small amounts of pentose especially in derivatives of adenylic acid. Z. Physiol. Chem. 258, 117.

Meltzer, H. L. and Kassir, S. (1983). Inhibition of calmodulin-activated Ca^{2+} -ATPase by propranolol and Nadolol. Biochim. Biophys. Acta, 755, 452.

Metcalf, J. C. and Kornberg, H. L. (1978). In "Biochemistry of cell walls and membranes". Int. Rev. Biochem. 19, 1-62.

Miller, R. R. and Ilson, H. G. (1975). Propranolol-withdrawal rebound phenomenon - Exacerbation of coronary events after abrupt cessation of antianginal therapy. N. Engl. J. Med., 293, 416.

Mielk, J. B. and Garrett, E. R. (1969). Kinetics and mechanism of drug action on microorganisms. Chemotherapy. 14, 337.

Minkley, E. G. Jr. (1984). Purification and characterization of Pro-TraTp, the signal sequence containing precursors of a secreted protein encoded by the F sex factor. J. Bacteriol. 158, 464.

Mitchell, P. (1979). Keilins respiratory chain concept and its chemiosmotic consequences. Science, 206, 1148.

Miura, T. and Mizushimas (1968). Separation by density gradient centrifugation of two types of membranes from spheroplast membrane of Escherichia coli K12. Biochim. Biophys. Acta. 150, 159.

Mizuno, T. (1979). A novel peptidoglycan-associated lipoprotein found in the cell envelope of Pseudomonas aeruginosa and Escherichia coli. J. Biochem. (Tokyo) 86, 1059.

Mühlradt, P. and Goleck, S. (1975). A symmetrical distribution and artifactual reorientation of lipopolysaccharide in the outer membrane bilayer of Salmonella typhimurium. Eur. J. Biochem. 51, 343.

Mullins, S. C. (1954). Some physical mechanisms in narcosis. *Chem. Rev.* 54, 289.

Munton, T. J. and Russel, A. D. (1970). Effects of glutaraldehyde on the outer layers of Escherichia coli. *J. Appl. Bacteriol.* 35, 193.

Mutoh, M. and Frukawa, H. (1978). Role of lipopolysaccharide and outer membrane protein of Escherichia coli K12 in the receptor activity for bacteriophage T4. *J. Bacteriol.* 136, 693.

Mutoh, M. and Inokuchi, K. (1982). Amino acid sequence of the signal peptide of OmpF, A major outer membrane protein of Escherichia coli. *FEBS. Lett.* 137, 171.

Nachlas, M. V. and Margulies, S. I. (1960). Sites of electron transfer of tetrazolium salts in the succinoxidase systems. *J. Biol. Chem.* 255, 2739.

Narayanum, B. P. and Minkley, E. G. (1984). The product of the F-sex factor TraT surface exclusion gene is a lipoprotein. *J. Biol. Chem.* 259, 5357.

Nayler, W. G. and Stone, J. (1969). An effect of quinidine sulphate on the lipid-facilitated transport of calcium ions in cardiac muscle. *Am. Heart J.* 71, 363.

Noris, M. J. and Rogers, D. T. (1985). Cell envelope composition and sensitivity of Proteus mirabilis, Pseudomonas aeruginosa and Serratia marcescens to polymyxin and other antibacterial agents. *Lett. Appl. Microbiol.* 1, 3.

Novikoff, A. B. (1961). In "The cell (eds. J. Brachet and A. E. Mirsky). Vol. 2. pp. 423. Academic Press, New York.

Ohkawan, I. and Webster, R. E. (1981). The orientation of the major coat of bacteriophage F1 in the cytoplasmic membrane of Escherichia coli. *J. Biol. Chem.* 256, 9951.

Osborn, M. J. and Gander, J. E. (1972). Mechanism of assembly of the outer membrane of Salmonella typhimurium. *J. Biol. Chem.* 247, 3962.

Overath, P. and Schairer, H. U. (1970). Correlation of In vivo and In vitro phase transitions of membrane lipids in Escherichia coli. Proc. Natl. Acad. Sci. USA. 67, 606.

Parker, J. O. and West, R. O. (1968). Haemodynamic effects of propranolol in coronary heart disease. Am. J. Cardiol. 21, 11.

Peats, S. Turvey, J. R. (1958). Polysaccharides of Bakers Yeast. The presence of 1:6 linkages. J. Chem. Soc., 386, 8.

Postman, P. N. and Roseman, S. (1976). The bacterial phosphoenolpyruvate sugar phosphotransferase system. Biochemica. et. Biophysica. Acta, 457, 213.

Powell, C. E. and Slater, J. H. (1958). Blocking of inhibitory adrenergic receptors by a Dichloro analog of isoproterenol. J. Pharmacol., 122, 480.

Rauls, D. O. and Baker, J. K. (1979). Relationship of nonspecific antiarrhythmic and negative inotropic activity with physicochemical parameters of propranolol analogues. J. Med. Chem. 22, 81.

Reed, R. L. and Cheney, C. B. (1974). Propranolol therapy throughout pregnancy. A case report. Anaesth. Analg. 47, 161.

Renooij, W. and Van Golde, L. M. G. (1977). The transposition of molecular classes of phosphatidyl choline across the rat erythrocyte membrane and their exchange between the red cell membrane and plasma lipoproteins. Biochem. Biophys. Acta. 470, 465.

Rhoads, D. P. and Epstein, W. (1978). Energy coupling to net K^+ transport in Escherichia coli K12. J. Bio. Chem. 252, 1394.

Rogers, H. J. (1963). In "The structure and function of the membranes and surface of cells". 22nd Biochem. Soc. Sym. Cambridge University Press, England.

Rogers, H. J. and Perkins, H. R. (1980a). Biosynthesis of cell walls and cell membranes. London: Chapman and Hall, pp. 478-507.

Rogers, H. J. and Perkins, H. (1980). In "Microbial cell membranes". (eds. Chapman and Hall, London, New York.

Rose, A. H. (1976). In "The filamentous fungi". Bio-synthesis and metabolism (eds. Smith, J. E. and Bazzzy, R., London). pp. 308.

Rosenkranz, H. S. and Carr, H. S. (1965). Phenyethyl alcohol: Effect on macromolecular synthesis of Escherichia coli. J. Bacteriol., 89, 1354.

Rothman, J. E. and Engelman, D. M. (1972). Molecular mechanism for the interaction of phospholipid with cholesterol. Nature, 237, 44.

Rye, R. M. and Wiseman, (1964). Release of phosphorous-32 containing compounds from Micrococcus lysodiekcticus treated with chlorhexidine. J. Pharm. Pharmacol., 16, 516.

Rye, R. M. and Wiseman, D. (1966). Cell Viability and Phosphorous-32-release in Escherichia coli, effect of chlorhexidine. Ibid. 18, Suppl, 1145.

Salt, W. R. (1976). Induced turbidity changes in non-grow cultures of Escherichia coli. Ibid. 28, 263.

Salt, W. G. and Traynor, J. D. (1979). Interactions between anethocaine (tetracaine) and non-growing cultures of Escherichia coli. Ibid. 31, 41.

Salt, W. G. and Wiseman, D. (1970). The oclation between the uptake of cetyltrimethyl ammonium bromide by Escherichia coli and its effect on cell growth and viability. Ibid. 22, 261.

Salton, M. R. J. (1951). The adsorption of cetyltrimethylammonium bromide by bacteria, its action in releasing cellular constituents and its bacterial effects. J. Gen. Microbial., 5, 391.

Salton, M. R. J. (1960). In: "The bacteria". Vol. 1: Structure, (Eds. Gunsalus, I. C. and Stainer, R. Y), Academic Press, New York and London.

Salton, M. R. J. (1967). Structure and functions of bacterial cell membranes. *A Rev. Microbiol.*, 21, 417.

Salton, M. R. J. (1968). Lytic agents, cell permeability and monolayer penetrability. *J. Gen. Physiol.*, 52, 227.

Salton, M. R. J. (1971). Bacterial membranes. *Crit. Rev. Microbiol.*, 1, 161.

Sato, T. and Ito, K. (1977). Membrane proteins of *Escherichia coli* K12, Two-dimensional polyacrylamide gel electrophoresis of inner and outer membranes. *Eur. J. Biochem.*, 78, 557.

Schaller, K. and Dreher, P. (1981). Structural and functional properties of colicin M. *J. Bacteriol.* 146, 54 - 63.

Schmidt, R. M. and Rosenkranz, H. S. (1970). Antimicrobial activity of local anaesthetics: Lidocaine and Procaine. *J. Infect. Diseases*, 121, 597.

Schmiedeberg, F. and Lemmer, B. (1972). Conformational changes in membrane by β -sympatholytic agents. *Arc. Pharmacol.*, 274, Suppl. 125.

Schneider, H. (1968). The intramembrane location of alcohol anaesthetics. *Biochim. Biophys. Acta*, 163, 451.

Schneider, H. Rosenbush, J. P. L. (1981). Matrix protein in planar membranes. Clusters of channels in a native environment and their functional reassembly. *Proc. Natl. Acad. Sci., USA*, 78, 2302.

Seeman, P. M. (1966). Membrane stabilization by drugs: tranquilizers, steroids and anaesthetics. *Int. Rev. Neurobiol.* 9, 144.

Seeman, P. M. (1972). The membrane actions of anaesthetics and tranquilizers. *Pharmacol. Rev.* 24, 583.

Seeman, P. M. and Roth, S. (1971). The membrane concentrations of alcohol anaesthetics. *Biochimica. et. Biophysica. Acta*. 225, 171.

Sekeya, T. and Nazawa, Y. (1983). Reorganization of membrane Ergosterol during cell fission. Events of *Candida albicans*: A freeze fracture study of distribution of Filipin-Ergosterol complexes. *J. Ultrastructure. Res.* 83, 48.

Seppala, A. J. and Saris, N. W. (1971). Inhibition of phospholipase A induced swelling of mitochondria by local anaesthetics and related agents. *Biochem. Pharmacol.* 20, 305.

Shaw, M. and Dinglinger, F. (1969). The structure of an acylated inositol mannoside in the lipids of propionic acid bacteria. *Biochem. J.* 112, 769.

Shinshick, E. I. and McConnell, H. M. (1973). Lateral phase separation in phospholipid membranes. *Biochemistry*, 12, 2351.

Shigematsu Jun Uno and Arail, T. (1982). Effect of ketoconazole on isolated mitochondria from Candida albicans. *Anti. Microb. Agents. et Chemother.* 21, 919.

Silva, M. T., and Sousa, J. (1979). "Effect of local anaesthetic on Bacterial Cells". *J. Bacteriol.* 1979. 137, 461.

Simon, F. J. (1964). Inhibition of bacterial growth by drugs of morphine series. *Science.* 144, 543.

Singer, S. J. and Nicholson, G. L. (1972). The fluid-mosaic model of the structure of cell membranes. *Science*, 175, 720.

Smith, H. J. (1982). The need to redefine membrane stabilizing activity of Beta-adrenergic receptor antagonists. *J. Mol. et Cell. Cardiol.* 14, 495.

Smith, R. J. and Sabin, C. (1976). Effect of anti-inflammatory drugs on lysosomes and lysosomal enzymes from rat liver. *Biochem. Pharmacol.* 25, 2171.

Somani, P. and Luns, B. K. B. (1965). The antiarrhythmic actions of beta-adrenergic blocking agents. *J. Pharmacol. Exptl. Ther.*, 147, 194.

Stainer, Y. G. and Adelberg, E. A. (1976). In "General Microbiology". (4th eds. R. Y. Stainer, M. Doudoroff, E. A. Adelberg). Macmillan Press, London.

Unger, S. H. (1980). In "Chemical Structure: Biological activity relationships: Quantitative approaches". (Eds. C. F. Carvas), Akademiai Kiado, Budapest, pp. 3.

Umbreit, W. W. and Burns, R. H. (1972). In "Manometric biochemical techniques". (5th Eds). Minnesota: Burgess Publishing Company. pp. 27.

Vanderkooi, G. and Green, D. E. (1970). Biological membrane structure, I. The protein crystal model for membranes. Proc. Natl. Acad. Sci. USA, 66, 615.

Venables, P. and Russells, A. D. (1972). Some effects of nystatin on Saccharomyces cerevisiae. Microbios, 6, 239.

Wei, Yau-Huei and Lin, Teng-Nan. (1975). Inhibition of the mitochondrial Mg^{2+} -ATPase by Propranolol. Biochem. Pharmacol. 34, 911.

Weiss, B. and Prozialeck, W. (1980). Pharmacological regulation of Calmodulin activated Mg^{2+} -ATPase. Ann. N.Y. Acad. Sci., 356, 319.

Whelman, E. (1979). Stabilization of lysosomes in anoxic myocardium by Propranolol. Br. J. Pharmac. 65, 479.

White, D. A. and Lenarz, N. J. (1971). Distribution of lipids in the wall and cytoplasmic membrane subfractions of the cell envelope of Escherichia coli. J. Bacteriol., 109, 686.

Wiethhold, G. and Lemmer, B. (1972). Conformational changes in membranes by β -sympatholytic agents. Naunyn-Schme. Arch. Pharmacol. 274, 12.

Wilm, K. and Stahl, J. C. (1983). Effects of Econazole nitrate on yeast cells and mitochondria. Biochem. Pharmacol., 32, 1825.

Wilson, J. A. (1979). Principles of animal physiology (2nd eds. J. A. Wilson). Macmillan, Publishers, New York and London, pp. 25.

Wilson, V. G. and Luftig, R. B. (1970). Interaction of bacteriophage T4 tail fibre components with a lipopolysaccharide fraction from Escherichia coli. J. Mol. Biol., 51, 423.

Yu, F. and Mizushima, S. (1982). Roles of lipopoly-saccharide and outer membrane protein OMPC of Escherichia coli K12 in the receptor function for bacteriophage T4. J. Bacteriol. 151, 718.

Yu, F. and Yamada, A. (1981). Role of lipopolysaccharide in the receptor functioning for the bacteriophage T4 in Escherichia coli. Ibid. 148, 712.

Van Zwieten, P. A. (1969). Decrease in ionic permeability of the cell membrane in guinea-pig arterial tissue by treatment with anti-fibrillatory agents and hexobarbitone determined by means of 86 Rb. Br. J. Pharmacol., 35, 103.

