INTERACTIONS BETWEEN OLIGOAMINES AND SUPEROXIDE ANION

by

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THESIS

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SUMMARY

1_ Oligoamines and EDTA inhibited the reduction of cytochrome_C and nitroblue tetrazolium (NBT) induced by the hypoxanthine/xanthine oxidase superoxide anion generating system in the following order of effectiveness :
putrescine> diaminopropane> spermidine> EDTA> spermine> cadaverine
2_ Oligoamines and EDTA did not affect the rate of urate formation from the hypoxanthine/xanthine oxidase system.
3_ Oligoamines and EDTA inhibited the reduction of cytochrome_C induced by stimulated PMNL's in the same order of effectiveness as mentioned before.
4_ Oligoamines and EDTA inhibited luminol dependent stimulated PMNL's chemiluminescence.
5_ Oligoamines and EDTA inhibited the aerobic photoreduction of NBT.
6_ Oligoamines-copper sulphate complexes inhibited the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase system more effectively than oligoamines or copper sulphate individually.
7_ Superoxide anion, hydrogen peroxide and hydroxyl radical induced breakdown of isolated intact guinea pig liver lysosomes.
8_ Oligoamines and EDTA protected isolated intact guinea pig liver lysosomes from the lytic effect of superoxide anion generated either by the hypoxanthine/xanthine oxidase system or by stimulated PMNL's.
9_ Oligoamines and EDTA have no stabilizing effect on isolated intact guinea pig liver lysosomes.
10_ The uptake of oligoamines by lysosomes was in the following order putrescine> spermidine> spermine.
11_ Oligoamines were metabolised into aldehyde compounds either by the hypoxanthine/xanthine oxidase system or stimulated PMNL's.
12_ Oligoamines and EDTA have no effect on the activities of free lysosomal enzymes (acid phosphatase and β_glucosaminidase).
13_ Oligoamines and EDTA inhibited lipid peroxidation in guinea pig liver lysosomes induced either by the hypoxanthine/xanthine oxidase or ascorbic acid_ferrous sulphate.
14_ Oligoamines and EDTA have no effect on the release of PGE_2 from stimulated peritoneal guinea pig PMNL's.
15_ Oligoamines increased the uptake of (^3H)thymidine and (^3H)leucine by stimulated peritoneal guinea pig macrophages in the following order of effectiveness:
spermine> spermidine> putrescine> cadaverine.
16_ PGE_2, dibutyryl Cyclic AMP, and theophylline inhibited luminol dependent stimulated peritoneal guinea pig PMNL's chemiluminescence.

Key Words : Oligoamines, Superoxide anion, Lysosomes.

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ABBREVIATIONS

The following abbreviations have been used in the text :-

α_1_PI	α_1_Protease inhibitor
C_1, C_3, C_{3a}, C_5, C_{5a}, C_6, C_7,	Complement components
Cyclic AMP	Adenosine 3', 5'- cyclic monophosphate
DMSO	Dimethyl sulphoxide
EDTA	Ethylene diamine tetra-acetic acid
HBSS	Hanks Balanced Salt Solution
MPO	Myeloperoxidase
NBT	Nitro blue tetrazolium
PMA	Phorbol myristate acetate
PMNL's	Polymorphonuclear leucocytes
RA	Rheumatoid arthritis
SAMD	S-adenosyl methionine decarboxylase
SLE	Systemic lupus erythematosus
SOD	Superoxide dismutase
TBA	Thiobarbituric acid

1. INTRODUCTION

1.1 The Inflammatory Process

Inflammation protects the host from foreign invaders by initiating events which destroy bacteria, viruses, and other agents. It is a highly complex process involving many types of cells, enzymes, and physiologically active substances that alter local blood flow and cell behaviour. In many of the rheumatic diseases, the offending agent causing inflammation is unknown e.g. rheumatoid arthritis. In other rheumatic diseases the inciting agent is recognized e.g. sodium urate crystals in gouty arthritis.

Two excellant reviews of the inflammatory process have been published recently (Moore and Weiss, 1985; Rodnan et al, 1984).

Inflammation may occur in different forms involving different mechanisms. Although the detailed mechanisms of the inflammatory reaction are not fully understood, these reactions are certainly dependent on a number of chemical signals or mediators.

Inflammatory mediators are primarily active in three areas as, vasoactive substances, chemotactic factors, and agents leading to cell and tissue damage. Some overlap between these three areas occur.

1.1.1 Vasoactive Substances

These substances cause vasodilation and increase vascular permeability. They affect the contractile elements in the endothelial and periendothelial cells, leading to dilation of blood vessels and the opening of junctions between cells in postcapillary venules causing oedema. Examples of vasoactive substances are discussed below:

1.1.2 Vasoactive Amines

Histamine is stored in basophils and tissue mast cells and is released

and

by the interaction of antigen IgE with these cells during immediate hypersensitivity reactions. Serotonin released from stores in blood platelets may also contribute to vasopermeability (Fearon and Austen, 1979).

1.1.3 Anaphylatoxins

These substances (C_{3a} and C_{5a}) are polypeptide fragments of approximately 10,000 molecular weight. They are derived from the complement components C_3 and C_5 either by standard pathways of complement activation or through action of proteolytic enzymes, such as plasmin. These components are potent mediators of vascular permeability acting either directly on vessels or through the release of histamine from mast cells (Fearon and Austen, 1979).

1.1.4 Kinins

Bradykinin is the most important kinin. It is a nonapeptide with potent vasodilator activity and a promotor of vascular permeability (Fearon and Austen, 1979). Bradykinin is formed as a result of a complex cascade of proteolytic reactions outlined below :

Hageman Factor → negatively charged surfaces → Active Hageman Factor

Prekallikrein → Kallikrein

Plasma Kininogens → Bradykinin

Hageman factor (coagulation factor xii) activation begins the sequence leading to the production of bradykinin by binding to negatively charged surfaces, such as sodium urate or calcium pyrophosphate

crystals. This probably involves proteolytic cleavage. Activated Hageman factor in turn activates the conversion of prekallikrein to kallikrein. Kallikrein then cleaves bradykinin from precursor kininogens in plasma. Kallikrein also activates plasminogen to plasmin and the protein plasminogen activator appears to be a modified form of kallikrein. In addition to a direct effect on vasopermeability, bradykinin has the ability to stimulate histamine release from mast cells and to stimulate prostaglandin synthesis in a variety of tissues (Fearon and Austen, 1979).

1.1.5 Prostaglandins

Their vital role in inflammation will be discussed later in a separate chapter.

1.1.6 Chemotactic Factors

Inflammatory cells, including neutrophils, eosinophils, and monocytes, migrate to sites under the influence of chemotactic factors which are substances causing increased cell motility and directed cell movement (Synderman and Goetzl, 1981).

Certain fragments of complement components have chemotactic properties. The most important of these are the anaphylatoxins, C3 and the soluble complexes C_5, C_6, and C_7. In addition, neutral protease such as plasmin may generate other chemotactic peptide fragments from C_3 and C_5. The C_{5a} fragment may also increase neutrophil adhesiveness, allowing margination and escape of these cells from blood vessels (Fearon and Austen, 1979). Other proteins with chemotactic activity include kallikrein or plasminogen activator, peptides derived from fibrin and collagen, and bacterial products. Goetzl and Sun, (1979) have shown that several lipoxygenase products such as leukotrienes are active chemotactic factors.

1.1.7 Degradative Enzymes

In addition to vasoactive and chemotactic factors a large number of enzymes are released in inflammatory exudates that catalyze the hydrolysis of tissue components, including proteins, carbohydrates, and lipids. Inflammatory cells contain large quantities of these hydrolases, primarily in lysosomal granules. The function of these enzymes may be primarily bactericidal since lysosomes fuse with phagosomes containing internalized particles. Other components, such as immune complexes may be destroyed in this manner.

Many of the hydrolytic enzymes are active at acid pH apparently in the phagolysosome. However, these enzymes are released in part from leucocytes during phagocytosis and may exert their effect on host tissues. Although enzymes with activities limited to acid pH values may be of little consequence at the pH of the extracellular environment, some enzymes, primarily proteases are active at neutral pH and so may attack and destroy host tissues (Smolen and Weissmann, 1979). Collagenases are important extracellular degradative enzymes. Collagenases hydrolyse native collagen by cleaving the triple helical structure into two fragments. One fragment representing three quarters of the original molecule contains the terminal amino group, the second fragment a quarter of the original molecule contains the terminal carboxyl group (Harris and Krane, 1974). Since collagens are major constituents of connective tissues collagenase activity is likely to mediate the destruction of articular cartilage, tendons, joint capsules, bone and other inflammatory processes.

1.1.8 Other Neutral Proteases

Two proteases are secreted by polymorphonuclear leucocytes, and

macrophages which account for much of the extracellular proteolytic activity in rheumatoid arthritis and other inflammatory states. The first is elastase, a protease of broad substrate specificity, which attacks a wide range of proteins. Elastase degrades proteoglycan subunits into several fragments. Elastase is also capable of degrading elastin of blood vessels and many other proteins including collagen (Smolen and Weissmann, 1979; Krane, 1979).

The second important extracellular protease is cathepsin G, a chymotrypsin-like enzyme active at neutral pH against proteoglycans and other proteins. Other cathepsins, such as cathepsin D, are inactive at neutral pH and therefore are probably insignificant in extracellular locations. Cathepsin D may be important in the intracellular degradation of proteoglycans in phagolysosomes at acid pH. Other lysosomal enzymes degrade carbohydrates and proteins but appear to be active only at the acid pH of the phagolysosome (Smolen and Weissmann, 1979; Krane, 1979).

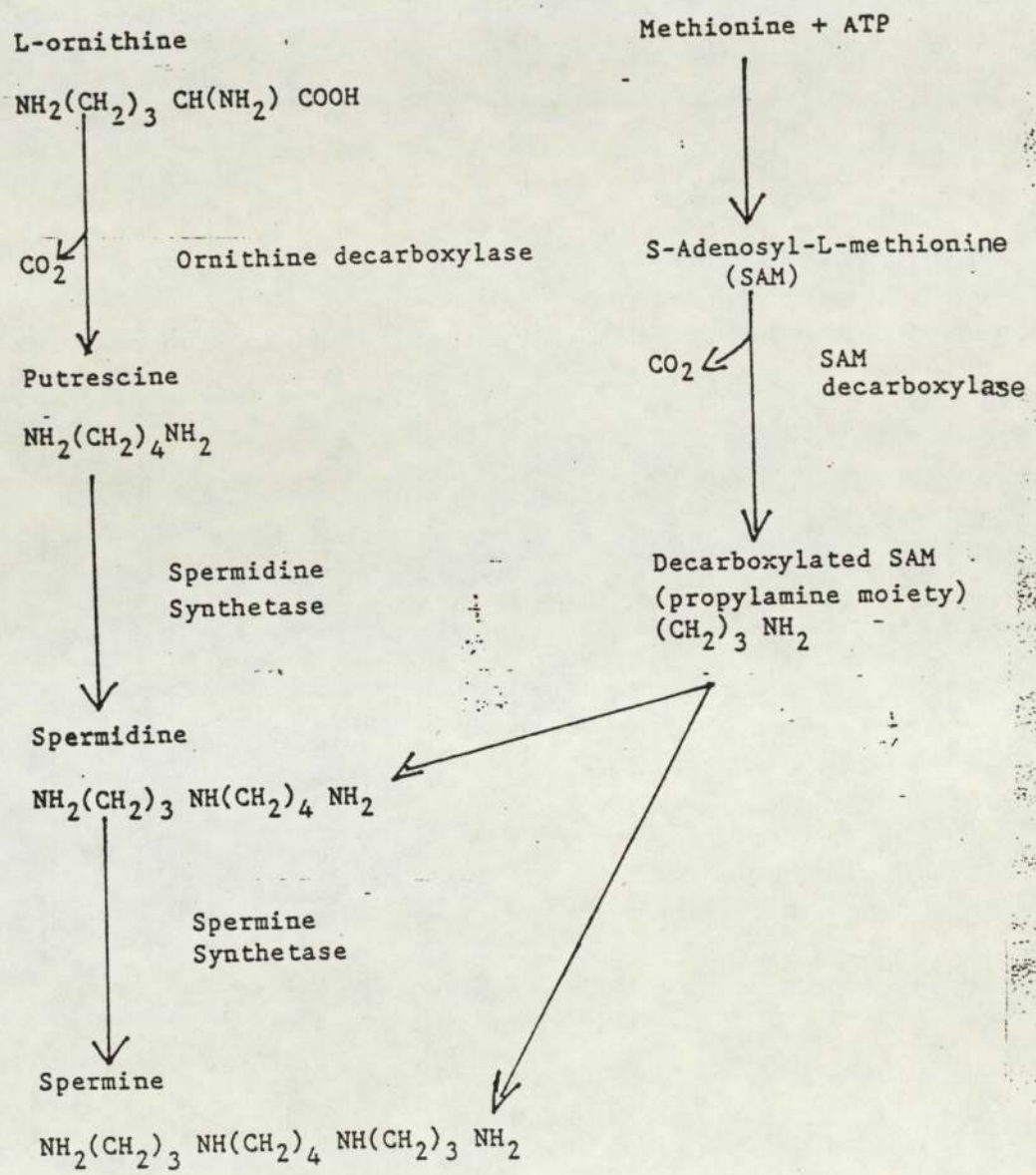
Proteolytic enzymes may also participate in inflammation by releasing mediators from complement components and from plasma kininogens. The neutral protease, plasmin, is generated by the action of plasminogen activator on the zymogen, plasminogen. Plasmin acts on several substrates, including fibrinogen and fibrin, releasing biologically active peptide fragments. It may also lead to the activation of Hageman factor, activation of collagenase from its latent procollagenase forms and activation of C_1 and C_3 by proteolytic reactions (Smolen and Weissmann, 1979; Krane, 1979).

1.2 Endogenous Oligoamines

The natural oligoamines putrescine spermidine and spermine are intracellular constituents of all living organisms. The name putrescine refers to putrefaction and is derived from observations that putrescine is rapidly formed in animal tissues undergoing bacterial decomposition. The name spermidine and spermine refer to semen where spermine was first discovered (Mann, and Lutwak_Mann, 1981). It is only recently that the role of these physiologic organic cations has been appreciated including their role in medicine. The fields of medicine in which the results of oligoamines research have an immediate relevance include cancer chemotherapy, treatment of hyperproliferative skin diseases, treatment of psoriatic diseases, clinical chemistry of a variety of diseases, development of new antigestional principles, new antiviral remidies and recently in inflammatory diseases.

1.2.1 The Biosynthesis of Oligoamines

The biosynthetic pathway of oligoamines in mammalian cells is well characterized (Tabor and Tabor, 1984; Pegg and McCann, 1982).
The biosynthetic pathway for oligoamines (collective name for di, tri and tetra_ amines) is outlined below:



The Biosynthetic Pathway of Polyamines in Eucaryotes

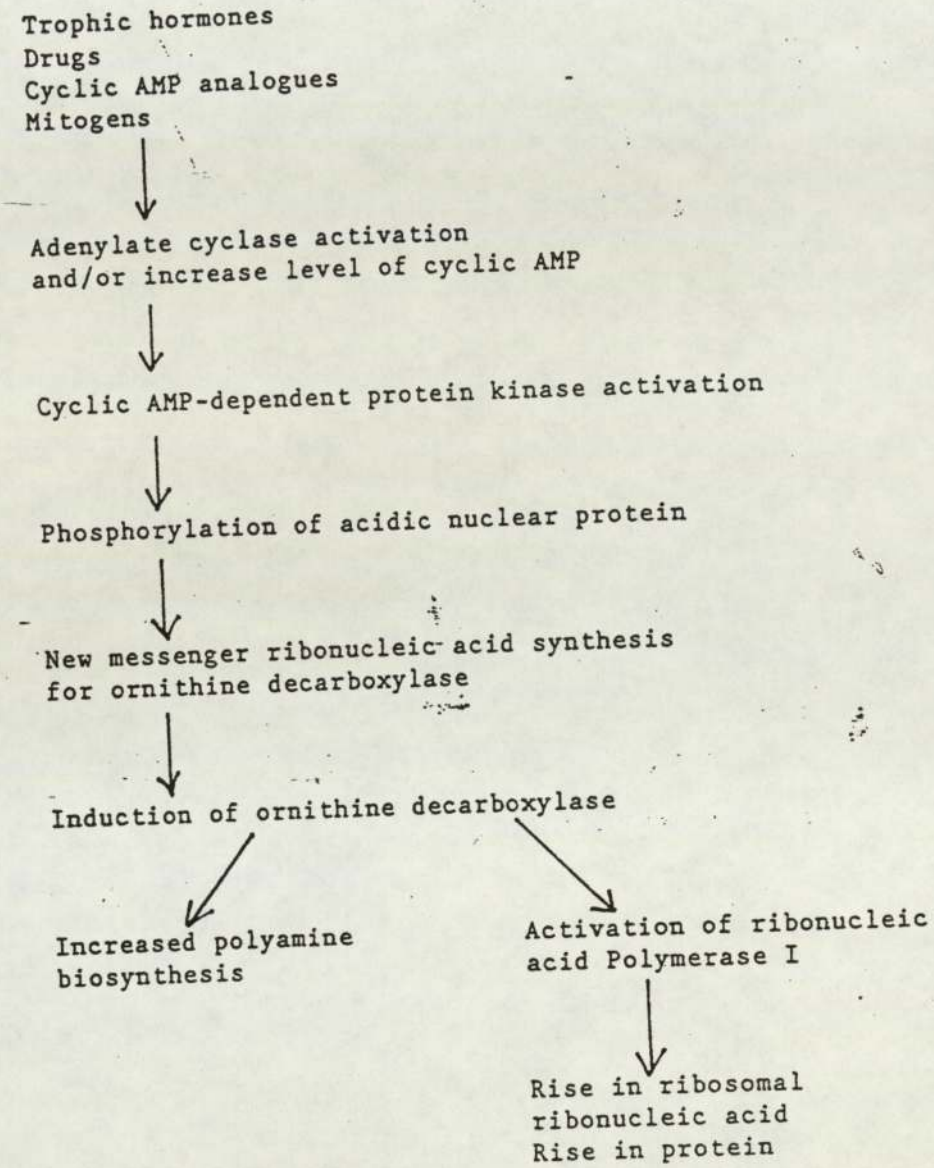
Although the figure is self explanatory, there are several features of worthy of note. The pathway illustrated is only applicable to eukaryotes. The main differences between the eukaryote and prokaryote pathways are that prokaryotes have an additional route to putrescine from arginine. Prokaryote S_adenosyl methionine decarboxylase (SAMD) require magnesium ions for activity, but are not activated by putrescine. Prokaryotes seem generally incapable of spermine biosynthesis. The n-propylamine group of spermidine and spermine arises from methionine. This is an essential amino acid in higher animals but it is synthesized from aspartic acid in plants and microbes.

The rate limiting steps in the biosynthetic pathway of oligoamines are generally considered to be ornithine decarboxylase (ODC) and S-adenosyl methionine decarboxylase (SAMD) (Mitchell et al, 1985). Ornithine decarboxylase has a molecular weight of 55,000 (Obenrader and Prouty, 1977) and has a central role in differentiation and growth regulation. The enzyme is inhibited by various ornithine analogues and requires the presence of thiol groups (Ono et al, 1972) and pyridoxal phosphate as co-factors.

The turn over rate of mammalian ODC is much faster than that of any other mammalian enzyme; the half life is 10 to 30 minutes (Tabor and Tabor, 1984).

A large number of stimuli cause a rapid increase of 10 to >200 fold in the level of ODC both in vivo and in cultured mammalian cells (Tabor and Tabor, 1984). The activity of this enzyme is elevated in rapidly growing organs and in tumour cells (Russell and Snyder, 1969; Williams et al, 1972) and the activity also rises as a result of the

administration of various drugs and hormones (Bachrach, 1984; McCann, 1980; Bachrach, 1975; Richman et al, 1971). In addition to hormones, endogenous cyclic nucleotides or dibutyryl cyclic AMP stimulates ODC activity in different organs and cells in tissue culture (Bachrach, 1980). It has been suggested that cyclic nucleotides mediate ODC induction (Russell et al, 1976). Prosser et al (1984) have shown that cyclic AMP mediated induction of ODC, and the induction of ODC was followed by rise in RNA and protein synthesis. It was proposed that ODC, and the products of its action, the oligoamines, might be the link between hormones, cyclic AMP, RNA and protein biosynthesis. This is summarised in the following diagram:



A Schematic Diagram of Major Sequential Steps in a Trophic Response

One of the most outstanding advances in the study of ODC regulation was the demonstration of ODC antizyme (Canellakis et al, 1978). ODC antizyme has a molecular weight of 260,500 daltons and is induced by exposing cells to oligoamines. It reacts specifically with ODC and neutralizes its activity. ODC antizyme has a relatively short half life. It has recently been postulated that ODC antizyme is normally attached to subcellular components (nucleus and ribosomes) and that oligioamines at millimolar concentrations cause its release (Fujisawa and Kitani, 1985; Heller et al, 1977). Therefore oligoamines may regulate ODC activity and control additional biosynthesis.

Purified SAMD from lactating mouse mammary gland was found to have a molecular weight of between 50,000 and 52,000 (Oka et al, 1978). This enzyme was stimulated markedly by putrescine which also protected the enzyme against inactivation (Pegg, 1984). In contrast spermine inhibited the activity of the enzyme while spermidine had no effect. The hormone insulin and hydrocortisone were found to increase SAMD.

1.2.2 Interconversion of Oligoamines

Persson et al, (1985) have reviewed the interconversion of oligoamines.

The spermidine synthase and spermine synthase reactions are effectively irreversible, but it has been known for many years that conversion of spermine into spermidine and spermidine into putrescine can occur in vivo. This interconversion takes place by the action of two enzymes, spermine N acetyl transferase and polyamine oxidase. The former enzyme needs acetyl Co A to covert spermidine into N acetyl spermidine and will also acetylate spermine forming N acetyl spermine (Pegg et al, 1981). These acetyl derivatives are extremely good

substrates for the enzyme polyamine oxidase, which cleaves at the internal nitrogen to yield N_acetyl propionoaldehyde and putrescine or spermidine depending on the substrate (Bolkenius and Seiler, 1981; Seiler et al, 1981). Although polyamine oxidase can oxidize polyamines themselves in vitro, the reaction requires unphysiological levels of aldehyde activators. In contrast the acetyl derivatives are very rapidly oxidized under physiological conditions (Seiler et al, 1981). Therefore it appears probable that acetylation is the limiting step in this interconversion. It has been shown that spermidine N_acetyl transferase is rapidly induced and increased many fold after exposure to toxic agents that enhance the conversion of spermidine into putrescine and spermine into spermidine (Pegg et al, 1981). Only very small amounts of the acetylated derivatives are present in rat tissues even at the peak of induction of the much greater activity of polyamine oxidase. In the mouse, larger amounts of N_acetyl spermidine and , N_acetyl spermine can be found because mouse tissues have twenty times less polyamine oxidase than the rat (Poso and Pegg, 1982; Seiler et al, 1981). However, even in mice the acetylated derivatives are degraded within a few hours (Poso and Pegg, 1982).

1.2.3 Degradation of Oligoamines

Putrescine can be oxidized by diamine oxidase, yielding γ_aminobutyraldehyde, which can be further oxidized to γ_aminobutyrate , or Δ_pyrroline (the spontaneously cyclized form of (γ_aminobutyraldehyde) may be converted into 2_pyrrolidone and 5_hydroxy_2_pyrrolidone (Seiler et al, 1981; Tabor and Tabor, 1976) Putrescine can also be acetylated by a microsomal enzyme and the monoacetyl putrescine oxidised by monoamine oxidase. This pathway can also give rise to γ_aminobutyrate and is important in γ_aminobutyrate

formation from putrescine in tissue like brain that has little diamine oxidase (Seiler, 1980).

Acetylation of putrescine, spermine and spermidine can also be brought about by a nuclear enzyme that, with spermidine as a substrate, forms predominantly the N_acetyl spermidine derivative. The only known metabolic fate for this conjugate is deacetylation by an enzyme that can also degrade monoacetyl putrescine but not N_acetyl spermidine (Seiler et al, 1981). Acetylated polyamines are found in blood and urine, but in quite small amounts (Seiler et al, 1981). Suzuki et al, (1985) have shown that acetylated oligoamines were oxidized by human diamine oxiodase .

The importance of acetylation in polyamine excretion is unclear. Takenoshita et al, (1984) have reported an elevation of the N_acetylspermidine level in the tissue of colorectal carcinoma. Extracellular oxidation of polyamines by serum oxidases yields aldehyde derivatives from the primary moieties. The aldehydes are unstable and can librate putrescine and acrolein but can also be taken up into the cell and further metabolised producing putrescine, N_(2_carboxy ethyl)_spermidine and possibly spermic acid (Seiler et al, 1981). These derivatives have been found in small amounts in normal tissue and urine but may be produced in higher concentrations in experiments using non physiological levels of polyamines.

Our knowledge of cadaverine function in higher vertebrates is scanty. Until recently little was known concerning the formation of cadaverine in mammalian tissue, its formation from lysine was shown to occur in the kidneys of mice after the injection of anabolic steroid (Henningsson et al, 1976; Perrson, 1977). Bardsley et al, (1970) have

shown that cadaverine is one of the two known substrates for the diamine oxidase enzyme, the other substrate is putrescine. Henningsson and Henningsson, (1983) have shown that cadaverine is metabolised by diamine oxidase into Δ^1-piperidine, γ-aminovaleric acid, carbon dioxide and some unidentified compounds.

1.2.4 Polyamine Oxidase

Polyamine oxidase (PAO) may be defined as an amine oxidase capable of catalysing the oxidation of polyamines, spermine and spermidine (Morgan, 1981; Morgan, 1980). Polyamine oxidizing enzymes have been identified in a range of mammalian tissues (Seiler et al, 1980). In the presence of the polyamines spermine and spermidine, the enzymes react with molecular oxygen to generate hydrogen peroxide, an oxidant implicated in the killing of some protozoan parasites (Arrick et al, 1981;) and aldehydes that react with biologic molecules possibly in a manner analogous to the lipid oxidation products produced by oxygen stress in erythrocytes and other cell types (Tappel, 1975). The bovine serum enzyme acts on the primary amino groups of spermine and spermidine to form an aminodialdehyde and aminoaldehyde, respectively. These are unstable and undergo β-elimination to form acrolien. In contrast, the rat liver enzyme cleaves the polyamines at secondary amino groups and results in the formation of aminopropionaldehyde. Macrophages and human pregnancy polyamine oxidases have been shown to oxidise polyamines at secondary amino groups with the formation of aminopropionoaldehyde too (Morgan, 1983; Morgan, 1981).

1.2.5 Oligoamines and Inflammation

At the present time, the evidence for a direct involvement of oligoamines in inflammation is limited. The anti-inflammatory effects of dexamethasone have been shown to be mediated by polypeptides

synthesized following binding of the steroid to glucocorticoid receptors (Tsurufuji et al, 1979). Bartholeyns et al, (1981a) suggested that the diamine putrescine was necessary for the biosynthesis of these anti-inflammatory mediators. These workers found that dexamethasone induced rises in hepatic ornithine decarboxylase activity (the enzyme responsible for the synthesis of putrescine) and putrescine concentration which were maximal at 4h. The concentrations of spermidine and spermine were unchanged. Using an irreversible inhibitor of ornithine decarboxylase (α_difluoromethylornithine) the effects of dexamethasone could be inhibited, but the effects of indomethacin were unaltered. Thus these investigators concluded that de novo synthesis of putrescine was an essential factor in the induction of new protein synthesis known to be involved in the anti-inflammatory action of dexamethasone. This study was extended by Bartholeyns et al, (1981b) who found that pretreatment of rats with aminoguanidine sulphate, an inhibitor of putrescine catabolism sixteen hours before the administration of α_difluoromethyl ornithine or actinomycin D completely prevented blockade of the anti-inflammatory effect of dexamethasone by α _difluoromethyl ornithine, but left unaltered the suppressive effects of actinomycin D. Polyester sponge exudate was found to contain polyamines (Bird and Lewis, 1979). The direct anti-inflammatory effect of oligoamines has been shown in animal models of inflammation (Bird and Lewis, 1979). Bird et al, (1983) have shown that putrescine was ten times as potent as spermidine as an anti-inflammatory agent in the carrageenan rat model of inflammation and was also anti-inflammatory in the adjuvant arthritic rat. Mohd_Hidir, (1985) showed that putrescine inhibited carrageenan and serotonin induced oedema in rat. Serotonin paw oedema

of mice and carrageenan paw oedema of rats were inhibited by putrescine, spermidine and spermine (Oyanagui, 1984).

Oligoamines have various biological actions which are very close to that of glucocorticoids. Spermine and spermidine serve to suppress lymphocyte proliferation in vitro and are believed to inhibit the release of inflammatory mediators (Theoharides, 1980). Spermine is present in the placenta and reacts with serum polyamine oxidase to form aldehydes that inhibit lymphocyte proliferation which inhibits foetal rejection (Morgan and Iliei, 1980).

Pregnancy serum contains higher levels of polyamine oxidases which produce aldehydes which act as immunoregulatory factors inhibiting abortion (Gaugas and Curzen, 1978).

The biological activity of thymic inhibitor " Chalone " is reported to depend for its activity on spermine which binds to chalone peptide (Allen et al, 1977). Milk protein synthesis by cultured mammary epithelium in vitro was enhanced by hydrocortisone, which could be replaced by spermidine (Oka and Perry, 1976).

Vanella et al, (1979) have shown that oligoamines have an inhibitory effect on the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase superoxide generating system. Vanella et al, (1980a) have demonstrated that spermine has scavenging effect on superoxide anion. Kafy and Lewis (1983) have reported that oligoamines protected isolated guinea pig liver lysosomes from superoxide induced lysis. Putrescine has been shown to inhibit superoxide production from stimulated PMNL's (Mohd_Hidir, 1985; Haigh et al, 1984). Haigh et al, (1985) have shown that putrescine was metabolised by phorbol myristate acetate (PMA) stimulated PMNL's into ɣ_aminobutyric acid and ɣ_aminobutyraldehyde. Cell proliferation is arrested by oxidized

polyamines (Gaugas and Dewey, 1978) and this property may be important in chronic inflammation where cellular proliferation is characteristic.

Prevention of auto-haemolysis of erythrocytes by oligoamines showsthe protective action exerted by oligoamines against cellular injury (Vanella et al, 1980b). Kimura et al, (1981) observed superoxide dismutase like activities from copper polyamine complexes using the hypoxanthine/xanthine oxidase superoxide generating system. It has been shown that oligoamines have an inhibitory action on lipid peroxidation (Tadolini et al, 1984; Khanna, 1982; Kitada et al, 1979) This may be relevant to their possible role as anti-inflammatory agents.

Sechi et al, (1978) have shown that spermidine, spermine, and putrescine have an inhibitory action on phospholipase A_2 and demonstrated that oligoamines protected phospholipid and mitochondrial membranes against the action of exogenous phospholipases. Nahas and Graff, (1982) have shown an inhibitory activity of oligoamines on phospholipase_C from human platelets.

Rennert et al, (1976) have shown that oligoamines inhibit platelet aggregation. Subbarao and Frostier (1977) have also shown that oligoamines inhibit platelet aggregation and inhibit the release of serotonin from them too.

Genedani et al, (1984) have shown that putrescine has analgesic properties.

Oligoamine levels in the serum from patients suffering from rheumatoid arthritis have been determined by Partsch et al, (1978). There was no

significant differance between normal serum and serum from rheumatoid arthritis patients.

1.2.6 Ornithine Decarboxylase and Inflammation

Although a connection between ODC, and oligoamines and inflammation is tenuous several properties attributed to ODC and oligoamines may be relevant. It has previously been described that liver disease has an ameliorating effect on inflammation (Hench, 1933). ODC and oligoamines have a considerable role in liver damage. Janne and Raina, (1968) observed that after partial hepatectomy there was a marked stimulation of ODC activity which resulted in a rapid accumulation of endogenous putrescine. This increase in putrescine favours an increase in spermidine since putrescine acts as a substrate in the spermidine synthase reaction and stimulates SAMD, but competitively inhibits spermine synthesis (Hannonen et al, 1972; Janne et al, 1978). The administration of the hepatotoxin carbon tetrachloride which has been found to inhibit inflammation (Mielens et al, 1969) also causes a marked activation of oligoamine synthesis (Holta et al, 1973). These workers also made the observation that during early liver generation and after carbon tetrachloride treatment there was a conversion of spermine to putrescine. This conversion was suggested to occur due to the action of peroxisomal polyamine oxidase in the liver.

The stimulation of ODC was also found to be due to various other treatments. It was found that infusion of hypertonic glucose or mannitol stimulated ODC and DNA labelling while 3.6% (w/v) saline had no effect. In addition the intraperitoneal injection of celite, a chemically inert mechanical irritant, caused a fifty fold increase in ODC after four hours (Schrock et al, 1970). Oligoamine synthesis during the formation of granulation tissue induced by implanting

viscose cellulose sponges subcutaneously in the rat has been studied by Riana et al, (1973). It was found that the activities of the oligoamine synthesizing enzymes rose steadily during the period of rapid proliferation. An increase in RNA was also noted. A rapid increase in ODC activity and accumulation of RNA and oligoamines, notably spermidine has also been demonstrated in healing wounds in rat skin (Mizutani et al, 1974).

Pregnancy, which is known to cause remissions in rheumatic diseases, (Fagerhol and Laurell, 1970) is also known to alter oligoamine levels. In both the human and rat the levels of oligoamines are elevated during pregnancy. It is notable that in the rat the elevation of putrescine is the greatest and spermine shows little change (Andersson et al, 1978; Russell et al, 1978). Warren et al, (1978) have shown increased levels of ODC and polyaminesfollowing an inflammatory stimulus.

Prostaglandins which are thought to be involved in the inflammatory process (Ferreira and Vane, 1974) also affect ODC levels. Verma and Boutwell, (1980) while studying the mechanism of skin tumour promotion by TPA (12_0_tetradecanoyl phorbol_13_acetate) found that prostaglandins may play an important role in the induction of ODC activity. This induction of ODC activity could be depressed by prior treatment with prostaglandin synthetase inhibitors and this inhibition could be counteracted by treatment with PGE_1 or PGE_2 but not with $PGF_1\alpha$ or $PGF_2\alpha$. Prostaglandin PGE and dibutyryl cyclic AMP have been shown to stimulate ODC activity (Prosser et al, 1984).

1.3 Sources of Superoxide

Several reactions are known to produce substantial amounts of

superoxide. The autoxidation of hydroquinones, leukoflavin (Misra and Fridovich, 1972a), catecholamines (Misra and Fridovich, 1972b), thiols (Misra, 1974). A number of enzymes (Fridovich, 1975) including xanthine oxidase, aldehyde oxidase and flavin dehydrogenase produce superoxide as do fragments of subcellular organelles such as mitochondria (Loschen et al, 1974) and large amounts of superoxide are liberated during the respiratory burst which accompanies active phagocytosis of particles or bacteria by neutrophils (Babior et al, 1973; Curnutte and Babior, 1974), monocytes (Sagone et al, 1976) and macrophages (Drath and Karnovsky, 1975). Reduced ferrodoxins are also subject to spontaneous oxidation which produces superoxide (Misra and Fridovich, 1971). Haemoglobin and myoglobin in their oxygenated forms do slowly liberate superoxide as they are converted to methaemoglobin and metmyoglobin (Misra and Fridovich, 1972c). The production of methaemoglobin is sufficiently large to account for the fact that erythrocytes contain a methaemoglobin reductase which reverses the process.

1.3.1 Cytotoxicity of Superoxide

Superoxide toxicity may fall into two categories, damage and evoked tissue response (McCord, 1983). Damage is the result of direct or indirect attack at the molecular level by superoxide or secondarily generated radicals. The metal catalyzed Haber_Weiss reaction

$$O_2^{-\cdot} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^-$$

provides a mechanism for converting the relatively unreactive superoxide radical into the potently oxidizing hydroxyl radical (McCord and Day, 1978). This appears to be the mechanism that accounts for the superoxide dependent degradation of hyaluronic acid (McCord, 1974) as well as for the cytotoxicity of superoxide shown towards

neutrophils *in vitro* (Saline and McCord, 1975). On the other hand, a free radical chain oxidation of enzyme bound nicotinamide adenine dinucleotide is initiated and propagated by superoxide per se (Beilski and Chan, 1973). In general a broader spectrum of determinal reactions may be expected when the hydroxyl radical is produced secondarily. The second category of superoxide induced toxicity may be described as evoked tissue response. The nature of this response is not yet well documented and is speculative. The rational why tissues may have evolved the ability to respond to superoxide [illegible] due to the fact that the radical plays key metabolic roles in the phagocyte mediated capillary permeability is advantageous by facilitating the infiltration by phagocytes into an infected tissue. If so then the tissue's ability to recognize superoxide (which signals the presence of activated phagocytes) and its response by lowering its permeability barriers is entirely appropriate. A key feature that distinguishes such a response from actual damage is rapid resolution or reversibility when the stimulus (O_2^-) disappears.

1.3.2 Superoxide and Inflammation

The role of superoxide in inflammation has been reviewed elsewhere (Fridovich, 1986; Weiss, 1986). Because a small fraction of the oxygen reduced by aerobic cells is converted to superoxide through normal metabolic channels, all such cells are equipped with a protective enzyme, superoxide dismutase (SOD), which eliminates the radical by catalyzing its disproportionation $O_2^{-\cdot} + {}^{-\cdot}O_2 + 2\,H^+ \rightarrow O_2 + H_2O_2$ (McCord & Fridovich, 1969; McCord *et al*, 1971). Although traces of superoxide dismutase are detectable in extracellular fluids, the enzyme is an intracellular enzyme. It was discovered empirically nearly twenty years ago that superoxide dismutase has anti-inflammatory activity

when injected into animals. At that time its enzymatic activity was unknown and it was given the name "Orgotein" (Menander and Huber, 1977). This observation was biochemically inexplicable until Babior et al, (1973) reported that phagocytosing neutrophils produce superoxide probably for bactericidal purposes. Johnston et al, (1975) confirmed the role of superoxide in the destruction in phagocytes of ingested microbes, by recognizing that inflammatory cells produce potentially cytotoxic radicals derived from superoxide when they exhibit bactericidal activity. These findings led to a rational hypothesis for the anti-inflammatory activity of superoxide dismutase. When an inflammed tissue was infiltrated by active phagocytes it was vulnerable to radical damage. However, if superoxide dismutase was present in the extracellular fluid it scavenged superoxide producing radicals and protected the tissue (McCord, 1974).

McCord and Wong, (1979); Petron et al, (1980) have examined the anti-inflammatory activity of intravenously injected SOD in three laboratory models of induced inflammation, the reverse passive Arthus reaction in the rat, carrageenan induced foot oedema in the rat, and passive immune complex induced glomerulonephritis in the mouse. In all three models treatment with SOD produced substantial suppression of the inflammatory response. They made several observations however and suggested that the working hypothesis was insufficient to explain all the data. The first was that the enzyme did not cause complete suppression of symptoms. Therefore partial protection by SOD could be explained because phagocytes possess some cytotoxic mechanisms that do not involve superoxide, Secondly although catalase and mannitol had been found to prevent cytotoxicity in in vitro experiments presumably by preventing hydroxyl mediated damage they had no effect in vivo.

Their third observation showed that on histological examination a dramatic decrease in the extent of inflammatory cell infilteration into tissues occurred in SOD treated animals which was not consistent with an anti-inflammatory action. Finally the intradermal injection of a superoxide generating system (xanthine oxidase and a purine) did not produce the grossly observable signs of inflammation expected from superoxide production but it did result in a histologically observable heavy infiltration of neutrophils to the injection site. These latter observations were reconciled with the formulation of a new modified hypothesis which could be tested experimentally in that the normal accumulation of neutrophils specifically requires the generation of extracellular superoxide. McCord (1983) has reported that extracellular fluids contain a latent but superoxide activable chemoattractant. The fact that the first cells (neutrophils) to react to an inflammatory stimulus would begin to liberate superoxide into the microenvironment. This superoxide, by activating a latent chemotactic factor would serve as a chemical beacon to draw additional cells to the site. The newly arriving cells would in turn be stimulated to produce more chemoattractant and there by amplify or maintain the gradient until the stimuli decayed. The existence of a hypothetical latent chemotactic factor in plasma was verified experimentally (Petron et al, 1980). Unlike most chemoattractants for neutrophils e.g. (complement fragment C_{5a} or N-formyl- methionyl peptide) the superoxide induced factor did not stimulate degranulation or superoxide production. If it had, it would have catalysed its own self generation by a cascade process. However the factor is solely chemotactic and is dependent on a stimulus of the respiratory burst to effect its production. The superoxide

dependent chemotactic factor appears to play a major role in the development of neutrophil mediated inflammatory responses. The primary mechanism of the anti-inflammatory activity of SOD therefore appears to be in the prevention of the activation of this factor by removing superoxide in the extracellular fluid. Superoxide may have other pro-inflammatory properties. Handin et al, (1977) have shown that superoxide induced the secretion of serotonin and intiated the aggregation of platelets.

The implication of superoxide in various diseases has been reviewed by Marklund, (1984).

1.3.3 Role of Superoxide in Rheumatoid Arthritis (RA)

Since synovial fluid which is the natural joint lubricant has little SOD or catalase activities (Blake et al, 1981; McCord, 1974), oxygen derived species are not detoxified and react with joint components to cause tissue damage. Greenwald et al, (1976) have shown that the degradation of cartilage proteoglycans and collagen is due in part to superoxide in vitro. Greenwald and Moy (1980) has shown that exposure of hyaluronic acid solution to an enzymatically generated superoxide flux not only reduced the viscosity of solution but also rendered hyaluronic acid susceptible to further degradation by lysosomal enzymes. They also emphasized the importance of this finding by stressing the lack of convincing evidence for the existence of hyaluronidase in the pathogenic joint fluid despite frequent findings of reduced synovial viscosity in inflammatory joint disease (Kafoed and Barcelo, 1978). They emphasized the relevance of their in vitro findings to changes occuring in inflammed joints by demonstrating a

reduction of the viscosity of hyaluronic acid solution after exposure to stimulated PMNL's and also by demonstrating impairment of collagen gelation.

Burkhardt et al, (1986) have shown that cartilage slices (bovine nasal cartilage) altered by elastase treatment were more susceptible to oxygen derived radical attack than were intact tissue specimens. Degradation was inhibited by SOD. In the same studies they showed that coincubation of latent collagenase from PMNL's with a superoxide generating system led to activation of collagenolytic activity, resulting in marked degradation of the bovine cartilage slices.

It has been shown that free radical reactions generated by U.V light or activated PMNL's can denature IgG. Such changes in the structure of IgG were identical to those found in IgG isolated from rheumatoid serum and synovial fluid (Lunec and Blake, 1984). Lunec et al, (1985) further showed that IgG not only undergoes free radical denaturation but that in so doing it can itself become a stimulus for PMNL's to generate more free radicals. This observation may be of considerable importance since it describes a self perpetuating mechanism of radical release and tissue damage mediated by PMNL's. Conversion of complement induced by the free radical denatured IgG could be seen to further amplify this destructive mechanism, an observation which is consistent with the finding of complement depletion in rheumatoid serum and synovial fluid.

Biemond et al, (1986) have demonstrated that PMNL's isolated from rheumatoid arthritis patients produced more superoxide compared with PMNL's of healthy individuals.

1.3.4 Superoxide and Auto-immune Diseases

In various types of autoimmune disease e.g. systemic lupus

erythematosus (SLE), progressive systemic sclerosis and rheumatoid arthritis increased breakage of chromosomes in lymphocytes has been demonstrated (Emerit, 1982). Plasma from such patients has been shown to contain a chromosome breaking clastogenic factor. Breaks also occur in normal lymphocytes when they are cocultivated with lymphocytes from patients with autoimmune disease. The clastogenic factor can be induced by U.V irradiation of plasma from SLE patients. In all models SOD in the culture medium protects the lymphocytes against the increased chromosome breakage (Emerit, 1982). This finding may implicate superoxide either directly or indirectly as a causative agent of these diseases. The clastogenic factor has a molecular weight between 1000and 10,000 but its chemical structure is not known. Evidence is also begining to appear for important direct associations between the immune system and reactive oxygen species. It has been suggested that tolerance to self antigen is actively maintained by T_suppressor cells and that the precursors of such cells are especially sensitive to oxygen free radicals (Halliwell, 1981). There is also evidence that suggests that oxygen radical generation by monocytes is essential for subsequent lymphocyte activation (McKeown et al, 1984). Allan et al, (1986) have shown that reactive oxygen species are toxic to lymphocytes. T_cells were more susceptible to reactive oxygen species than B_cells. In the same study they showed that T_8 cells (suppressor/cytotoxic) are more susceptible to the toxic action of oxygen radicals than T_4 cells (helper/inducer), and suggested that reactive oxygen species may be responsible for the increased T_4:T_8 ratio in RA synovial fluid and synovial tissue.

1.3.5 Superoxide and Lung Damage

Leucocytes accumulate in the lung under many pathologoical conditions

with resultant damage to the lungs. The phenomenon can be induced by complement activation in several ways (Till and Ward, 1983) e.g. by immune complexes (McCormick et al, 1981) and by skin burn damage (Tilland Ward, 1983). In all the above models parenteral administration of SOD has been shown to protect the lung thus implicating superoxide in the mechanism leading to damage.

1.3.6 Superoxide and Cancer

Any substance that reacts with DNA is potentially carcinogenic. Exposure of DNA to a superoxide generating system causes extensive strand breakage and degradation (Rowley and Halliwell, 1983; Brown and Fridovich, 1981). This effect has been shown in vitro to be due to the formation of hydroxyl radicals. Both bacteria and animal cells in culture suffer DNA damage on exposure to superoxide generating systems, an effect which has been shown to be mutagenic (Weitburg et al, 1983; Weitzman and Stossel, 1981). It is therefore tempting to attribute the increased risk of the development of cancer in chronically inflammed tissues to the generation of oxygen radicals by phagocytic cells. There is no direct evidence of this but interest was generated by reports that cancer cells grown in culture and taken from some transplantable tumours in animals were defecient in SOD activity, especially in their mitochondria (Oberley, 1982). The relevance of these studies to human cancer is not clear, especially since human tumours biopsied during surgery show no defect in SOD activity (Marklund et al, 1982; Westman and Marklund, 1981).

1.3.7 Miscellaneous Effects of Superoxide

Oncogenic transformation of cells induced by X_rays, bleomycin, miconidazol and other nitromidazols, is significantly inhibited by SOD, even if the enzyme is given after the inducing agents (Bork and

Troll, 1983; Miller et al, 1982). Protection by SOD against pyelonephritis damage (Roberts et al, 1982) and against oedema after burn damage (Bjork and Arturson, 1983) has been shown which may implicate superoxide as a pathogenic agent in these conditions.

1.4 Hydrogen Peroxide Formation

The generation of hydrogen peroxide can occur by many mechanisms, of which I shall outline three. The first two are superoxide dependent, and the last involves the direct reduction of molecular oxygen (McCord et al, 1971) e.g. glucose oxidase.

1.4.1 Spontaneous Dismutation of Superoxide into Hydrogen Peroxide

Superoxide is unstable compared to hydroxyl radical and hydrogen peroxide and can form these products by spontaneous dismutation. There are three dismutation reactions that can occur. Superoxide is the conjugate base of HO_2 which has a pKa of 4.8 (Fridovich, 1983). Spontaneous dismutation of superoxide occurs most rapidly when the pH equals to the pKa (reaction I). At pH 4.8 and at equilibrium, HO_2 and superoxide are at equal concentrations and the rate of the reaction is at a maximum. Under physiological conditions most of superoxide anion is in the non protonated form and reaction (III) is unfavourable due to electrostatic repulsion in forming a complex consisting of two O_2^- (Fridovich, 1983).

I $\quad HO_2^- + HO_2 \rightarrow H_2O_2 + O_2$

II $\quad H + HO_2 + O_2^- \rightarrow H_2O_2 + O_2$

III $\quad 2H + O_2^- + O_2^- \rightarrow H_2O_2 + O_2$

At a neutral pH, enzymatically catalyzed dismutation of superoxide by SOD is the more important mechanism for hydrogen peroxide generation compared with spontaneous dismutation (Fridovich, 1983).

1.4.2 Direct Formation of Hydrogen Peroxide

The primary product of reduction of superoxide by various oxidases is hydrogen peroxide via a two electron transfer pathway (Halliwell, 1984). Hydrogen peroxide has long been thought to be toxic. It was found to cause lysis of human erythrocytes (Stocks and Dormandy, 1973). Hydrogen peroxide has been shown to be cytotoxic to human lymphocytes (Allen et al, 1986). It can cause oxidation of proteins and lipids (Stauffer and Etson, 1969) but only at concentrations which greatly exceeded the in vivo concentrations. Therefore hydrogen peroxide may not directly attack cellular components but may react with other compounds to form more reactive species.
Ohmuri et al, (1979) have demonstrated that hydrogen peroxide activates histamine secretion from mast cells.

1.5 Hydroxyl Radical Formation

It has been demonstrated in many in vitro studies that hydroxyl radical (OH) generation can occur by :

1_A Fenton modified type Haber_Weiss reaction where superoxide is acting as a reducing agent.

2_A Superoxide dependent reaction catalysed by transferrin.

3_An ascorbic acid dependent reaction where ascorbic acid is acting as as reducing agent.

1.5.1 Hydroxyl Radical Formation by the Fenton Modified Haber Weiss Reaction

The presence of OH radical has been shown by studies using a xanthine/xanthine oxidase system which is able to oxidise the methanol producing ethylene gas as an end product (Beauchamp and Fridovich,

1970). It was shown that neither superoxide nor hydrogen peroxide was responsible for ethylene production and the formation of OH radical *via* the Haber_Weiss reaction is able to oxidise methanol. In subsequent studies (Halliwell, 1981) has demonstrated that Haber_Weiss reaction although energetically favourable has a rate of reaction which is too slow to explain the results. To resolve this conflict the formation of the OH from the interaction of superoxide and hydrogen peroxide was postulated to occur by the Fenton type modified Haber_Weiss reaction (Motohashi and Mori, 1983). In this type of reaction superoxide first reacts with an oxidised form of trace metal (probably iron in biological systems) causing reduction of the metal and generation of oxygen. The reduced form of metal reacts with hydrogen peroxide resulting in the regeneration of the initial oxidized metal and the formation of OH and OH

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^- + OH^{\cdot}$$

Modified Haber_Weiss Reaction (Fenton Reaction)

$$O_2^{\cdot-} + Me^{N+1} \rightarrow Me^{N} + O_2$$

$$Me^{N} + H_2O_2 \rightarrow Me^{N+1} + OH^{\cdot} + OH^-$$

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^{\cdot} + OH^-$$

1.5.2 Formation of Hydroxyl Radical by Transferrin

An area of interest is the possibility of OH radical formation in biological systems by iron containing proteins such as lactoferrin (Winterbourn, 1983) and transferrin (Bannister *et al*, 1982a). Studies by Bannister *et al*, (1982b) have shown that transferrin is being used as a catalyst in hydroxyl radical generation following superoxide production by neutrophil NADPH oxidase in the presence of hydrogen peroxide. Motohashi and Mori, (1983) have demonstrated that

transferrin in the hypoxanthine/xanthine oxidase system may induce hydroxyl radical generation through a Fenton type Haber_Weiss reaction.

The hydroxyl radical is one of the most reactive species known and can react with biommolecular molecules e.g. depolymerization of hyaluronic acid (McCord, 1974).

1.5.3 Formation of Hydroxyl Radical by Ascorbic Acid

In the postulated Fenton type reaction, superoxide is acting as a reducing agent and it has been demonstrated that it may be replaced by other compounds which act as reducing agents. Iron_EDTA was shown to catalyse hydroxyl radical production from hydrogen peroxide and ascorbate by a mechanism largely independent of superoxide when ascorbate and superoxide were both present. The ascorbate mechanism was more important than superoxide as a source of hydroxyl radical, and would appear to be more biologically significant (Winterbourn, 1979).

1.6 Defence Mechanisms Against Oxygen Derived Free Radicals

The defence mechanisms can be probably broadly devided into two main groups based on location. The anti_oxidant systems which are responsible for the intracellular mechanisms include catalase, glutathione peroxidase and vitamin E, whereas the main extracellular anti_oxidant system is provided by the plasma protein called caeruloplasmin.

1.6.1 Intracellular Antioxidant Enzyme Systems

The first line of defence mechanism to combat excessive superoxide formation is provided by SOD. Whereas, catalase and glutathione peroxidase are responsible for the removal of hydrogen peroxide and other oxygen derived species and thus provide the second line of

defence. However, vitamin E is the main non enzymatic chain breaking anti_oxidant.

1.6.1.1 Superoxide Dismutase

The intracellular SOD can protect cells from the potentially injurious superoxide by scavenging it thereby preventing the production of hydrogen peroxide. Thus SOD is able to prevent the formation of the more lethal radicals such as hydroxyl radical.

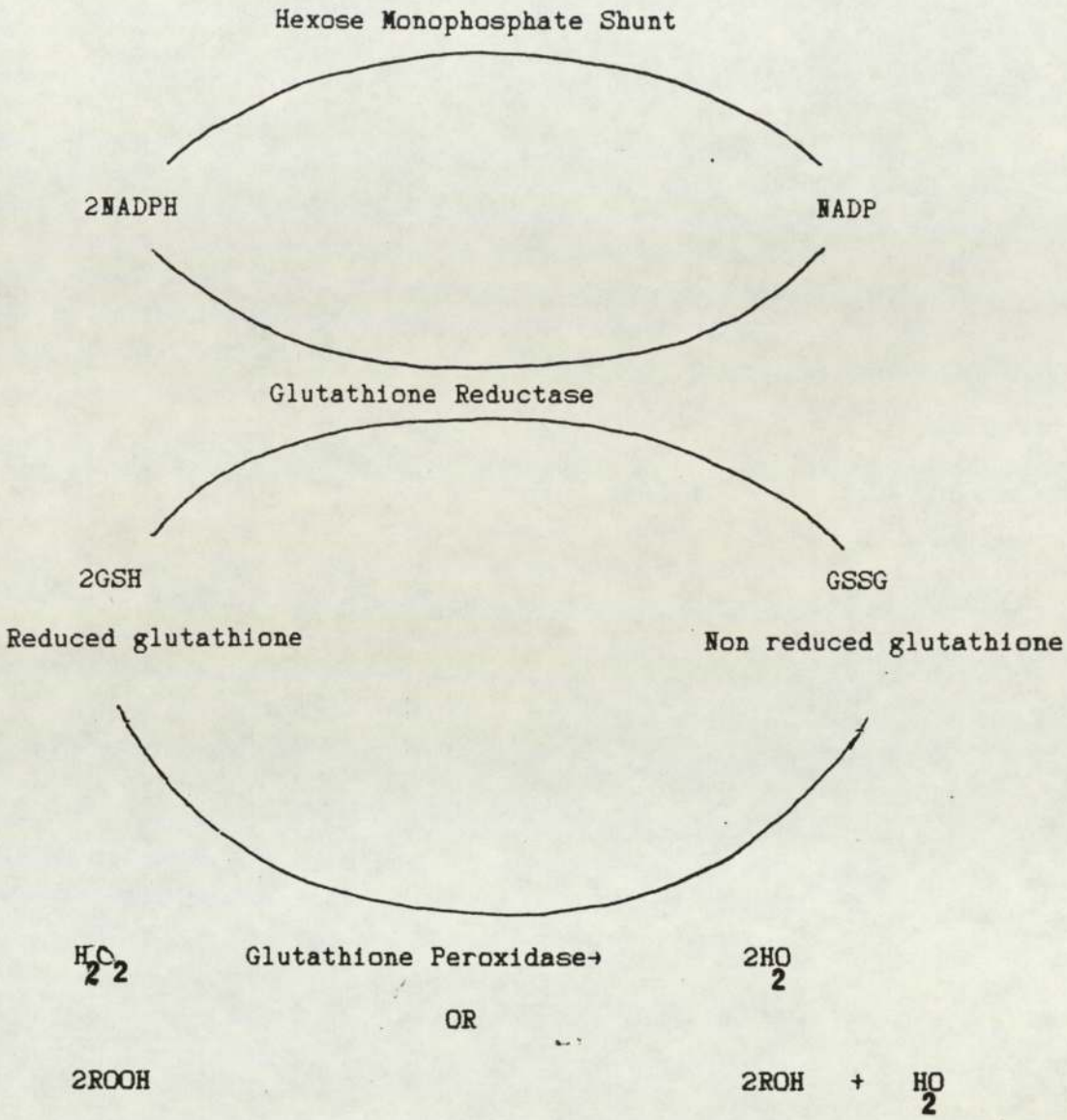
$$2H^{+} + 2O_2^{-} \xrightarrow{SOD} H_2O_2 + O_2$$

1.6.1.2 Catalase and Glutathione Peroxidase

During the respiratory burst hydrogen peroxide is secreted into extracellular environment and may diffuse into back the cytoplasm of PMNL's potentially injuring these cells. Two hydrogen peroxiode anti_oxidants have been identified, these are catalase and glutathione peroxifdase (Fridovich, 1976). Catalase, a cytoplasmic enzyme, catalyses the divalent reduction of hydrogen peroxide to water when the concentration of hydrogen peroxide is high.

Glutathione peroxidase, is present in the cytoplasm of the cell and is able to detoxify hydrogen peroxide to water by catalysing the oxidation of reduced glutathione (Fridovich, 1976; Lawrence, 1976).

$$2H_2O_2 \xrightarrow{\text{catalase}} O_2 + 2H_2O$$



The oxidized form of glutathione may then be reduced by a second enzyme, glutathione reductase with NADPH as a cofactor (Lawrence et al, 1978). The glutathione peroxidase is also able to metabolise hydrogen peroxide or hydroperoxides depending upon the activity of glutathione reductase and the availability of NADPH. NADPH is generated from the activation of hexose monophosphate shunt.

1.6.1.3 Vitamin E a Cellular Non Enzymatic Antioxidant

Despite the existence of biological anti-oxidant systems preventing the initiation of intracellular peroxidation as discussed previously they are unable to completely remove all the oxygen derived radicals

and therefore lipid membranes can undergo auto-oxidation. If these are left unchecked, then oxidation of lipid membranes could eventually spread through the entire cell (Hornsby and Crivello, 1983). The major cellular non enzymatic chain breaking anti_oxidant is vitamin E. The active biological component of vitamin E is α_tocopherol (Witting, 1980). Tocopherol is believed to function by terminating free radical reactions by competing for peroxy free radicals (RO), resulting in the formation of tocopherol dimers (Fanton and Ward, 1982). α_tocopherol is inactivated during lipid peroxidation and may be regenerated from the reduced form by cytosolic anti-oxidants principally ascorbic acid (Packer et al, 1979)

1.6.2 Extracellular Antioxidants

As outlined before, cells are protected under normal conditions against damage by the oxygen derived free radicals by the enzymes SOD, catalase and glutathione peroxidase. Extracellular fluids, however are generally poor in these enzymes (McCord, 1974) and yet can be subjected to oxygen derived free radicals produced by activated PMNL's. It has been shown that extracellular fluids do contain effective anti-oxidants of their own which are different from those found intracellularly i.e. caeruloplasmin (Gutteridge, 1983).

1.6.2.1 Caeruloplasmin

Caeruloplasmin is a copper containing α_glycoprotein and has an approximate plasma concentration of 300 µg/100 ml in the human adult (Scheinberg and Sternlieb, 1963). It has been proposed that caeruloplasmin functions as a copper transport protein (Scheinberg, and Morell, 1957) but caeruloplasmin possesses other properties :

1_Circulating anti-oxidant (Yammashoji and Kajimoto, 1983; Gutteridge et al, 1979)

2_Superoxide scavenger (Goldstein et al, 1979)

3_An acute phase reactant (Stocks et al, 1974)

1.7 Lipid Peroxidation

Lipid peroxidation reflects the interaction between oxygen derived free radicals and polyunsaturated fatty acids (PUFA), since PUFA are an integral part of the membranes, alteration to PUFA results in structural changes to the membrane function (Tappel, 1973). It has been demonstrated that oxygen radicals can damage the membranes of erythrocytes (Fanton and Ward, 1982), human PMNL's (Saline and McCord, 1975) and cultured human endothelial cells (Sacks et al, 1978) by initiatiating lipid peroxidation. Lipid peroxidation has been reviewed elsewhere (Weiss, 1986; Hornsby and Crivello, 1983; Fanton and Ward, 1982).

1.7.1 Lipid Peroxidation and Lysosomal Enzyme Release

Early reports on the inter-relation between lipid peroxidation and the release of lysosomal enzymes came from studying the effects of radiation on various tissue. It was found to increase the release of hydrolytic enzymes from lysosomes (Goutier and Goutier, 1962). Also chemically induced peroxidation was shown to increase the release of lysosomal enzymes (Fong et al, 1973). NADPH dependent microsomal lipid peroxidation in the presence of ADP/Fe + was shown to produce a factor, probably hydroxyl radical, which promotes lysis of lysosomes. These in vitro studies indicate that agents which promote lipid peroxidation will give rise to a release of lysosomal enzymes, and agents which inhibit lipid peroxidation can prevent the release of lysosomal enzymes (Younes and Seigers, 1984)

1.8 The Role of Lysosomes in Inflammation

It has been shown that lysosomal enzymes are selectively released when PMNL 's or macrophages phagocytose particles such as immune complexes, micro-organisms or zymosan fragments (Wright and Malwista, 1973; Weissmann et al, 1971)

When a polymorphonuclear leucocyte encounters a suitable ingestible particle, the surface membrane of the cell invaginates and surrounds it. The vesicle containing the particle, now called a phagosome or phagocytic vesicle, pinches off from the surface and is introduced into the cytoplasm of the cell (Zucker_Franklin and Hirch, 1964). Digestion of the engulfed particle is intiated by the fusion of the lysosomal membrane to form a phagolysosome. The lysosomessubsequently discharge their contents into the phagosome, a process called degranulation (Hirsch and Cohen, 1960)

It has been suggested that the lysosomal enzymes are not entirely kept to the phagolysosome but some are discharged into the surrounding tissue. This might happen if there is premature merging of a lysosome with a phagosome before the phagosome is entirely sealed off. This is termed 'regurgitation during feeding'. There is thus a route out of the cell leading to a loss of enzymes (Weissmann et al, 1972). Such a process has been observed by electron microscopy (Zucker_Franklin and Hirsch, 1964). Another mechanism which has been proposed considers that the membranes of the phagolysosomes might also merge with the outer cell membranes of during the process of digestion thus releasing the lysosomal constituents. This is termed 'reverse endocytosis' (Weissmann et al, 1972) or'frustrated phagocytosis' (Becker and Henson, 1973).

The large quantities of destructive enzymes present in PMNL's lysosome include at least 20 different substances such as acid phosphatase, lipase, β_glucosaminidase, sulphatases, esterases and proteinases (Weissmann, 1974). These enable the PMNL's to destroy a wide variety of foreign matter. Evidence that lysosomal enzymes are probably important mediators of both tissue destruction and inflammation was presented by Weissmann et al, (1969). They found that they were able to induce chronic inflammation and cartilage degradation by injecting lysates of purified lysosomes into the joints of experimental animals. With respect to the tissue destruction observsed in inflammatory reactions, the most important lysosomal enzymes are the proteinases (Weissmann, 1977). These proteinases such as collagenase and elastase are capable of degrading two major components of extra-cellular matrix : collagen and proteoglycan. These proteinases may also contribute to inflammatory reactions by generating chemotactic factors from complement C_5 (Ward and Hill, 1970) and releasing kinin like materials from kininogens (Movat et al, 1973).

Phospholipase A_2 released by lysosomes can interact with cell membrane phospholipids with a resultant increase in arachidonic acid (Andersson et al , 1971). This leads to an increase in the production of pro-inflammatory arachidonic acid metabolites, for example, prostaglandins and leukotrienes.

Polymorphonuclear leucocyte lysosomes also contain a cationic protein capable of increasing vascular permeability as a result of mast cell disruption (Janoff et al, 1965).

Larger quantities of lysosomal enzymes are found in rheumatoid synovial fluid compared with normal synovial fluid and other inflammed tissues (Andersson, 1970; Coppi and Borardi, 1968).

1.9 Possible Cooperative Role of Lysosomal Enzymes and Oxygen Derived Free Radicals

PMNL's migrating into an inflammatory site have the potential to mediate tissue injury by either generating cyto-toxic oxygen metabolites or releasing lysosomal proteases (Weiss, 1983; Travis et al, 1980). Defence mechanisms designed to control the steady state concentration of released oxygen metabolites are primarily confined to intracellular sites, while protease activities are regulated by a complex system of extracellular anti-protease (Weiss, 1983; Travis et al, 1980). Thus anti-proteases localized at an inflammatory site are exposed to a relatively uncontrolled oxidizing enviroment into which the target proteases are released. The importance of phagocyte derived oxidants in modifying the protease anti-inflammatory system have been demonstrated by the following studies. Carp and Janoff, (1979) showed that α-1-protease inhibitor (α-1-PI) was partially inactivated by exposure to phagocytosing PMNL's and the involvement of oxygen derived radicals and possibly myeloperoxidase (MPO) was suggested by the inhibition of α-1-PI inactivation, in addition of certain scavenging agents and inhibitors. Clark et al, (1981) have shown that α-1-PI is very sensitive to oxidative inactivation by secretory products of human PMNL's. Under the experimental conditions employed α-1-PI was nearly completely inactivated by exposure to either the isolated MPO-H_2O_2-halide system usung purified components or to activated human PMNL's. The involvement of α-1-PI could be due to the formation of

long lived oxidants e.g. N_chloramines or the generation of hypohalous acids (Weiss et al, 1983). Weiss and Ragiani, (1984) have demonstrated that PMNL's can negate the protective effects of the α-1-PI in order to attack a subendothelial matrix via the generation of an oxidant with characteristics similar to HOCl and the release of elastase. The triggered neutrophils can use both oxygen metabolites and proteases in a co-operative fashion to mediate tissue injury in certain pathological conditions.

Amruso and Johonston, (1981) have shown that lactoferrin (an iron binding protein present in specific granules of PMNL's) can increase hydroxyl radical production presumably by its ability to provide iron as a catalyst for the production of hydrogen peroxide by superoxide. Varcelloti et al, (1985) have reported that superoxide and hydrogen peroxide are not sufficient to mediate erythrocyte lysis, but requires lactoferrin which generates hydroxyl radical which, in turn induced lysis of erythrocytes.

Therefore the lysosomal components can potentially contribute to the generation of oxygen derived free radicals.

1.10 Prostaglandins

Lewis (1983) has reviewed the role of prostaglandins in inflammation.

1.10.1 Sources of Prostaglandins in Inflammation

After the initial observation that PGE is released in leucocyte culture during mitogen and antigem stimulation (Ferraris and De_Rubertis, 1974) many investigations have been initiated to identify the most prominent PGE producing leucocytes. It appears that although all the major immune-inflammatory cell types, except lymphocytes, are capable of generating PGEs after an appropriate stimulus. In general

the macrophages seem to be the major source. Experiments with mixed lymphocyte cultures between allograft donor and recipient cells, suggested that PGE producing cells belong to the macrophage system (Dy et al, 1980). Human macrophages produce mainly PGE_2 and thromboxane A_2 as a result of stimulation by various materials such as phagocytic stimulants (e.g. zymosan, antigen_ antibody complexes), mitogens (e.g. phytohaemagglutinin, phorbol myristate acetate and Con A), lymphokines and bacterial endotoxins e.g. lipopolysaccharides. In a comparison of the activity of neutrophils and macrophages ($5x10^6$) stimulated with zymosan particles the cells were found to produce 1 ng, 1.5 ng and 5 ng PGE_2 respectively whereas lymphocytes produced little or no PGE when exposed to several stimuli (Gemsa et al, 1981). A few reported findings of PGE production by lymphocytes, may have been caused by monocyte contamination. The production of PGEsby neutrophils is relatively short lived which might indicate a some what different function e.g. vasodilation, to that of the PGE release from macrophages which is sustained over a much longer period.

Humes et al, (1977) showed that not only PGE_2but also prostacyclin is released from cultures of macrophages when exposed to stimulatory agents such as zymosan. In the presence of 200mg/ml zymosan, there was a 24 fold increase of PGE and a 15 fold increase in 6_oxo_PGE_1 (the stable breakdown product of prostacyclin) release. The phagocytosis of particles by macrophages is not sufficient to activate the AA cascade. Certain surface components of the phagocytosing particles play an importane role. Gemsa et al, (1981) showed although that latex particles are avidly phagocytosed they failed to induce PGE synthesis. In contrast in the presence of E. Coli, the latex particles induced some PGE synthesis and if the bacteria were coated with IgG the rate

of synthesis increased. An even greater enhancement was achieved on the addition of complement. They also found that in the case of IgG it appears to be the Fc portion which is the activating factor and among the complement components it appears to be C_{3b} which was involved in potentiating PGE release. They also have found indications that a C_5 related leucotactic factor may suppress PGE generation. Stimler et al, (1982) have shown that leukotrienes are released from guinea pig lung by C_{5a}. Therefore there could well be several links between complement system factors and the production of AA metabolites, which will develop on further investigation. An important role in PGE release has also been suggested for the microtubules of the cytoskeleton. Using colchicine and vinblastin, Gemsa et al, (1981) found that disaggregation of microtubules initiated along lasting and high PGE release from macrophages. This might be a factor involved in the anti-arthritic activity of colchicine.

1.10.2 Prostaglandins in Acute Inflammation

Until 1970, the evidence for the role of the prostaglandins in the development of inflammatory response was indirect and circumstantial. It consisted mainly of the detection of PG like sub stances in various inflammatory states and on this basis the suggestion was made that PGs are mediators of certain phases of inflammation (Willoughby and DiRosa, 1971). However although PGEs were known to be potent vasodilator agents and there were some reports of some PGs possessing chemotactic activity there was little reference to PGs playing a major role in inflammation, this was in part due to that it was generally accepted that they did not produce two important cardinal signs of inflammation e.g. increased vascular permeability and pain. Two major findings established a role of PGs in acute inflammation. Non

steroidal anti_inflammatory agents inhibited the formation of PGs from arachidonic acid by inhibiting cyclo-oxygenase. Many publications have confirmed these findings, indicating that non steroidal anti-inflammatory drugs reduce the vasodilation, oedema and pain of inflammation by inhibiting PG synthesis. The problem remained as to how inhibitors of PG synthesis could reduce oedema and pain while PGs themselves did not directly produce effects. The answer came with the finding by Ferriera (1972) in respect of pain and Williams & Morely, (1973) and Moncada et al, (1973), in respect of plasma exudation. It was shown that, although they did not act directly the PGs enhanced enormously the oedema and pain producing effects of other mediators such as histamine, bradykinin and complement components C_{5a}. However, there were also reports of an anti-inflammatory or anti-arthritic action for certain PGs. PGEs have been reported to inhibit pannus formation in adjuvant arthritic rats (Zurier and Quagliata, 1971) and PGE_2 to inhibit cotton pellet granulomas in rats (Bonta and Parnham, 1978). In actue models of inflammation, on the other hand, anti-inflammatory activity was reported only for PGE_2. Thus the view emerged in the early 1970s of being involved in the development of inflammation on the one hand, whilst on the other they might act as anti-inflammatory agents depending on the type of PG involved, the site of formation and release and the concentration of a given PG.

1.10.3 Prostaglandins in Chronic Inflammation

PGE_2 and PGE_2 have been reported in 8:72 h exudates of carrageenan induced granuloma. Later at 8 days in addition to a PG like material, thromboxane B_2, the stable metabolite of thromboxane A_2 was also detected (Chang et al, 1977). In kaolin induced granuloma PGE like activity reaches a maximum at 6 h, high levels being maintained for up

to 96 h (Lewis et al, 1976). Dumonde and Glynn (1962) developed a model of monoarticular arthritis which resembles rheumatoid arthritis by injecting antigen into the knee joint of sensitized rabbits, levels of PGE in the synovium of the affected joint reached a peak after 19 h, declining to low levels again during the chronic phase for up 46 days (Blackham et al, 1974). These authors found that, while indomethacin inhibited the early increase in PGE levels, there was only a moderate decrease in joint swelling . This and several other studies show that there is no simple correlation between chronic inflammation and PG levels. In surgically induced chronic arthritis in rabbits, the concentration of PGE in the synovial fluid plus cartilage of the operated joint was the same as that of the non-operated joint (Swinson et al, 1976). However, Parnham et al, (1978) have shown by a perfusion technique in rat paws that during the development of polyarthritic lesions of adjuvant arthritis the PGE levels in the perfusate of the hind paws of arthritic rats have paralleled the paw volume. Furthermore they showed that, between 18 and 22 days when the PGE levels were highest, there were also increased cyclic AMP levels in the paws. In in vitro investigations, it has been easier to show correlations between activated cells and PG production. Floman and Zor, (1976) showed that, synovial tissue taken from rats with monoarthritis induced by injection of cell free streptococcus extract, released 5-8 times as much PGE as that released from synovial tissue from non arthritic rats. Furthermore Robinson et al, (1975) showed an increased production of PGE in cultured RA cells and this was associated with increased cyclic AMP levels. Several studies have shown the presence of PGs in the synovial fluid from the joints of arthritic patients (Bonta and Parnham, 1978). PGE_2 appears to be most prominent, although PGE_1, $PGF_2\alpha$ and low concentrations of thromboxane B_2 have been reported.

1.11 Aims of Research

In a search for endogenous compounds with anti-inflammatory activity it was found that putrescine, spermidine and spermine accumulated in inflammatory exudates (Bird and Lewis, 1981). Putrescine and spermidine were found to be anti-inflammatory when tested against carrageenan induced inflammation in rat and putrescine was also anti-inflammatory against adjuvant induced arthritis in rat (Bird et al, 1983). The mode of action of oligoamines is unknown. Superoxide is produced from stimulated PMNLs and other phagocytes during inflammation. Superoxide induces the formation of other active oxygen species and is itself a powerful chemotactic agent (Petrone et al, 1980). Since oligoamines accumulate in inflammatory exudates it is possible that these substances may modulate superoxide action by scavenging this anion. The present study was designed to establish whether oligoamines have a scavenging action on superoxide produced both by the hypoxanthine/xanthine oxidase reaction and by stimulated PMNLs. Since it is possible that PMNLs may modify oligoamines, the pattern of metabolites produced when oligoamines were incubated with stimulated PMNLs were investigated, and compared with those produced when oligoamines incubated with the hypoxanthine/xanthine oxidase reaction

Lysosomes, lysosomal enzymes and superoxide have all been implicated in the pathogenesis of various inflammatory diseases e.g rheumatoid arthritis, so the effect of superoxide anion and oligoamines on the stability of isolated intact guinea pig liver lysosomes were studied.

The relevance of oligoamines to the hypoxanthine/xanthine oxidase induced lipid peroxidation in guinea pig liver lysosomes was investigated.

Stimulated PMNLs release prostaglandins which have an essential role in inflammation. The effect of oligoamines on the release of PGE_2 from stimulated PMNLs was also studied.

Cyclic AMP has been shown to suppress carrageenan induced oedema in the hind paw of rats (Ichikawa et al, 1972). Theophylline inhibited the development of adjuvant arthritis in rats (Mohd_Hidir, 1985). PGE has been demonstrated to inhibit cotton pellet granulomas in rats (Bonta and Parnham, 1978). So the effects of cyclic AMP, theophylline and PGE_2 on superoxide production from stmiulated PMNL's were also investigated.

2. MATERIALS and METHODS

2.1 The Effect of Oligoamines and EDTA on the Reduction of Cytochrome C Induced by Hypoxanthine/Xanthine Oxidase Superoxide Generating System.

Oligoamines, putrescine, spermidine, spermine, cadaverine, diaminopropane, and EDTA (hydrochloride salts) (Sigma Chemical Co Poole, Dorset) were tested for their effects on the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase superoxide generating system. The method of McCord and Fridovich, (1969) was used.

A stock solution of phosphate buffer (0.1 M pH 7.4) containing 0.5 mM hypoxanthine (Sigma Chemical Co. Poole, Dorset) and 50 n moles cytochrome_C (Horse Heart type III, Sigma Chemical Co.) was prepared. The reaction mixture consisted of 3 ml of the stock solution containing the following concentrations of oligoamines or EDTA 20 mM, 40 mM, 80 mM, 100 mM, and 200 mM in plastic cuvettes. The reaction was initiated by the addition of 0.8 units (25 µl) xanthine oxidase (grade III from butter milk, chromatographically purified, Sigma Chemical Co.) to all cuvettes. Changes in absorbance at 550 nm at 37°C every 30 seconds for 10 min in the absence and presence of the above concentrations of oligoamines or EDTA were recorded with an SP 500 spectrophotometer (PYE Unicam LTD.). The blank was the same as the samples except for the omission of xanthine oxidase and its replacement by phosphate buffer (0.1 M, pH 7.4) (25 µl). Superoxide dismutase (Sigma Chemical Co.) was tested for its effect on the reduction of cytochrome_C by the hypoxanthine/xanthine oxidase superoxide

generating system as a positive control at the following doses 175, 350, 700, 1400, and 2800 units.

One unit of SOD inhibits the reduction of cytochrome_C by 50% per minute at pH 7.8 at 25°C (McCord and Fridovich, 1969).

2.2 The Effect of Oligoamines and EDTA on the Reduction of Nitro blue terazolium (NBT) Induced by the Hypoxanthine/Xanthine Oxidase Superoxide Generating System

The oligoamines and EDTA mentioned in section 2.1 were tested for their effects on the reduction of NBT by the hypoxanthine/xanthine oxidase system. The method of Beauchamp and Fridovich, (1971) was employed.

A stock solution of phosphate buffer (0.1 M, pH 7.4) containing 0.5 mM hypoxanthine and 1.2×10^{-4} M NBT (Sigma Chemical Co.) were prepared. The reaction mixture consisted of 3 ml of the stock solution containing the follwoing concentrations of oligoamines or EDT 20 mM, 40 mM, 80 mM, 100 mM, and 200 mM in plastic cuvettes. The reaction was intiated by the addition of 0.8 units of xanthine oxidase (25 µl) (grade III from butter milk, chromatographically purified, Sigma Chemical Co.) to all cuvettes. Changes at 560 nm, at 37°C every 30 seconds for 10 min either in the absence or presence of the above concentrations of oligoamines or EDTA were recorded with an SP 500 spectrophotometer (PYE Unicam LTD.). The blank was the same as the sample except for the omission of xanthine oxidase and its replacement by (25µl) phosphate buffer (0.1 M, pH 7.4). SOD was tried for its effect on the reduction of NBT induced by the same superoxide generating system as a positive control at the same doses mentioned in section 2.1

2.3 The Effect of Oligoamines and EDTA on Urate formation from the Hypoxanthine/Xanthine Oxidase Reaction

The oligoamines and EDTA studied in section (2.1, 2.2) were tried for their effects on urate formation from the hypoxanthine/xanthine oxidase system. A stock solution of phosphate buffer (0.1 M, pH 7.4) containing 0.5 mM hypoxanthine was prepared. The reaction mixture consisted of 3 ml of the stock solution containing the following concentrations of oligoamines or EDTA 0.1 mM, 1 mM, 100 mM, and 200 mM in quartz cuvettes. The reaction was initiatd by the addition of 0.8 units (25µl) of xanthine oxidase to all cuvettes. Changes in the absorbance at 290 nm (peak absorbance of uric acid) at 37° C were recorded every 30 seconds for 10 min in the absence and presence of the above concentrations of oligoamnes or EDTA. The blank was the same as the samples except for the omission of xanthine oxidase and its replacement by phosphate buffer (25µl) (0.1 M, pH 7.4). SOD at the same doses described in sections (2.1, 2.2) were tried for their effects on urate formation from hypoxanthine/xanthine oxidase system as a positive control.

2.4 The Effect of Oligoamines and EDTA on the Reduction of Cytochrome C Induced by PMA Stimulated Peritoneal Guinea Pig PMNL's

2.4.1 Preparation of Peritoneal Guinea Pig PMNL's

Peritonitis was induced by injection of 20 ml of 1% (w/v) aqueous oyster glycogen (Sigma Chemical Co.) into the peritoneal cavity of male Dunkin Hartley Strain guinea pig (400_500g body weight). Exudates were recovered from the peritoneal cavity 4 h later after injection by lavage with 60 ml of cold (4° C) Hanks Balanced Salt Solution (HBSS) containing 10u ml^{-} of preservative free heparin (Sigma Chemical Co).

The HBSS was sterilized by filtration through a millipore filter (0.22 μm pore size) prior to use. The leucocyte rich fluid was harvested using a pasteur pipette and collected in glass test tubes (100 mm x 16 mm) kept at 4° C. The cells from the lavage fluids were recovered by centrifugation at (200 g for 10 min at room temperature) pooled and washed 3 times in ice cold HBSS, then viability, total and differential count were performed. Finally the cells were resuspended in EMEM media (Flow Labs, Ayrshire, Scotland), supplemented with 2 mM glutamine (Sigma Chemical Co.) and 1% (v/v) heat inactivated foetal calf serum (Flow Labs, Ayrshire, Scotland) and diluted to the required concentration prior to use.

Halothane (ICI Pharmaceutical Division, Alderley, Macclesfield, Cheshire, U.K.) anaethesia was induced in the experimental animals using Boyle's apparatus prior to injecting glycogen and the lavage fluid. Flow of 200 L min and 100 L min were used for oxygen and nitrous oxide respectively. Animals were killed by an over dose of halothane before making an incision along the ventral line to facilitate leucocyte collection from lavage fluid collected by a pasteur pipette.

2.4.2 Cell Viability, Total and Differential Count

Cell Viability : was estimated by trypan blue (1% w/v in distilled water) exclusion.

Total Leucocyte Count : was estimated using the 'improved Neubauer' haemocytometer.

Differential Count : A smear of cell suspension was made and air dried.

The cells were fixed in methanol and stained with Wright's stain (BDH)

The cells were examined under an oil immersion lens at a magnification of x 1000 and approximately 500 cells were counted in 4 or 5 random fields.

2.4.3 Phorbol Myristate Acetate (PMA) (Sigma Chemical Co. LTD Poole, UK)

PMA was dissolved in dimethylsulphoxide (DMSO) at a concentration of 10^{-2} M. Aliquots of this (50 µl) were stored at _20°C in sterile Eppendorf tubes (Anderman Co., LTD, Central avenue, East Molsey, Surrey, U.K.). PMA was diluted prior to use with EMEM media to the required concentration.

The method of McCord and Fridovich, (1969) was employed for investigating the effect of oligoamines and EDTA on the reduction of cytochrome_C induced by PMA stimulated PMNL's. 1 ml of EMEM media containing 2 mM glutamine and 1% (v/v) heat inactivated foetal calf serum 50 n moles cytochrome_C, 10^{-8} M PMA and 10 µg SOD in a plastic cuvette was placed in the reference compartment, 1ml of EMEM media containing 2 mM glutamine, 1% (v/v) heat inactivated foetal calf serum, 50 n moles cytochrome_C, 10^{-8} M PMA and the following concentrations of oligoamines or EDTA, 20 mM, 40 mM, 80 mM, 100 mM, and 200 mM in plastic cuvettes were placed in sample compartment. The reaction was initiated by the addition of 5 x 10^5 cells to each cuvettes and the absorbance change at 550 nm, in the absence or presence of the above concentrations of oligoamines and EDTA, was recorded with an SP 500 spectrophotometer (PYE Unicam LTD.).

SOD was tried for its effect on the reduction of cytochrome_C induced by PMA stimulated PMNL's at the same doses described in section (2.1).

2.5 The Effect of Oligoamines and EDTA on Luminol Dependent PMA Stimulated Peritoneal Guinea Pug PMNL's Chemiluminescence

The oligoamines and EDTA studied in section (2.1) were tried for their effects on luminol dependent PMA stimulated PMNL's chemiluminescence. Peritoneal guinea pig PMNL's were isolated as described in section (2.4.1).

2.5.1 Luminol (Sigma Chemical Co.)

Luminol was dissolved in DMSO at a concentration of 2×10^{-2} M and stored at 4° C as a stock solution and was diluted to the required concentration with EMEM media prior to use.

PMA preparation and storage was as described in section (2.4.3)

2.5.2 Measurement of Chemiluminescence

Chemiluminescence was measured in a photometer (Luminometer 1250 LKB, Wallac) the reaction mixture consisted of 2×10^{-4} M luminol, 10^{-8} M PMA and the oligoamines and EDTA described in section (2.1) at the following concentrations 1 mM, 5 mM, 10 mM, 50 mM, 100 mM, and 200 mM in 0.9 ml EMEM media supplemented with 2 mM glutamine and 1% (v/v) heat inactivated foetal calf serum in polystyrene vials. 5×10^{5} Cells were added to the vials and the resulting chemiluminescence in mV was continuously recorded at 37° C, on a potentiometric chart recorder for 30 min in the absence or presence of the above concentrations of oligoamines and EDTA. Control vials were set up to investigate the effect of EMEM media (supplemented with 2 mM glutamine and 1% (v/v) foetal calf serum) alone on the chemiluminescence of PMA stimulated PMNL's. SOD was tested for its effect on luminol dependent PMA stimulated PMNL's chemiluminescence at the doses reported in section (2.1)

2.6 The Effect of Oligoamines and EDTA on the Aerobic photo reduction of NBT

The method of Beauchamp and Fridivich, (1971) was used. The reaction mixture consisted of 5×10^{-5} M NBT (Sigma Chemical Co.) , 1×10^{-2} M methionine (Sigma Chemical CO.), 2×10^{-5}M sodium cyanide, 1.17×10^{-6}M riboflavin (Sigma Chemical Co.) and the oligoamines or EDTA at the following concentrations 10 mM, 20 mM, 40 mM, 80 mM, 100 mM, and 200 mM in 3 ml of 0.05 M potassium phosphate buffer pH 7.8 in plastic cuvettes. Control cuvettes were the same except for the omission of oligoamines and EDTA. The cuvettes were then incubated at 30°C and illuminated over 6 min by a Shandon_Southern Photopol Lamp. Reduction of NBT was measured in terms of increased absorbance at 560 nm using the SP 500 spectrophotometer.

2.7 The Effect of Copper Sulphate on the Reduction of Cytochrome C Induced by the Hypoxanthine/Xanthine Oxidase System

The effects of the following concentrations of copper sulphate (BDH) 10^{-10}M, 10^{-8} M, 10^{-6} M, on the reduction of cytochrome_C by the hypoxanthine/xanthine oxidase system were investigated in the same way as described in section (2.1)

2.8 The Effect of Oligoamine Copper Sulphate Complexes on the Reduction of Cytochrome C by the Hypoxanthine/Xanthine Oxidase System

The following concentrations of putrescine, spermidine, spermine, cadaverine, and diaminopropane, 1 mM, 10 mM, 20 mM, 40 mM, mixed with 10^{-8} M copper sulphate were tried for their effects on the reduction of cytochrome_C by the hypoxanthine/xanthine oxidase system in the same way as described in section (2.1).

2.9 The Effect of Reactive Oxygen Species on the Stability of Isolated Intact Guinea Pig Liver Lysosomes

2.9.1 The Effect of the Hypoxanthine/Xanthine Oxidase Superoxide Generating Sytem on the Stability of Isolated Intact Lysosomes

2.9.1.1 Preparation of the Lysosomes

The method used by Symons et al, (1969) was employed. Male Dunkin Hartley Strain guinea pigs (400_500g body weight) were killed by cervical dislocations and their livers were immediately removed, weighed and placed in ice cold 0.25 M sucrose solution, the livers were then chopped with scissors and placed in 0.25 M sucrose solution in a Tri_R_ glass mortar (Tri_R_ Instruments INC) on cracked ice and homogenised by a single stroke of a teflon pestle turning at 100 rpm with a clearance of 0.15_0.25 mm. The pestle was powered by a 1/15 electric motor. This homogenising process ruptures the cells with relatively little damage to the lysosomes. The crude homogenate was adjusted to 10 per cent with cold sucrose solution (0.25 M) and then centrifuged at 600g for 10 min. This process sedimented the unbroken cells, cell debris and nuclei leaving most of the lysosomes and mitochondria in suspension and some free lysosomal enzymes. The pellets were discarded and the supernatants were centrifuged at 20,000g for 30 min at 4° C in an ultracentrifuge (MSE Model Super Speed 50). This process sedimented the lysosomes and mitochondria leaving smaller particles in suspension with free lysosomal enzymes in solution. The supernatants were discarded and the pellets were resuspended in ice cold sucrose solution (0.25 M) and then centrifuged at 4° C for 30 min at 20,000g. The supernatants were discarded and the process repeated once more and the washed pellets were finally resuspended in sucrose solution (0.25 M) containing 0.05 M tris acetate buffer (pH 7.4) at a

concentration equivalent to 1g of liver in 10 ml of buffered sucrose solution. The final concentration was used for the incubation procedure for the determination of the stability or lytic action of superoxide on the lysosomes.

Different fluxes of superoxide were generated by transferring the following doses of xanthine oxidase, 0.4, 0.8, 1.6, 2.4, 3.2 units to 50 ml stoppered conical flasks containing 5 ml of lysosomal suspension and 0.5 mM hypoxanthine. In addition the following control flasks were set up :

1_Buffered sucrose solution (pH 7.4) and lysosome suspensions.

2_Xanthine oxidase and lysosome suspensions.

3_Hypoxanthine and lysosome suspensions.

4_Uric acid and lysosome suspensions.

2.9.1.2 Incubation Procedure

The flasks were placed into a shaking reaction incubator (GallenKamp LTD.) and incubated at 37°C for 90 min. At the end of the incubation procedure the flasks were immmediately removed and cooled on cracked ice. When cold the suspensions were transferred to polyethlene tubes and spun at 20,000g at 4°C for 30 min to remove the intact lysosomes and lysosomal debris leaving the lysosomal enzymes in solution.

2.9.1.3 Enzyme Assay Procedure

The supenatants were examined for the presence of acid phosphatase by the method of Huggins and Talalay, (1945) and for β_N_acetylglucosaminidase by the method of Caygill and Pitkeathly (1966). Acid phosphatase activity was determined by incubating 100 µl of the supernatant from the incubation procedure with 0.5 ml of P_nitrophenyl phosphate (0.015 M) (Sigma Chemical Co.) and 0.5 ml of

citrate buffer (0.09 M)) (pH 4.8) at 37°C for 30 min. The reaction was stopped and the colour developed by the addition of 5 ml of 0.1N sodium hydroxide (Merck). The P-nitrophenol which was liberated in the reaction was determined at 410 nm spectrophotometrically. Blank contained distilled water.

β_N_acetylglucosaminidase was determined by incubating 100 µl of the supernatant from the incubation procedure with 0.5 ml of P_nitrophenyl_2_acetamide_2_deoxy_3_D_glucopyranoside (0.02 M) (Sigma Chemical Co.) and 0.5 ml of citrate buffer (0.09 M) (pH 4.8) for 30 min. The reaction was topped and the colour developed by the addition of 5 ml of 0.1 N sodium hydroxide. The P-nitrophenol liberated was determined at 410 nm spectrophotometrically. The reaction tubes were prepared in duplicate as for acid phosphatase. The blank contained distilled water.

2.9.1.4 Standard Curve of P Nitrophenol

A standard curve for P-nitrophenol was prepared by diluting 100 mM solution of a standard P-nitrophenyl phosphate solution (Sigma Chemical Co.) 200 times with 0.02 N sodium hydroxide. The following solutions were prepared in duplicate. The blank contained 0.02 N sodium hydroxide.

P-nitrophenol standard solution	(ml)	1	2	4	6	8	10
0.02 N sodium hydroxide	(ml)	10	9	7	5	3	1
Concentration	(ug/ml)	0.13	1.26	2.15	3.38	5.09	6.3

2.9.2 The Effect of Hydrogen Peroxide on the Stability of Isolated Intact Guinea Pig Liver Lysosomes

The following concentrations of hydrogen peroxide (BDH) 50 µM, 100 µM,

150 μM, 200 μM and 250 μM were tried for their effects on the stability of isolated intact lysosomes in the same way as described in section (2.9.1).

2.9.3 The Effect of Hydroxyl Radical on the Stability of Isolated Intact Guinea Pig Liver Lysosomes

Different increasing fluxes of hydroxyl radical were generated by the reaction of increasing concentrations of hydrogen peroxide with increasing concentrations of ferrous sulphate. The reaction of the following concentrations of hydrogen peroxide 50 μM, 100 μM, 150 μM, 200 μM and 250 μM with the following concentrations of ferrous sulphate 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} M respectively were tried for their effects on the stability of isolated intact lysosomes in the same way as described in section (2.9.1).

2.9.4 The Effect of Ferrous Sulphate on the Stability of Isolated Intact Guinea Pig Liver Lysosomes

The following concentrations of ferrous sulphate 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M were tried for their effects on the stability of isolated intact lysosomes in the same way as described in section (2.9.1).

2.10 The Effect of Oligoamines and EDTA on the Breakdown of Isolated Intact Guinea Pig Liver Lysosomes by Products of the Hypoxanthine/ Xanthine Oxidase System

The reaction mixture consisted of 5 ml of lysosome enriched suspensions (prepared as in section 2.9.1.1) in 0.25 M sucrose solution containing 0.05 M tris acetate buffer (pH 7.4), 0.5 mM hypoxanthine, putrescine, spermidine, spermine, cadaverine, diaminopropane or EDTA at the following concentrations 0.1 mM, 1 mM, 10 mM, 20 mM, 40 mM, 60 mM, 80 mM. 100 mM, and 200 mM. Control flasks

as decribed in section (2.9.1.1) were set up. The incubation procedure, acid phosphatase, β-glucosaminidase assays were the same as in sections (2.9.1.2, 2.9.1.3).

2.11 The Effect of Oligoamines and EDTA on the Stability of Isolated Intact Guinea Pig Liver Lysosomes

The following concentrations of the oligoamines studied and EDTA 0.1 mM, 1 mM, 10 mM, and 100 mM were tried for their effects on the stability of isolated intact lysosomes in the same way as described in section (2.9.1).

2.12 The Uptake of Radioactive Oligoamines by Isolated Intact Guinea Pig Liver Lysosomes

Materials :

(1,2-^{14}C) Putrescine dihydrochloride (specific activity 118 mCi/m mol, Amersham International)

(^{14}C) Spermidine trihydrochloride (specific activity 118 Ci/m mol, Amersham International)

(^{14}C) Spermine tetrahydrochloride (specific activity 118 Ci/m mol, Amersham International)

5, 10, 20, n mole of radiactive putrescine, spermidine and spermine were transferred to test tubes each containing 5 ml of lysosome enriched suspensions (prepared as in section 2.9.1.1). The tubes were then incubated for 1 h at 37°C. The lysosome suspensions were then centrifuged at 20,000 g for 30 min and then resuspended in 5 ml of 0.25 M sucrose solution containing 0.05 M tris acetate buffer (pH 7.4). After centrifugation to recover the lysosomes the washing procedure was repeated twice. Finally the lysosomes were lysed by the addition of 0.1 ml of 1% (v/v) Triton X100 (Koch-Light Laboratories,

Bucks, England) and the lysate transferred to scintillation vials containing 5 ml of scintillation fluid (NCS : OC5; 4:1 v/v Amersham International). The amount of radioactivity was determined by a Beckman Liquid scintillation Ls_230 counter.

2.13 The Effect of PMA Stimulated Peritoneal Guinea Pig PMNL's on the Stability of Isolated Intact Guinea Pig Liver Lysosomes

Peritoneal guinea pig PMNL's were prepared as described in section (2.4.1).

Isolated intact guinea pig liver lysosomes were prepared as described in section (2.9.1.1).

PMA preparation and storage as in section (2.4.3)

2.13.1 Cytochalasin B (Sigma Chemical Co)

1 mg of Cytochalasin_B was dissolved in 1 ml DMSO and aliquots of this were stored at _20^{0}C. Portions of the stock solution were diluted with EMEM media to the required concentration prior to use. PMNL's were incubated with cytochalasin_B (5 µg ml) for 10 min prior to use. Lysosome enriched suspensions (5 ml) were transferred to 50 ml stoppered conical flasks containing 5 x 10^{5}, 1 x 10^{6}, 2 x 10^{6}, 4 x 10^{6}, and 8 x 10^{6} PMNL's.

The reaction was initiated by the addition of 10^{-8} M PMA to all flasks. Control flasks to investigate the effect of EMEM media, PMA, and cytchalasin_B, were also set up. The incubation procedure, acid phosphatase, assays were the same as in section (2.9.1.2, 2.9.1.3).

2.14 The Effect of Oligoamines and EDTA on the Breakdown of Isolated Intact Liver Lysosomes by PMA Stinulated Peritoneal Guinea Pig PMNL's

Peritoneal guinea pig PMNL's were prepared as described in section (2.4.1) and incubated with cytochalasin_B (5μg ml) for 10 min prior to use. Isolated intact guinea pig liver lysosomes were prepared as described in section (2.9.1.1). The reaction mixture consisted of 5×10^5 PMNL's (which has been incubated with cytochalasin_B for 10 min), 5 ml of lysosome enriched suspensions, oligoamines or EDTA at the following concentrations, 1 mM, 10 mM, 20 mM, 40 mM, 60 mM, 80 mM, 100 mM, and 200 mM, in 50 ml stoppered conical flasks. The reaction was initiated by the addition of 10^{-8} M PMA to all flasks. Control flasks were the same as in section (2.13). The incubation procedure and acid phosphatase assay were the same as in sections (2.9.1.2, 2.9.1.3).

2.15.1 Chromatographic Studies of the Products of the Interaction of Oligoamines with PMA Stimulated Peritoneal Guinea Pig PMNL's

2.15.1.1 Chromatographic Studies of the Products of Interaction of (^{14}C) Putrescine with Stimulated PMNL's

Peritoneal guinea pig PMNL's were prepared as described in section (2.4.1). The reaction mixture consisted of 5×10^5 cells, 70 μl (^{14}C) putrescine, and 10^{-8} M PMA in 2 ml EMEM media (pH7.4) in glass test tubes, the tubes were incubated for 30 min at 37° C. The cells were lysed by freezing and thawing and centrifuged at 10,000g for 30 min at 4° C. Aliquots of the supernatant (50μl) were spotted on to (20 x 5cm) TLC plates (Merck) and then extracted with the following systems :

1-Propanol-water-concentrated HCl 60 20 20 v/v

Butanol-water-glacial acetic acid	77	17	6		v/v
1-Propanol-water-triethylamine	85	15	3		v/v
Butanol-water-glacial acetic acid-pyridine	40	20	10	10	v/v

All chemicals of the solvent systems were supplied by (BDH Chemicals) The plates were dried with hair dryer and then scanned for radioactivity in a windowless gas flow Tracer Lab radioactive chromatogram scanner (Tracer Lab) with 1000 cpm, chart speed 2 inch/h and slit width 0.050 units. Two standards were also spotted on to the plates (^{14}C) putrescine, and (^{14}C) γ_aminobutyric acid. After scanning, the plates were sprayed with Schiff's reagent to detect aldehydes and with 2% (w/v) ninhydrin in acetone (BDH Chemicals) to detect oligoamines.

2.15.1.2 Chromatographic Studies of the Products of Enzymatic (diamine oxidase) Oxidation of (^{14}C) Putrescine

This experiment was done to compare the products of the interaction of putrescine and stimulated PMNL's with those of the enzymatic oxidation (diamine oxidase) products of putrescine. The reaction mixture was the same as described in section (2.15.1.1), but diamine oxidase (Sigma Chemical Co.) (2 units) was substituted for PMNL's and PMA. Chromatographic studies were the same as described in section (2.15.1.1)

2.15.1.3 Chromatographic Studies of the Products of Interaction of (^{14}C) Spermidine with Stimulated PMNL's

Peritoneal guinea pig PMNL's were prepared as described in section (2.4.2). The reaction mixture consisted of 5 x 10^5 cells and 70 µl (^{14}C) spermidine and 10^{-8}M PMA in 2 ml EMEM media (pH 7.4)in glass test

tubes for 30 min at 37°C. The cells were lysed by freezing and thawing and centrifuged at 10,000g for 30 min. Aliquots of the supernatants (50µl) were spotted on to 20 x 5 cm TLC silica plates. The extraction, development and radioscanning of the TLC plates were the same as in section (2.15.1.1). Standards (^{14}C) spermidine was also spotted on to the plates.

2.15.1.4 Chromatographic Studies of the Products of the Interaction of (^{14}C) Spermine with Stimulated PMNL's

This experiment was done in the same way as for (^{14}C) spermidine. Standard (^{14}C) spermine was also spotted on to the plates.

2.15.1.5 Chromatographic Studies of the Products of Interaction of Cadaverine with Stimulated PMNL's

The reaction mixture consisted of 5 x 10^{5} cells, 1 µM cadaverine (Sigma Chemical Co.) and 10^{-8}M PMA in 2 ml EMEM media (pH 7.4) in glass test tubes. The tubes were incubated for 30 min at 37°C. The cells were lysed by freezing and thawing and centrifuged at 10,000g. Aliquots (50 µl) of the supernatants were spotted on to (20 x 5 cm) TLC silica plates which were developed as in section (2.15.1.1). Standard cadaverine was spotted on to the plates.

2.15.1.6 Chromatographic Studies of the Enzymatic (diamine oxidase) Oxidation Products of Cadaverine

This experiment was done to compare the enzymatic oxidation products of cadaverine with the products of interaction of cadaverine with stimulated PMNL's. The reaction mixture was the same as described in section (2.15.1.5), except that diamine oxidase (5 units) was substituted for PMNL's and PMA. The extraction and the development of the TLC plates were as in section (2.15..1.1).

2.15.2 Chromatographic Studies of the Products of the Interaction of Oligoamines with the Products of the Hypoxanthine/Xanthine Oxidase System

These experiments were done to investigate the products of the interaction of putrescine, spermidine, spermine, and cadaverine with the oxy_products of the hypoxanthine/xanthine oxidase system. These experiments were done as described in section (2.15.1) using the same concentrations of oligoamines apart from the omission of PMNL's and PMA and their replacement with hypoxanthine (0.5 mM) and xanthine oxidase (0.8 units) as the source of the oxy_products.

2.16 Qualitative Determination of the Aldehyde Metabolites

The method of Friedmann and Haugen (1943) was used. 1 ml of the incubation mixtures described in sections (2.15.1, 2.15.2) was transferred to glass test tubes containing 0.5ml dinitropheny-lhydrazine (BDH Chemicals) in 6 N Hcl and then incubated for 10 min at 37°C. Then 2ml of 3 N sodium hydroxide was added to all tubes, a red colour was formed indicating the presence of an aldehyde compound.

2.17 The Effe ct of Oligoamines and EDTA on the Activities of Lysosomal Enzymes

2.17.1 Preparation of Lysosomal Enzymes

Lysosome enriched suspensions were prepared as in section (2.9.1.1) 0.1% (v/v) Triton X100 was added to lysosome enriched suspensions (5 ml) to rupture the lysosomes and release the enzymes. Each suspension was centrifuged at 20,000g for 30 min to remove intact lysosomes and lysosomal debris leaving the lysosomal enzyme in the supernatant.The following concentrations of the oligoamines studied or

EDTA 0.1 mM, 1 mM, 10 mM, and 100 mM, were tried for their effects on the activities of acid phosphatase and β-glucosaminidase. The reaction mixture consisted of 5 ml of the lysosomal enzyme supernatants in 50 ml stoppered conical flasks. Incubation procedure and enzyme asssays were the same as described in section (2.9.1.2, 2.9.1.3).

2.18 The Effect of Oligoamines and EDTA on Lipid Peroxidation

2.18.1 The Effect of Oligoamines and EDTA on Lipid Peroxidation in Guinea Pig Liver Lysosomes Incubated in the Hypoxanthine/Xanthine Oxidase System

Lipid peroxidation was determined by the thiobarbituric acid method described by Wills (1969). The reaction mixture consisted of 0.5 ml of lysosome enriched suspensions (prepared as in section 2.9.1.1), in 0.25 M sucrose solution containing 0.05 M tris acetate buffer (pH 7.4) containing 0.5 mM hypoxanthine in glass test tubes. Putrescine, spermidine, spermine, cadaverine, diaminopropane and EDTA were added separately at the follwing concentrations 1 mM, 10 mM, 20 mM, 40 mM, 80 mM, and 100 mM. The reaction was intiated by the addition of 0.8 units xanthine oxidase. The tubes were incubated at 37°C for 10 min. The reaction mixture were then transferred to polyethylene tubes which were centrifuged at 20,000g to recover the lysosomes. The lysosomal pellets after washing with buffered sucrose solution (pH 7.4) at 4° C from which air has been displaced by nitrogen was lysed by freezing and thawing and the lysate extracted with equal volume of ether. The ether extract was evaporated to dryness and 0.5 ml of 10 % trichloracetic acid and 3.5 ml of 0.68 % w/v thiobarbituric acid solution added to the tubes containing the ether residues. The tubes were placed in a boiling water bath for 10 min and cooled in running

water and the absorbance measured at 532 nm spectrophotometrically.

The following control tubes were st up :

1_The reaction mixture without oligoamines or EDTA.

2_Hypoxanthine and lysosome enriched suspensions

3_Xanthine oxidase and lysosome enriched suspensions

4_Lysosome enriched suspensions in 0.25 M sucrose solution containing 0.05 M tris acetate (pH 7.4)

2.18.2 The Effect of Oligoamines and EDTA on Ascorbic Acid Ferrous Sulphate Induced Lipid Peroxidationin Guinea Pig Liver Lysosomes

This experiment was identical to that described in section (2.18.1) except that hypoxanthine and xanthine oxidase were replaced by ascorbic acid (300 μM) and ferrous sulphate (20 μM)

2.19 The Effect of Oligoamines and EDTA on the Release of PGE_2 from Stimulated Peritoneal Guinea Pig PMNL's

The oligoamines studied and EDTA were tested for their effects on the release of PGE_2 from stimulated PMNL's at the following concentrations 0.1 mM, 1 mM, 50 mM, 100 mM, and 200 mM. Peritoneal guinea pig PMNL's were prepared as described in section (2.4.1). The experiment was carried out using prostaglandin E_2 (^{125}I) RIA Kit (NEN) using 1×10^6 PMNL's which were stimulated by 10^{-8} M PMA.

2.20 The Effect of Oligoamines and EDTA on the Uptake of (^3H) Leucine and (^3H) Thymidine by PMA Stimulated Peritoneal Guinea Pig Macrophages

2.20.1 Preparation of Peritoneal Guinea Pig Macrophages

0.5 % (w/v) Aqueous oyster glycogen solution (20 ml) was injected into the peritoneal cavity of male Dunkin Hartley strain guinea pig (400_500gbody weight). The macrophages were collected after 5 days in

the same way as described for collection of PMNL's in section (2.4.1)

2.20.2 Measurement of the Uptake of (^{3}H) Leucine and (^{3}H) Thymidine by Stimulated Macrophages

The method of Mon et al, (1981) was employed. The reaction mixture consisted of 10 µl of (^{3}H) leucine (specific activity 147 Curies/m mol, NEN) or 10 µl of (^{3}H) thymidine (specific activity 21 Curies/m mol, Amersham International), 5 x 10^5 macrophages, the oligoamines studied or EDTA at the following concentrations, 1 mM, 10 mM, and 100 mM in 4 ml EMEM media (pH 7.4), in glass test tubes. The reaction was initiated by the addition of 10^{-8} M PMA to all tubes. Control tubes were the same except for the omission of oligoamines and EDTA. All tubes were incubated at 37°C for 15 min. At the end of the incubation period, the tubes were rapidly cooled to 4°C by immersion in an ice water bath. The cells were pelleted by centrifugation at 900g for 5 min, washed in 5 ml cold (4° C) EMEM media (pH 7.4) and repelleted by centrifugation at 900g for 5 min. The supernatants were decanted and the cells were resuspended in 4 ml of 20 % (w/v) trichloracetic acid at 4° C. The acid washed pellets were centrifuged at 900g for 5 min and the supernatant fractions were saved as the acid soluble fractions. The acid insoluble precipitate fractions were solubilized with o.1 % (v/v) Triton x100. Aliquots of both fractions were transferred to scintillation vials containing 5 ml of scintillation fluid (NCS : OC%5; 4:1 v/v; Amersham International). The amount of radioactivity (cpm) was determined by a

Beckman Liquid Scintillation LS. 230 Counter

2.21 The Effect of Dibutyryl Cyclic AMP, Theophylline, and PGE_2 on Luminol Dependent PMA Stimulated Peritoneal Guinea Pig PMNL's Chemiluminescence

Dibutyryl cyclic AMP, theophylline and PGE_2 were tested for their effects on luminol dependent stimulated PMNL's chemiluminescence. Dibutyryl cyclic AMP and theophylline were tested at the following concentrations 100 μM, 500 μM, 1000 μM. PGE_2 was tried at the following concentrations 10 nM, 100 nM and 200 nM. The method described in section (2.5) was employed.

3. RESULTS

3.1 The Effect of Oligoamines and EDTA on the Reduction of Cytochrome C Induced by the Hypoxanthine/ Xanthine Oxidase Superoxide Generating System.

Preliminary experiments showed that the oligoamines studied and EDTA have no effect on the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase reaction at 1 mM and 10 mM.

Figure 3.1 shows the effects of putrescine, spermidine, spermine, cadaverine, diaminopropane, and EDTA on superoxide output as measured by the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase system. All the oligoamines studied and EDTA inhibited the superoxide induced reduction of cytochrome_C. The order of reactivity was:

putrescine > diaminopropane > spermidine > EDTA > spermine > cadaverine

At 40 mM putrescine, diaminopropane, and spermidine significantly ($p < 0.05$) caused 38%, 29% and 18% inhibitory effects on the reduction of cytochrome_C respectively.

At 80 mM EDTA, spermine and cadaverine significantly ($p < 0.05$) caused 48%, 36% and 25% inhibitory effects on the reduction of cytochrome_C respectively.

SOD used as a positive control significantly inhibited the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase system as Figure 3.2 shows.

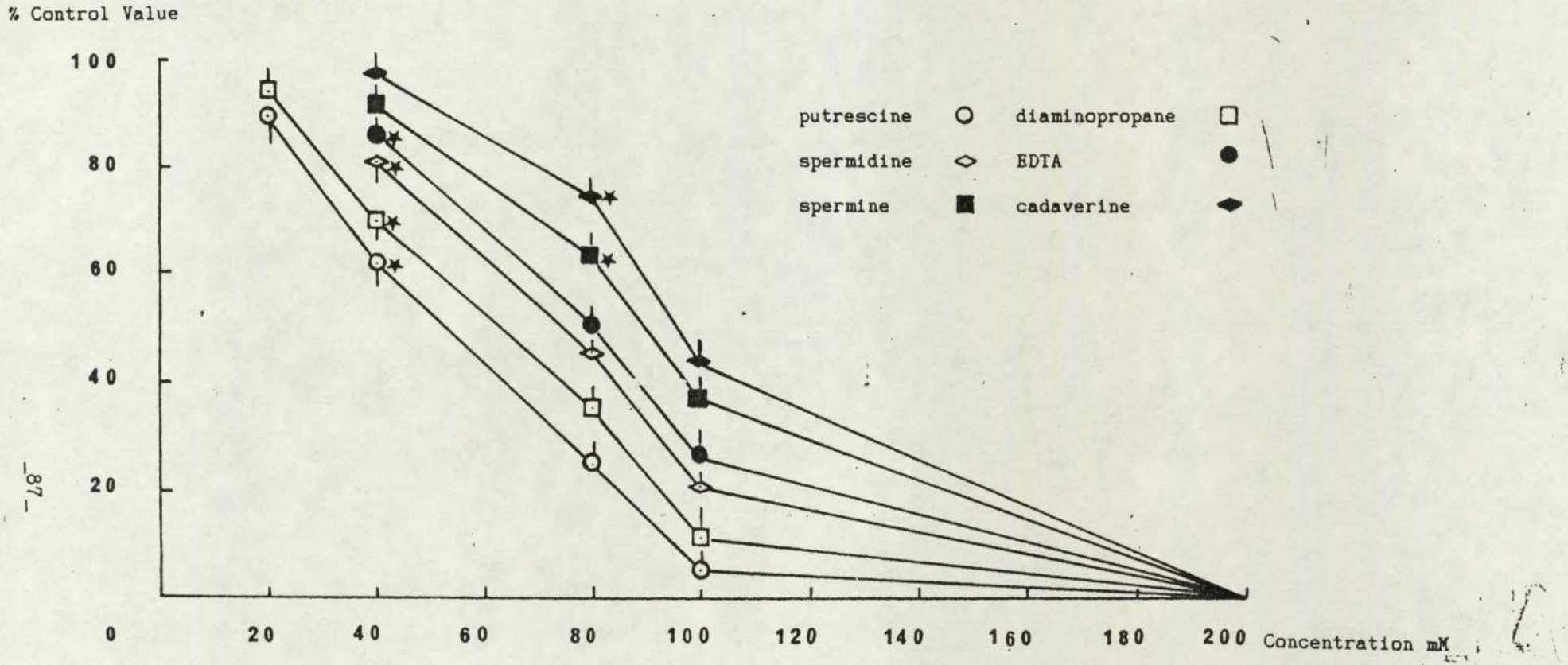


Figure 3.1 The effect of oligoamines and EDTA on the reduction of cytochrome_C by the products of hypoxanthine/xanthine oxidase reaction . Each result represents the mean of four determinations ± SEM. Significance was taken at $P < 0.05$ (★) (student t test) at the lowest effective concentrations.

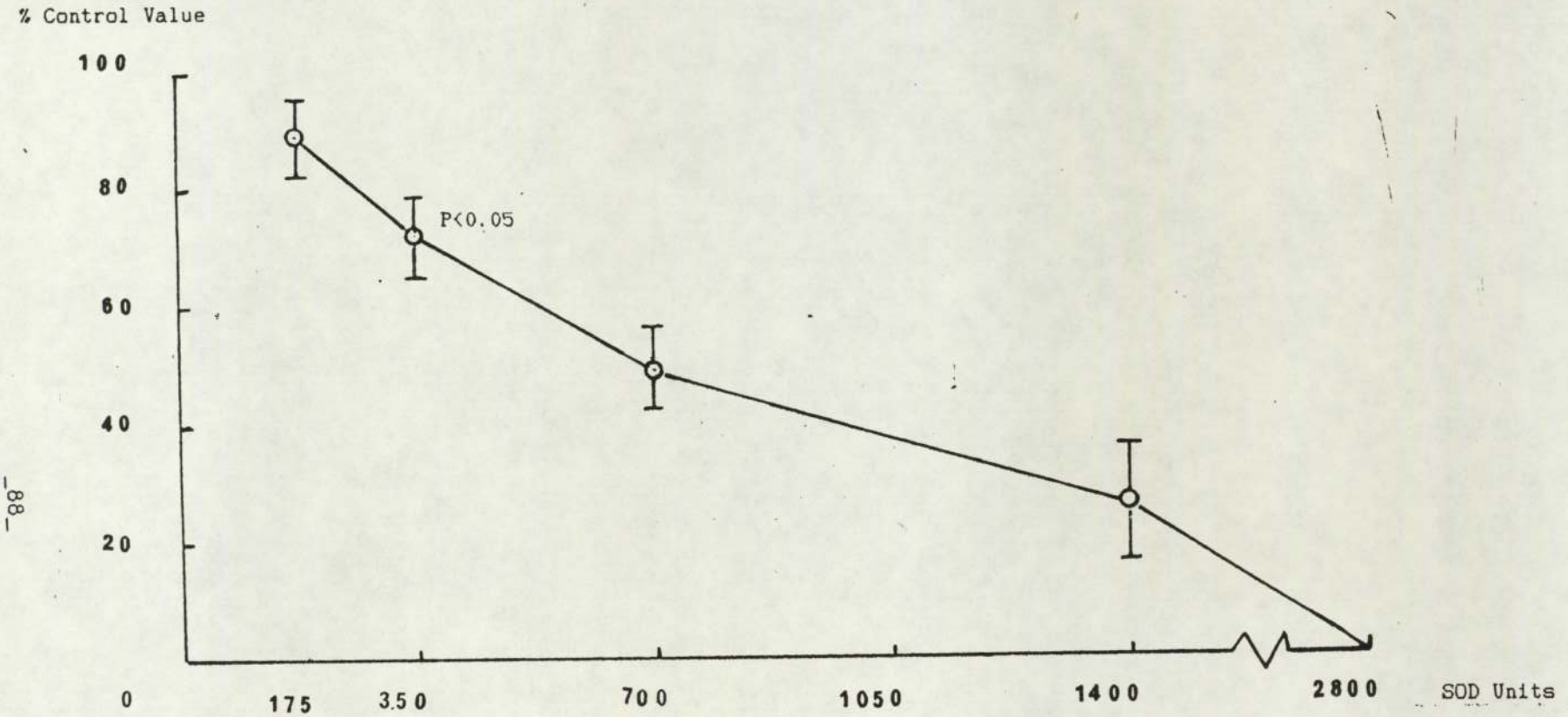


Figure 3.2 The effect of SOD on the reduction of cytochrome-C by the products of hypoxanthine/xanthine oxidase reaction.

Each result represents the mean of four determinations ± SEM.

3.2 The Effects of Oligoamines and EDTA on the Reduction of Nitro blue tetrazolium (NBT) Induced by the Hypoxanthine/Xanthine Oxidase Superoxide Generating System .

Preliminary experiments showed that at 1 mM and 10 mM the oligoamines studied and EDTA have no effect on the reduction of NBT by the hypoxanthine/xanthine oxidase system.

Figure 3.3 shows the effects of oligoamines studied in section 3.1 and EDTA on superoxide output as measured by the reduction of NBT. All the oligoamines studied and EDTA inhibited the reduction of NBT induced by hypoxanthine/xanthine oxidase system. The order of effectiveness was the same as in section 3.1.

At 20 mM putrescine and diaminopropane significantly ($p < 0.05$) caused 24% and 19% inhibitory effects on the reduction of NBT respectively.

At 40 mM spermidine and EDTA significantly ($p < 0.05$) caused 42% 29% and 17% inhibitory effects on the reduction of NBT respectively.

At 80 mM spermine and cadaverine significantly ($p < 0.05$) inhibited the reduction of NBT (59% and 27% respectively).

SOD significantly inhibited the reduction of NBT induced by hypoxanthine/xanthine oxidase system used as Figure 3.4 shows.

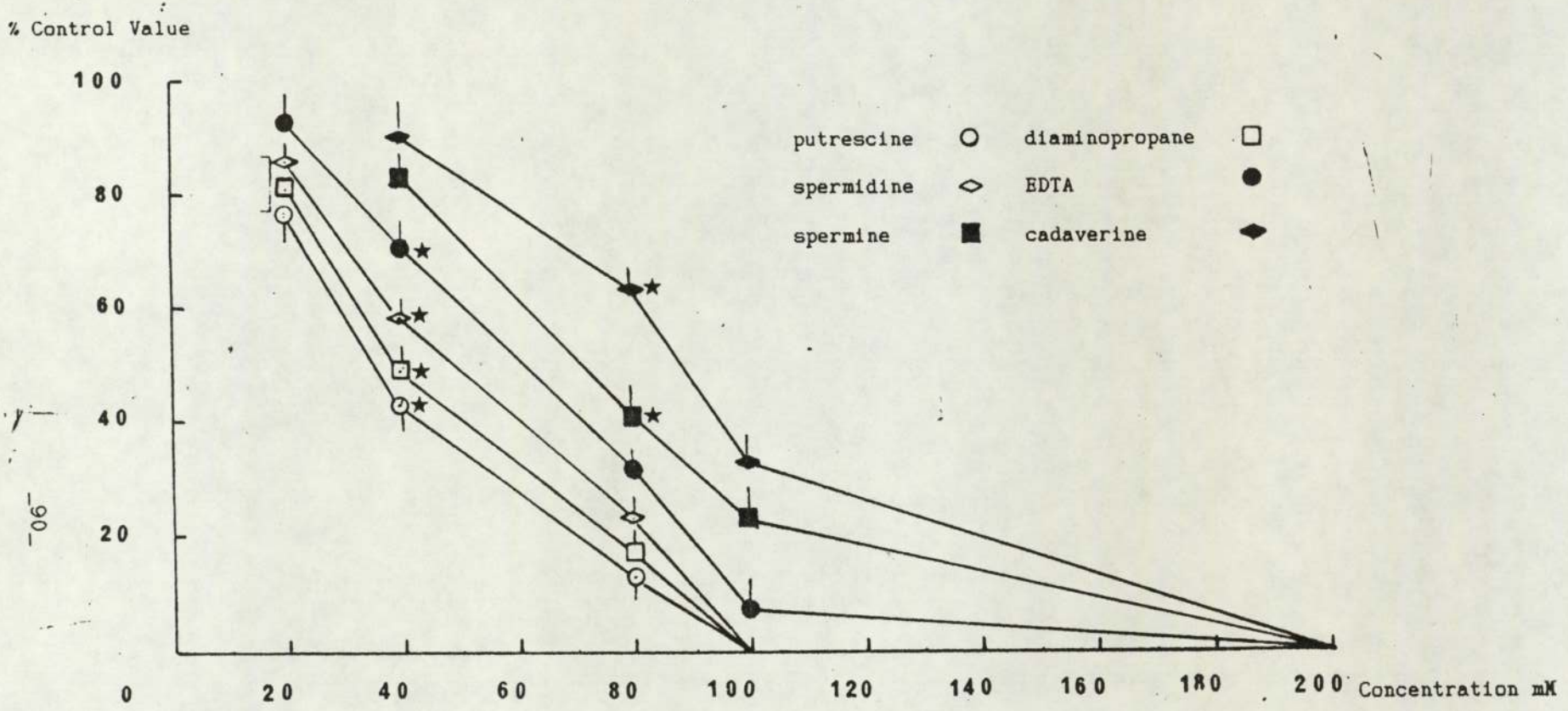


Figure 3.3 The effect of oligoamines and EDTA on the reduction of NBT by the products of hypoxanthine/xanthine oxidase reaction .

Each result represents the mean of four determinations ± SEM.

Significance was taken at $p < 0.05$ (★) (student t test) at the lowest effective concentration.

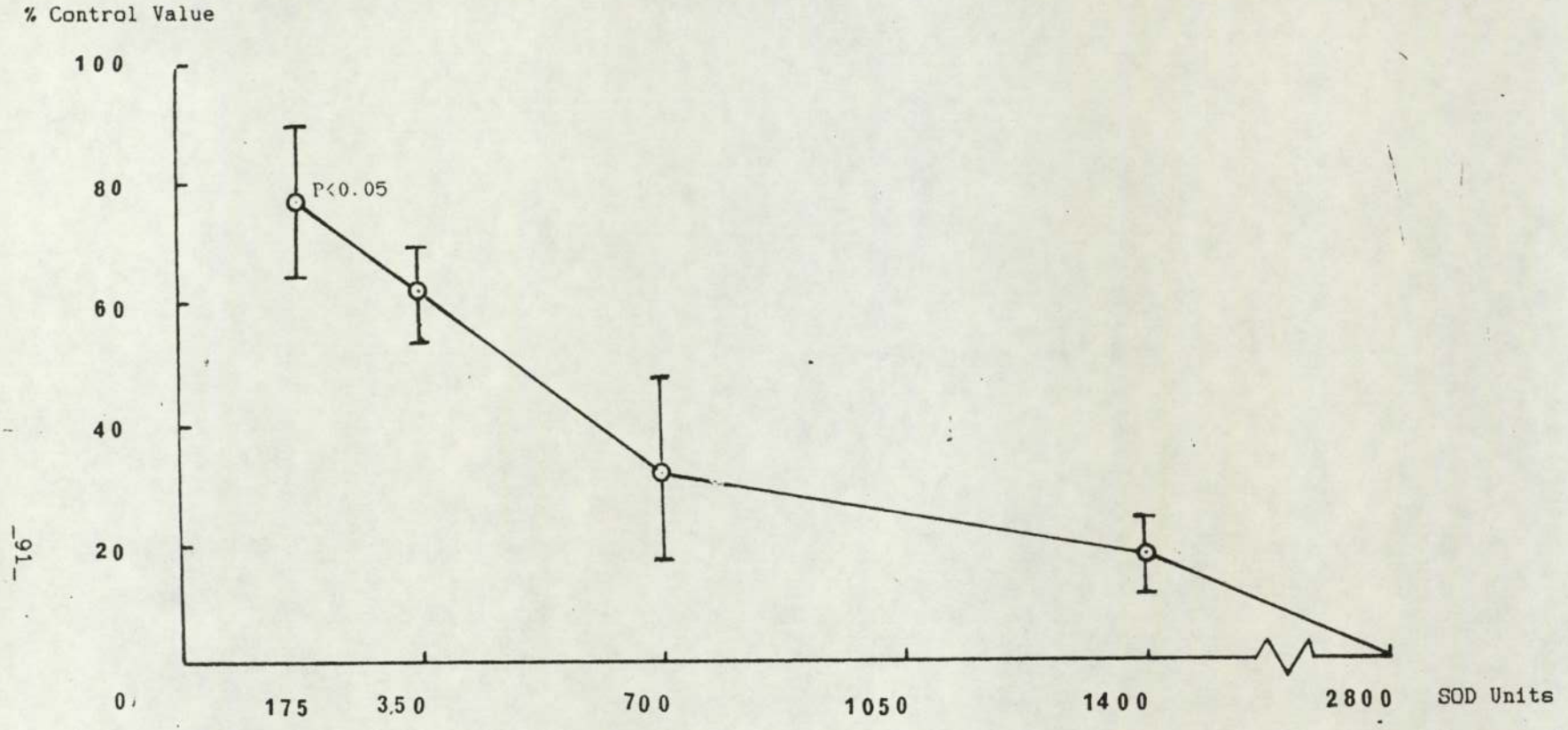


Figure 3.4 the effect of SOD on the reduction of NBT by the products of hypoxanthine/xanthine oxidase reaction.

Each result represents the mean of four determinations ± SEM.

3.3 The Effect of Oligoamines and EDTA on Urate Formation from the Hypoxanthine/Xanthine Oxidase System.

The inhibition by oligoamines and EDTA of the hypoxanthine/xanthine oxidase system induced reduction of cytochrome_C or NBT may be due directly to the inhibition of the catalytic activity of xanthine oxidase. This however, proved not to be the case since the oligoamines studied and EDTA did not inhibit the rate of urate formation from the hypoxanthine/xanthine oxidase system as Table 3.1 shows
SOD did not inhibit the rate of urate formation from hypoxanthine/xanthine oxidase system as Table 3.2 shows.

Table 3.1 The Effect of Oligoamines and EDTA on Urate Formation From the Hypoxanthine/Xanthine Oxidase System.

Concentration mM	Putrescine	Spermine	Spermine	Cadaverine	Diaminopropane	EDTA
0.1	103 ±1.8	108 ±1.2	109 ±3.5	102 ±2.2	102 ±1.3	103 ±1.6
1	107 ±3.1	105 ±2.1	103 ±2.4	109 ±3.3	105 ±2.4	102 ±4
100	108 ±1.1	103 ±1.7	110 ±1.9	104 ±4.3	102 ±2.2	110 ±2.5
200	102 ±0.9	106 ±29	102 ±2.4	101 ±1.5	101 ±1.8	99 ±1.2

The results are expressed as a percentage of the control values where oligoamines and EDTA were omitted. The actual readings were the mean of the absorbance (290 nm) values of four determinations ± SEM.

Table 3.2 The Effect of SOD on Urate Formation From the Hupoxanthine/Xanthine Oxidase System.

SOD units	% of control
700	103 ± 0.9
1400	105 ± 1.3
2800	101 ± 2.6

The results are expressed as a percentage of the control values where SOD was omitted. The actual readings were the mean of the absorbance (290 nm) values of four determinations ± SEM.

3.4 The Effect of Oligoamines and EDTA on Superoxide Output from PMA Stimulated PMNL's on Cytochrome C Reduction.

Preliminary experiments showed that the oligoamines and EDTA studied have no effect on the reduction of cytochrome_C by stimulated PMNL's at 1 mM and 10 mM.

The effects of oligoamines and EDTA on superoxide produced from stimulated peritoneal guinea pig PMNL's, as measured by the reduction of cytochrome_C is shown in figure 3.5. The results are very similar to those obtained when superoxide was generated from the hypoxanthine/xanthine oxidase system with same order of effectiveness. SOD as a positive control inhibited the reduction of cytochrome_C induced by stimulated PMNL's significantly in all doses used as Figure 3.6 shows.

3.5 The Effect of Oligoamines and EDTA on Luminol Dependent PMA Stimulated PMNL's Chemiluminescence.

Figure 3.7 shows that the oligoamines studied and EDTA inhibited stimulated peritoneal guinea pig PMNL's luminol dependent chemiluminescence. The results are similar to those obtained by cytochrome_C reduction induced by stimulated PMNL's, with the same order of effectiveness, but the method was more sensitive.

Evidence that chemiluminescence was produced by superoxide was obtained when SOD was substituted for oligoamines and EDTA in experiments. A dose responsive suppression was found and complete suppression at 2800 units as Figure 3.8 shows

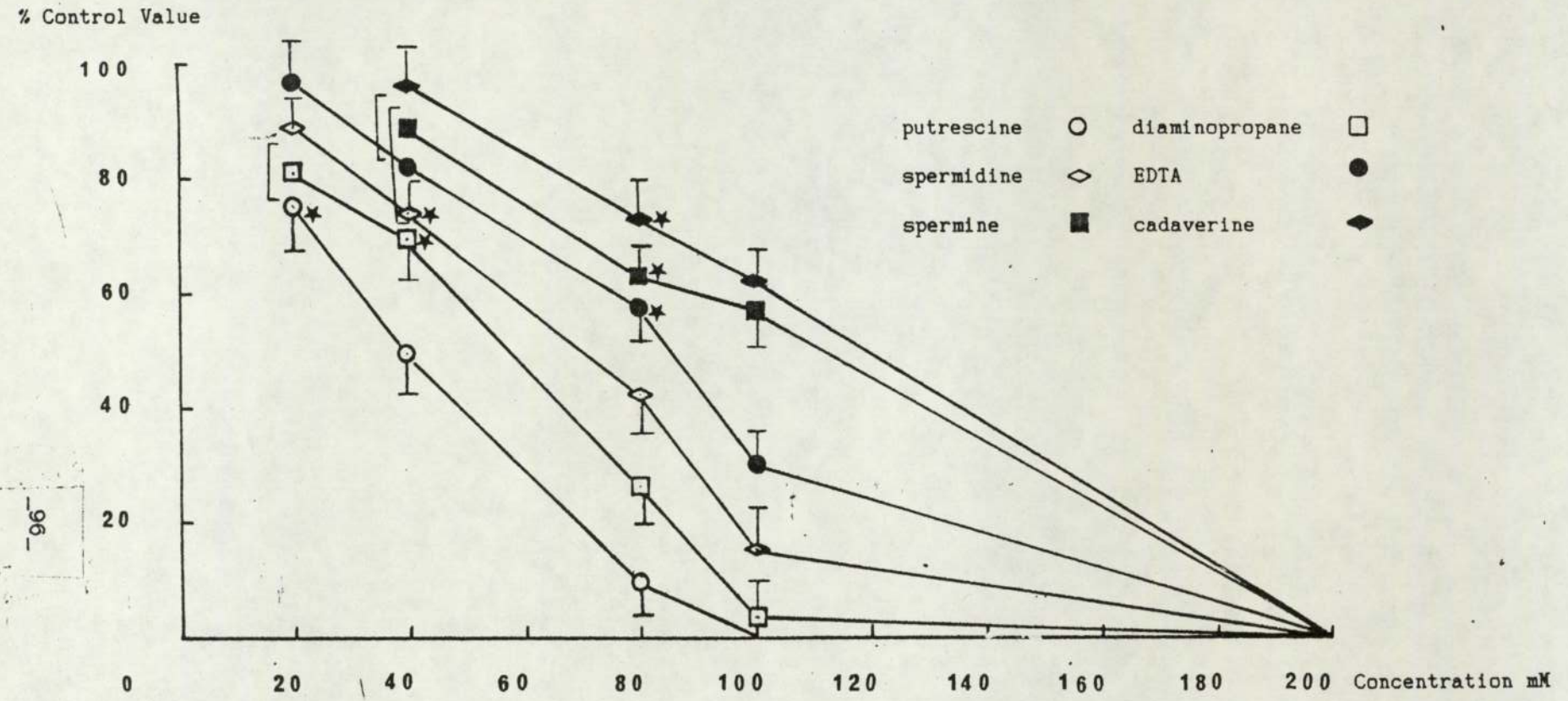


Figure 3.5 The effect of oligoamines and EDTA on cytochrome_C reduction in the presence of guinea pig PMNL's stimulated with PMA.
Each result represents the mean of four determinations ± SEM.
Significance was taken at $P < 0.05$ (★) (student t test) at the lowest effective concentration.

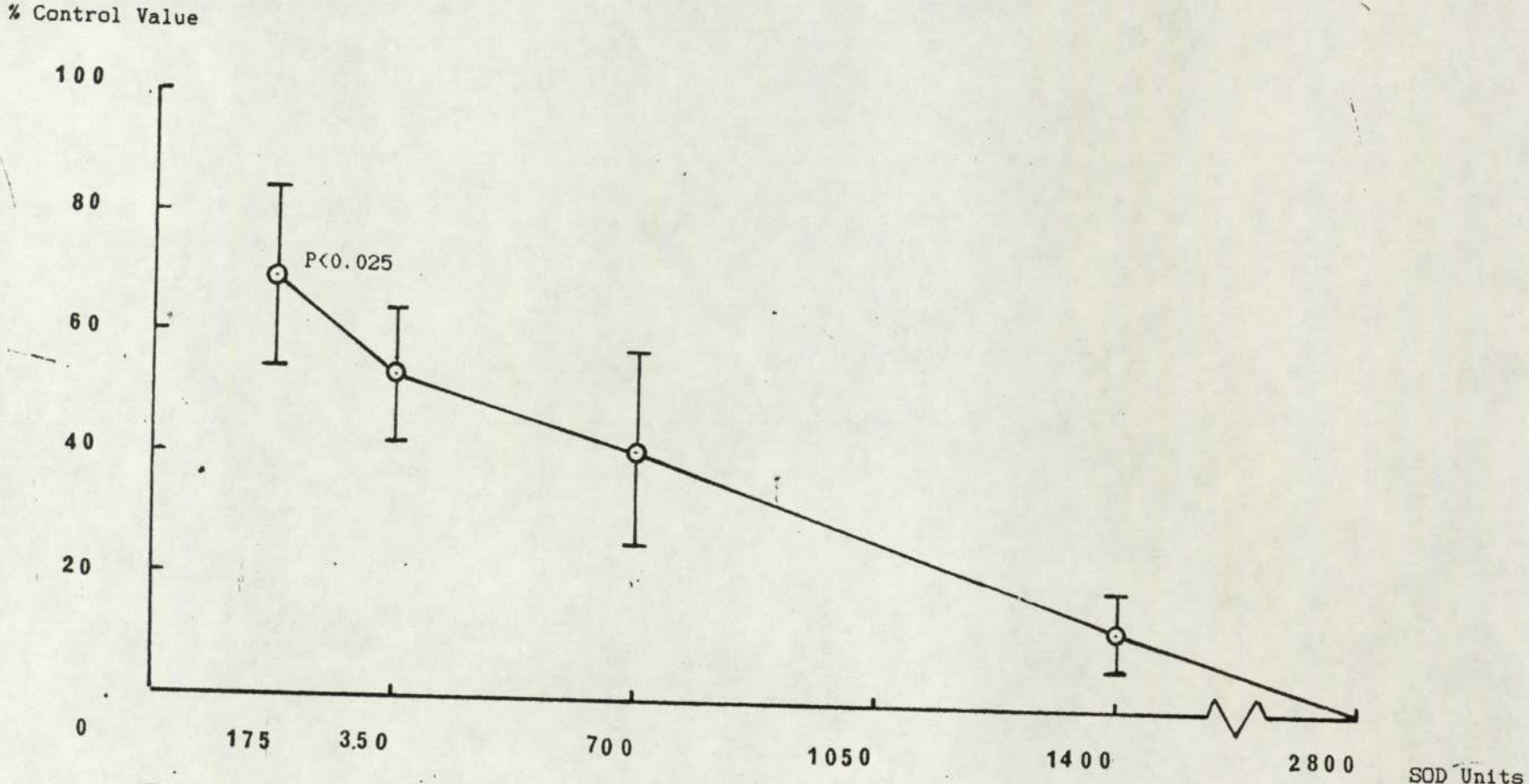


Figure 3.6 The effect of SOD on the reduction of cytochrome_C induced by PMA stimulated PMNL's

Each result represents the mean of four determinations ± SEM .

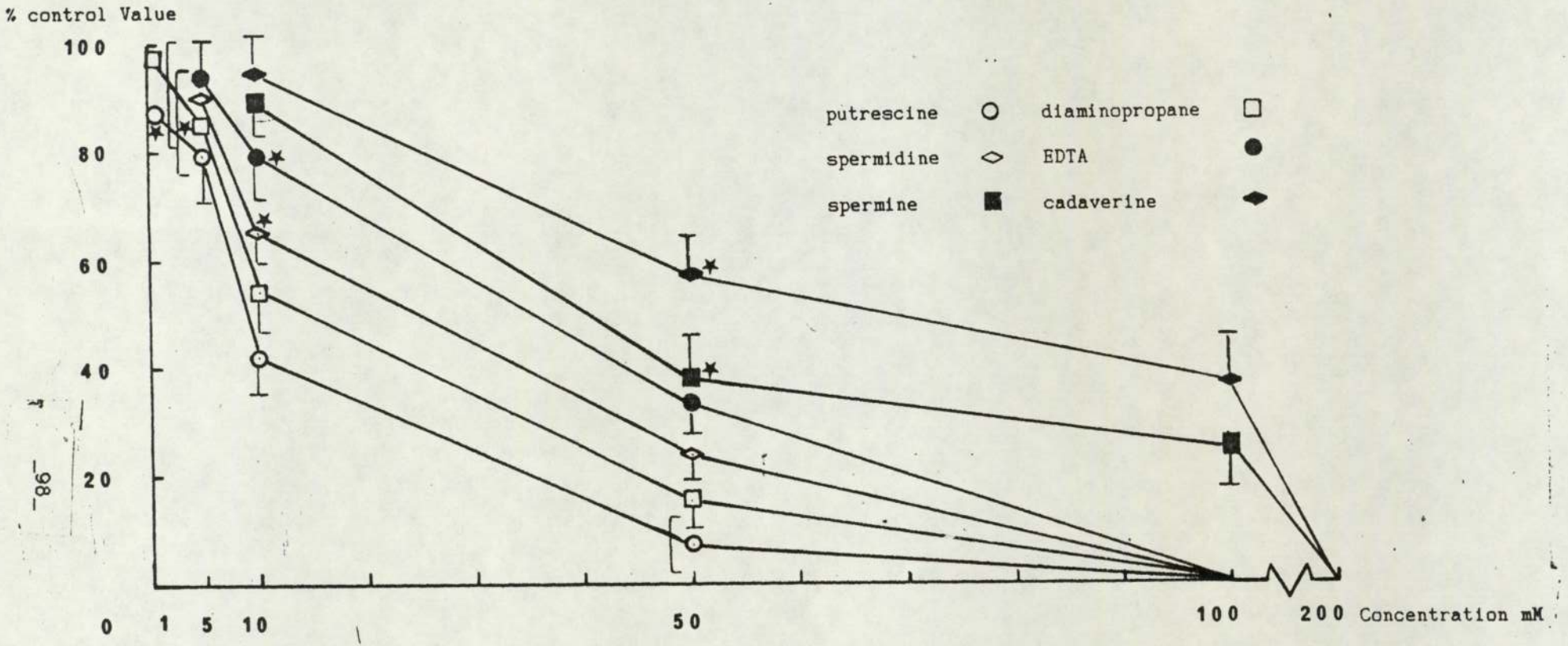


Figure 3.7 The effect of oligoamines and EDTA on luminol dependent PMA stimulated PMNL's chemiluminescence.

Each result represents the mean of four determinations ± SEM.

Significane was taken at $p < 0.05$ (★) (student t test) at the lowest effective concentration.

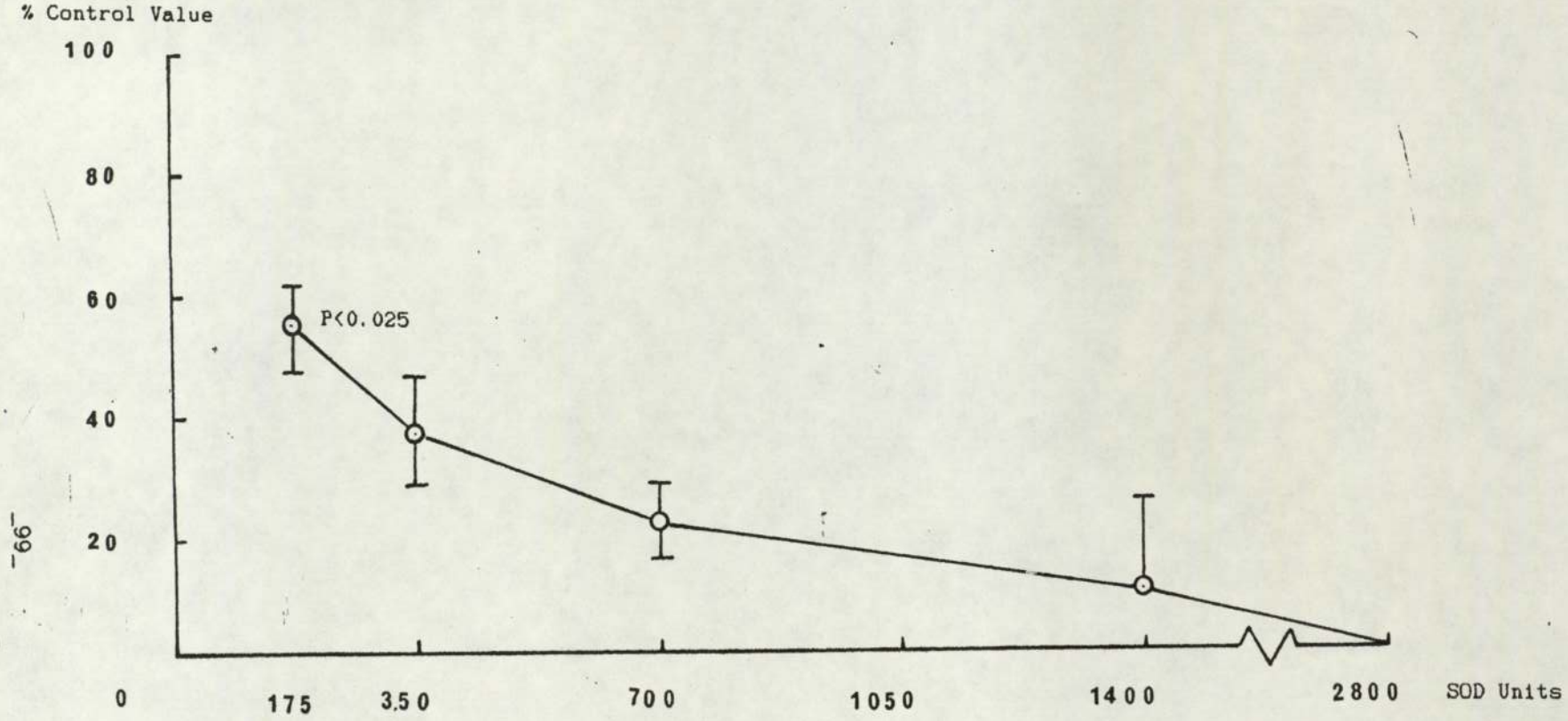


Figure 3.8 the effect of SOD on luminol dependent PMA stimulated PMNL's chemiluminescence.

Each result represents the mean of four determinations ± SEM.

3.6 The Effect of Oligoamines and EDTA on the Aerobic Photoreduction of NBT .

Figure 3.9 shows that the oligoamines studied and EDTA inhibited the aerobic photoreduction of NBT. The order of reactivity was the same as in sections 3.1, 3.2, 3.3, 3.4, 3.5 .

Figure 3.10 shows that SOD significantly inhibited the aerobic photoreduction of NBT.

3.7 The Effect of Copper Sulphate on the Reduction of Cytochrome C Induced by the Hypoxanthine/Xanthine Oxidase Superoxide Generating System

At 10^{-8}M copper sulphate significantly ($p < 0.05$) inhibited the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase system (21%) as Figure 3.11 shows.

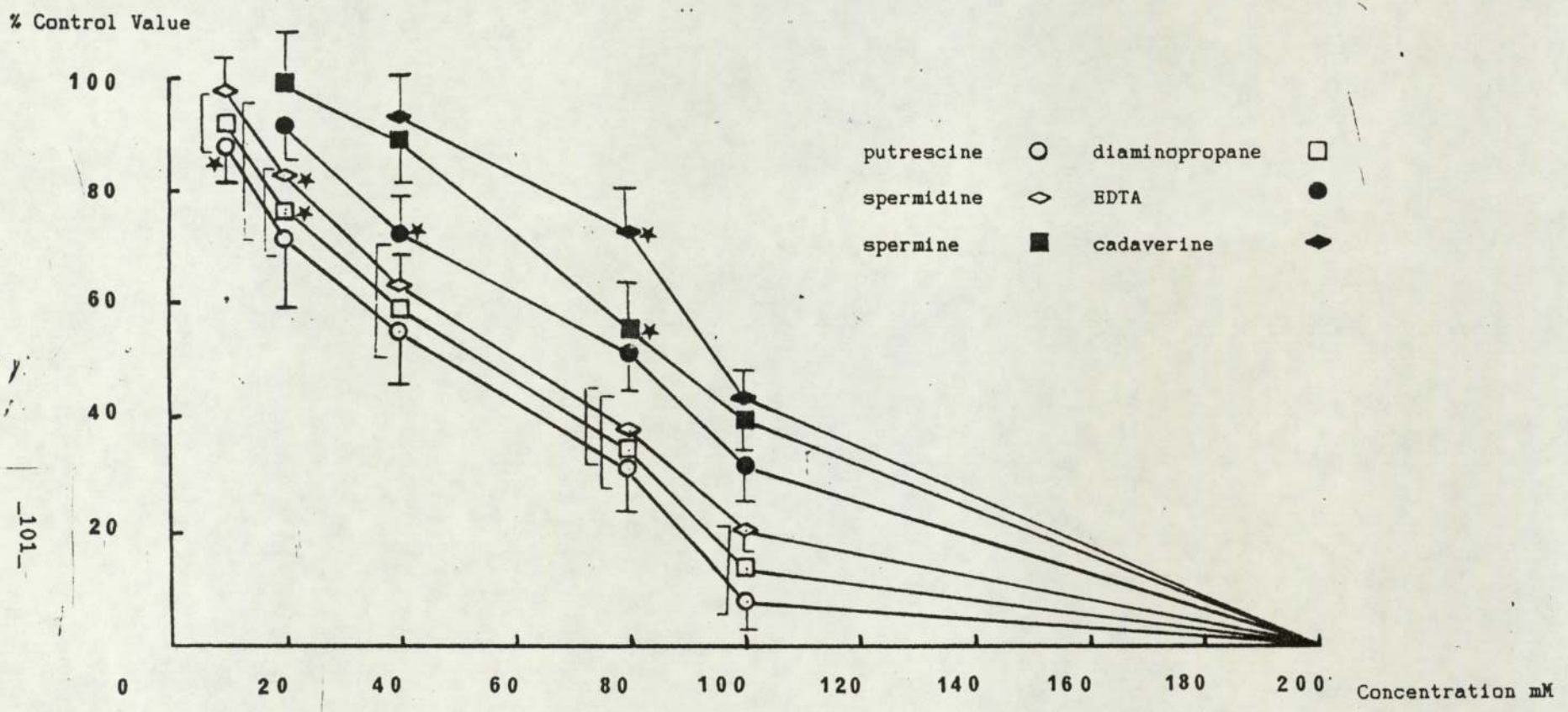


Figure 3.9 The effect of oligoamines and EDTA on the aerobic photoreduction of NBT .

Each rseult represents the mean of four determinations ± SEM.

Significance was taken at $p < 0.05$ (*) (student t test) at the lowest effective concentration .

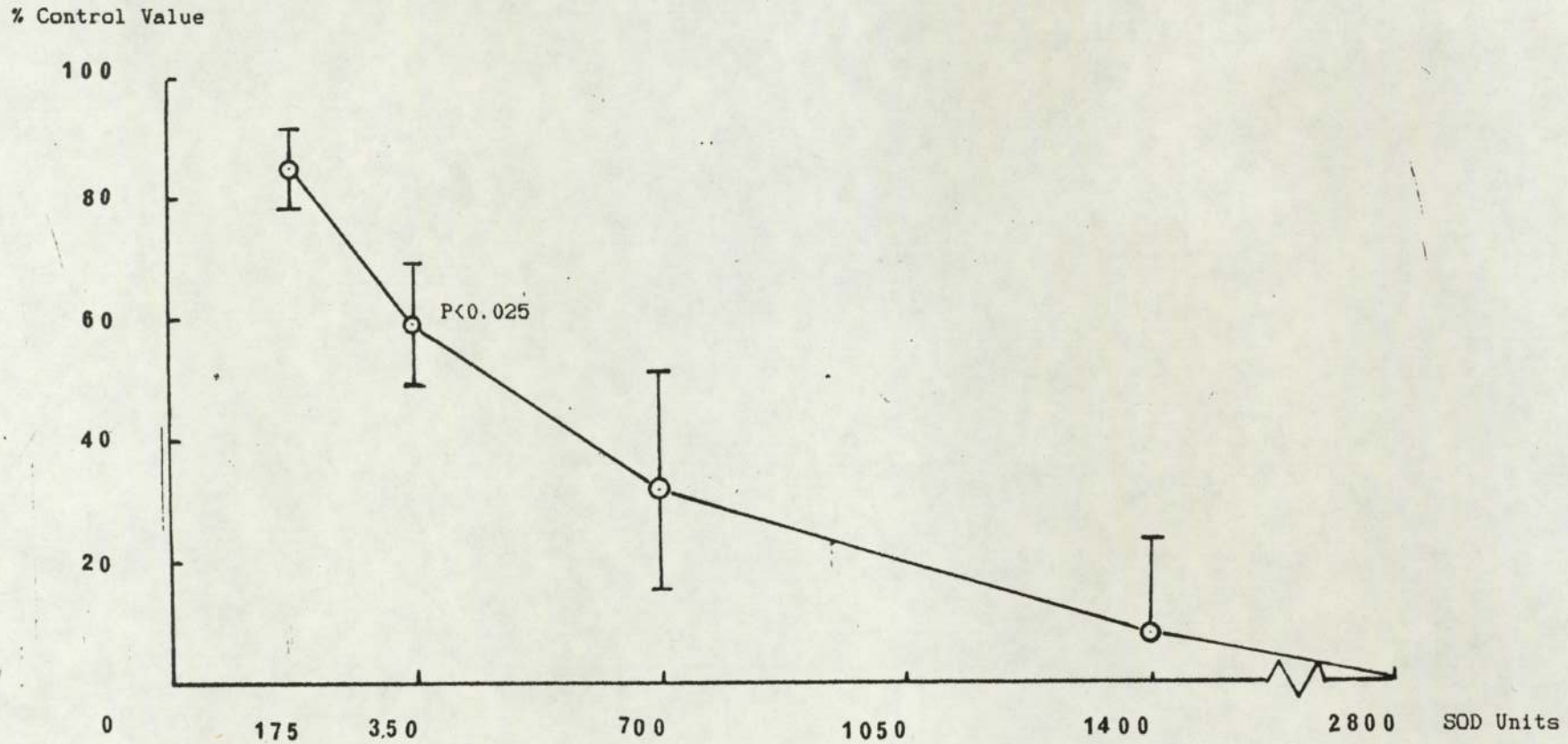


Figure 3.10 The effect of SOD on the aerobic photoreduction of NBT.

Each result represents the mean of four determinatios ± SEM.

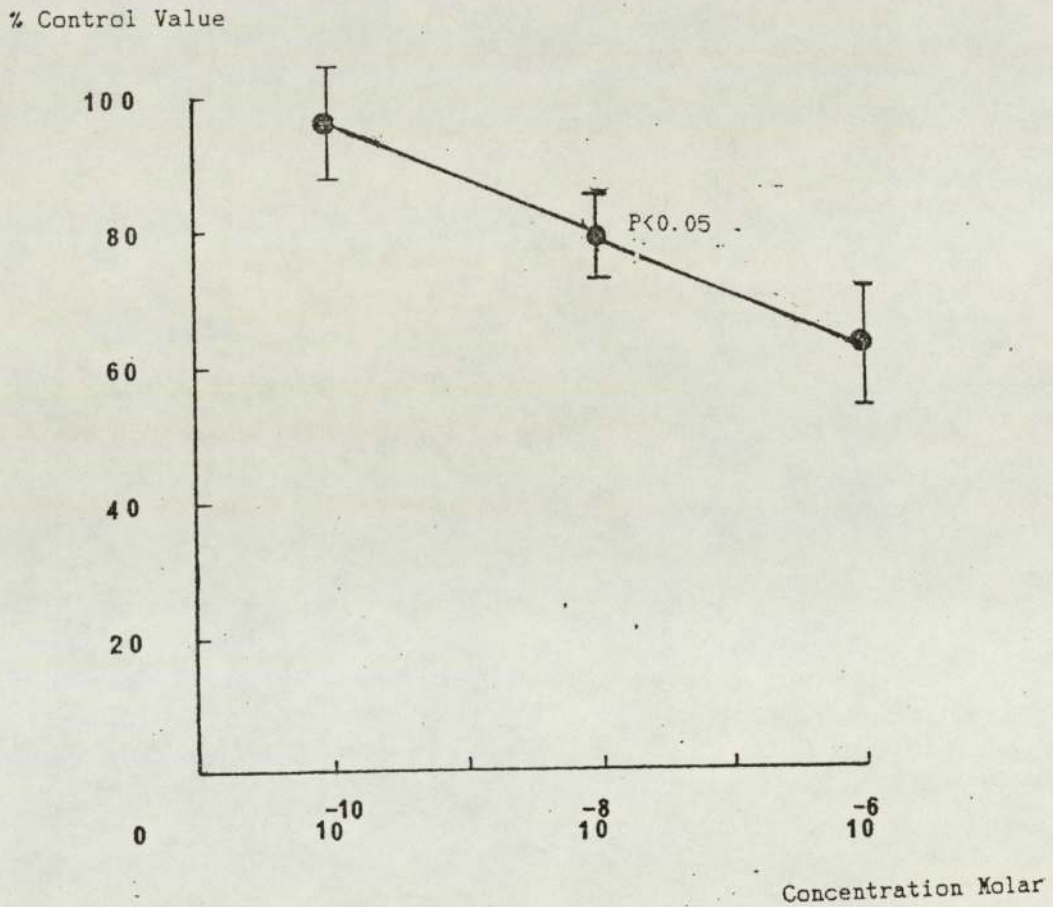


Figure 3.11 The effect of copper sulphate on the reduction of cytochrome-C by the products of hypoxanthine/xanthine oxidase reaction.

Each result represents the mean of four determinations ± SEM.

3.8 The Effect of Oligoamine.Copper Sulphate Complexes on the Reduction of Cytochrome C Induced by the Hypoxanthine/Xanthine Oxidase Superoxide Generating System.

Figure 3.12 shows that oligoamine_copper sulphate complexes significantly inhibited the reduction of cytochrome_C induced by hypoxanthine/xanthine oxidase system more efficiently than oligoamines or copper sulphate individually, suggesting that oligoamine_copper sulphate complexes may have SOD activities.

A complex of 10^{-8} M copper sulphate and 1 mM of either putrescine, or diaminopropane or spermidine or spermine or cadaverine caused 39%, 34%, 31%, 28% and 25% inhibitory effect ($P<0.05$) (student t test) on the reduction of cytochrome_C respectively.

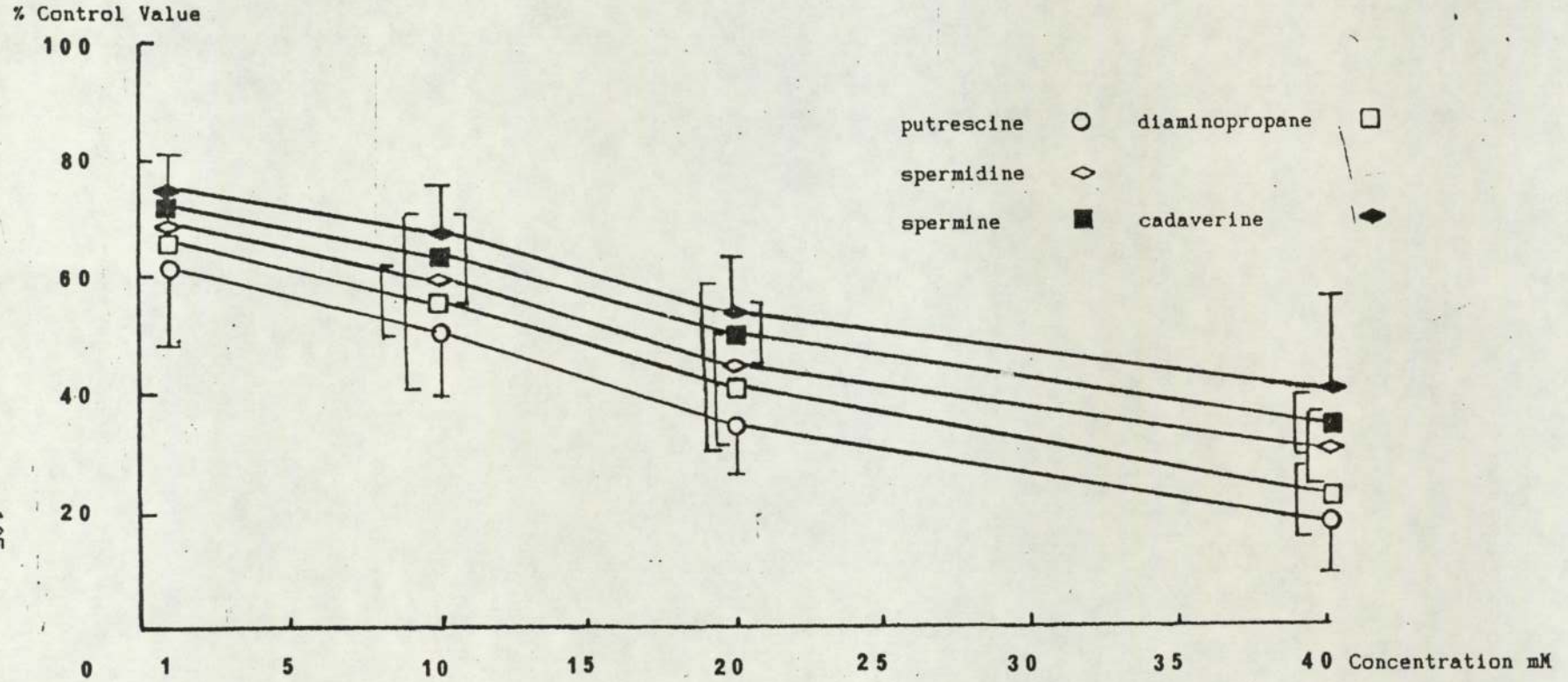


Figure 3,12 The effect of oligoamines_ copper sulphate complexes on the reduction of cytochrome-C by the products of hypoxanthine/xanthine oxidase reaction

Each result represents the mean of four determinations ± SEM.

3.9 The Effect of Reactive Oxygen Species on the Stability of Isolated Intact Guinea Pig Liver Lysosomes.

3.9.1 The Effect of the Hypoxanthine/Xanthine Oxidase Superoxide System on the Stability of Isolated Intact Lysosomes.

The effect of increasing the amount of xanthine oxidase (to increase the fluxes of superoxide generated) on the lysis of lysosomes was that lytic action increased.

Figure 3.13 shows that increasing the amount of xanthine oxidase significantly increased the release of acid phosphatase and β-glucosaminidase from intact lysosomes. When xanthine oxidase was boiled and added to the reaction mixture of lysosomes and hypoxanthine no effect was obtained indicating the need for an active enzyme.

3.9.2 The Effect of Hydrogen Peroxide on the Stability of Isolated Intact Lysosomes.

Figure 3.14 shows that increasing the concentrations of hydrogen peroxide above 150 μ M significantly increased the release of acid phosphatase and β-glucosaminidase from intact lysosomes. Concentrations below 150 μ M did not have a significant effect on the release of both enzymes from intact lysosomes.

3.9.3 The Effect of Hydroxyl Radical on the Stability of Isolated Intact Lysosomes.

Figure 3.15 shows that increasing the fluxes of hydroxyl radical generated by the reaction of increasing concentrations of hydrogen peroxide and ferrous sulphate significantly increased the release of acid phosphatase and β-glucosaminidase from intact lysosomes.

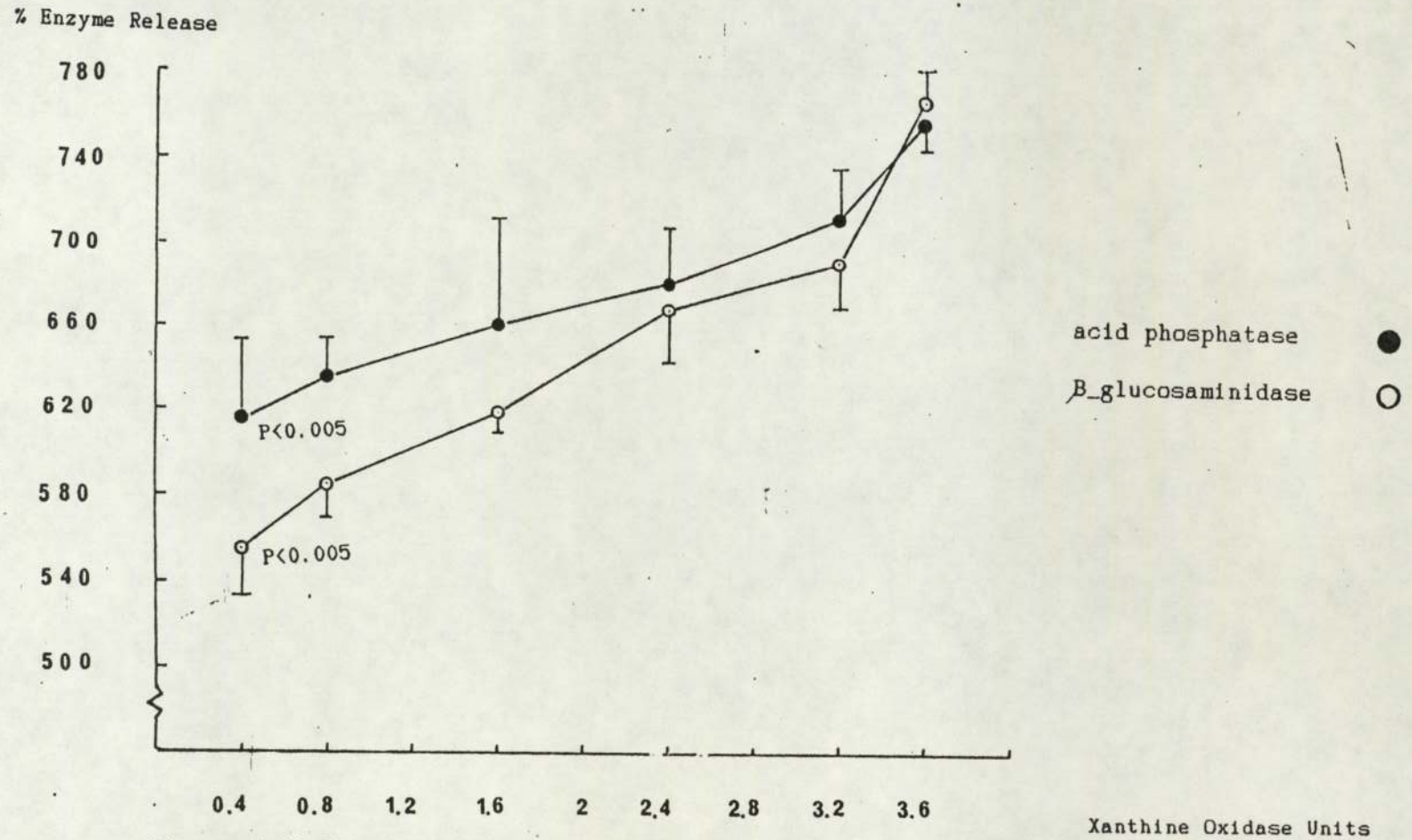


Figure 3.13 The effect of hypoxanthine/xanthine oxidase reaction on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

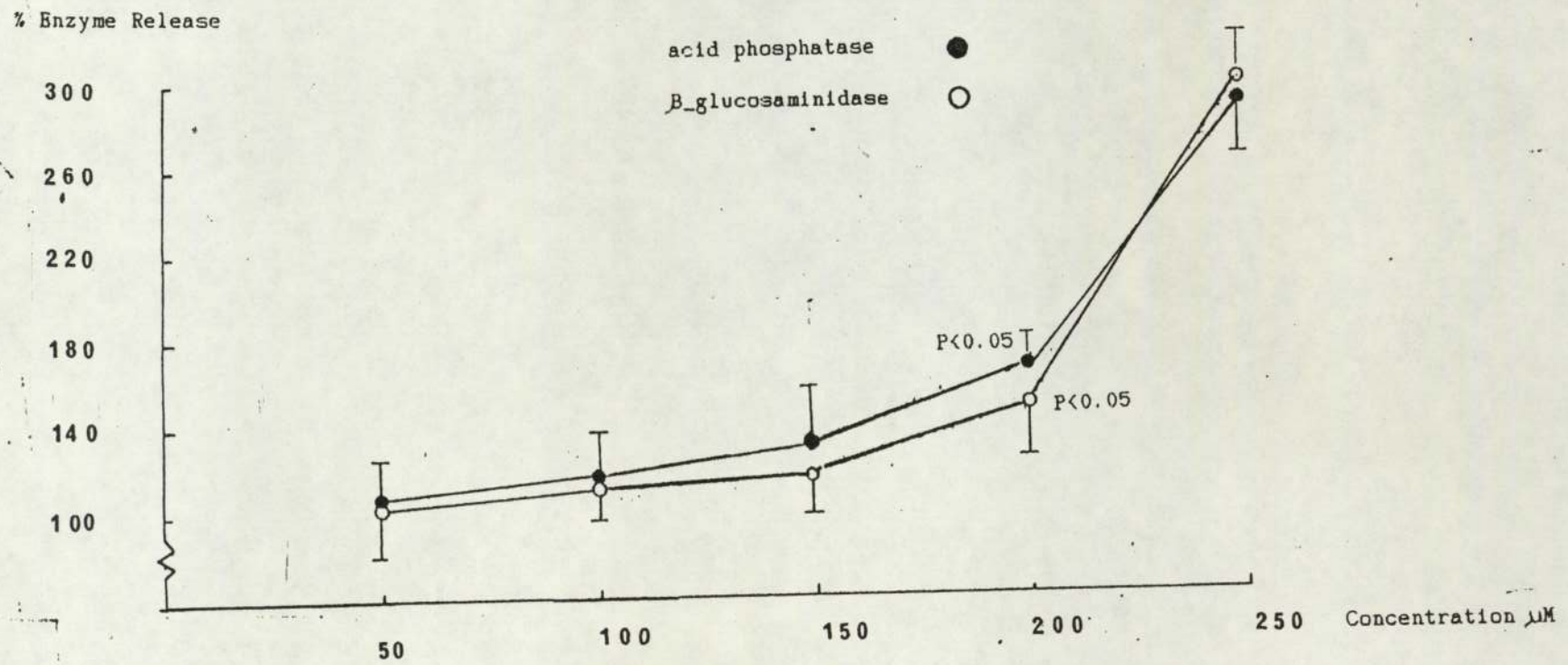


Figure 3.14 The effect of hydrogen peroxide on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

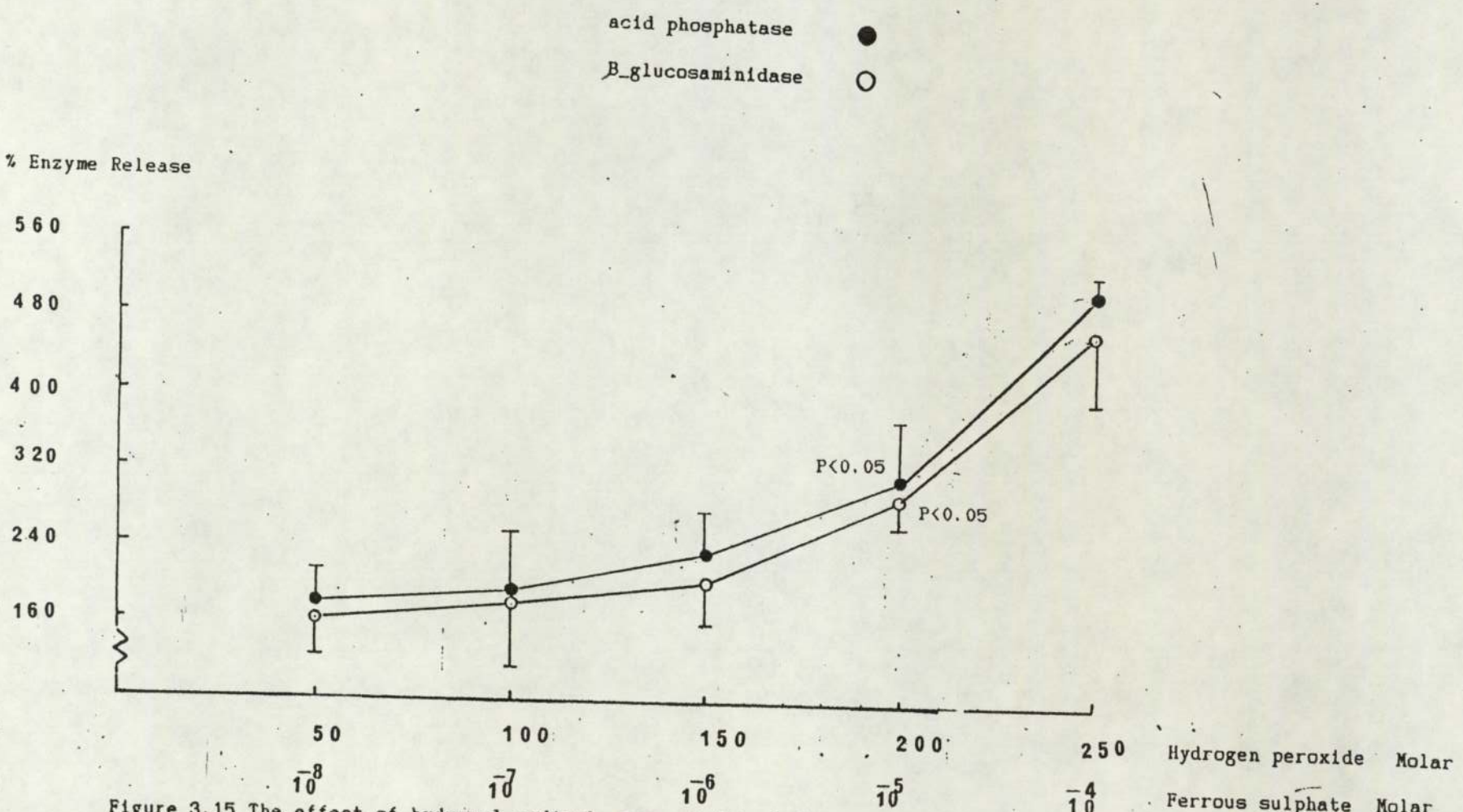


Figure 3.15 The effect of hydroxyl radical ,generated by hydrogen peroxide and ferrous sulphate, on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes Each result represents the mean of four deyterminations ± SEM.

3.9.4 The Effect of Ferrous Sulphate on the Stability of Isolated Intact Lysosomes.

Figure 3.16 shows that ferrous sulphate has no effect on the release of acid phosphatase and β-glucosaminidase from intact lysosomes.

3.10 The Effect of Oligoamines and EDTA on the Hypoxanthine/Xanthine Oxidase System Induced Breakdown of Isolated Intact Guinea Pig Liver Lysosomes.

Figures 3.17, 3.18, 3.19, 3.20, 3.21 and 3.22 show that putrescine spermidine, spermine, cadaverine, diaminopropane, and EDTA respectively, significantly inhibited the release of acid phosphatase and β-glucosaminidase from intact lysosomes incubated in the presence of the hypoxanthine/xanthine oxidase system with the same order of effectiveness as in section 3.1, indicating their protective effect against hypoxanthine/xanthine oxidase system induced breakdown of intact lysosomes.

SOD used as a positive control significantly inhibited the release of acid phosphatase and β-glucosaminidase from intact lysosomes in the presenece of the hypoxanthine/xanthine oxidase system as Figure 3.23 shows.

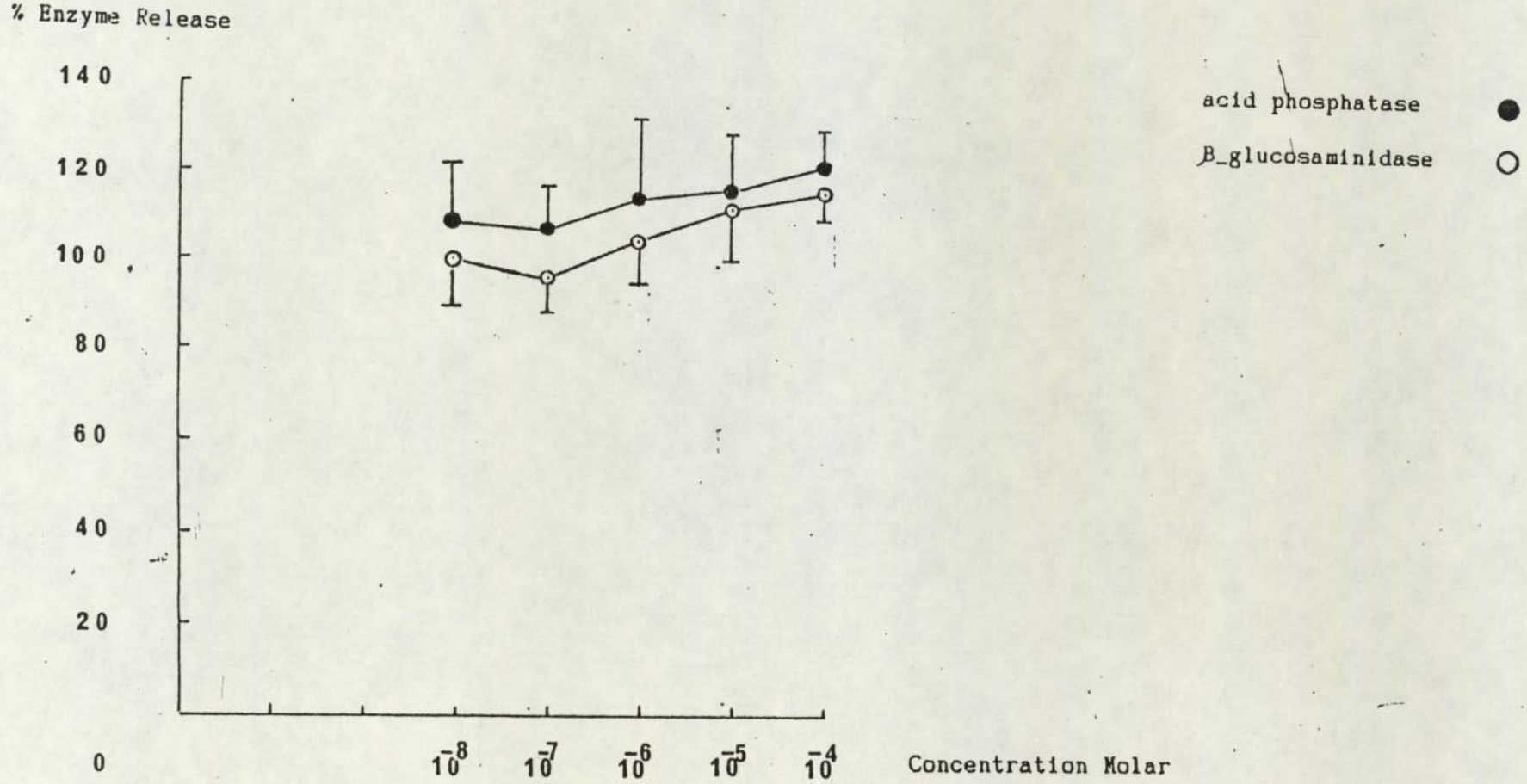


Figure 3.16 The effect of ferrous sulphate on the release of acid phosphatase and B-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

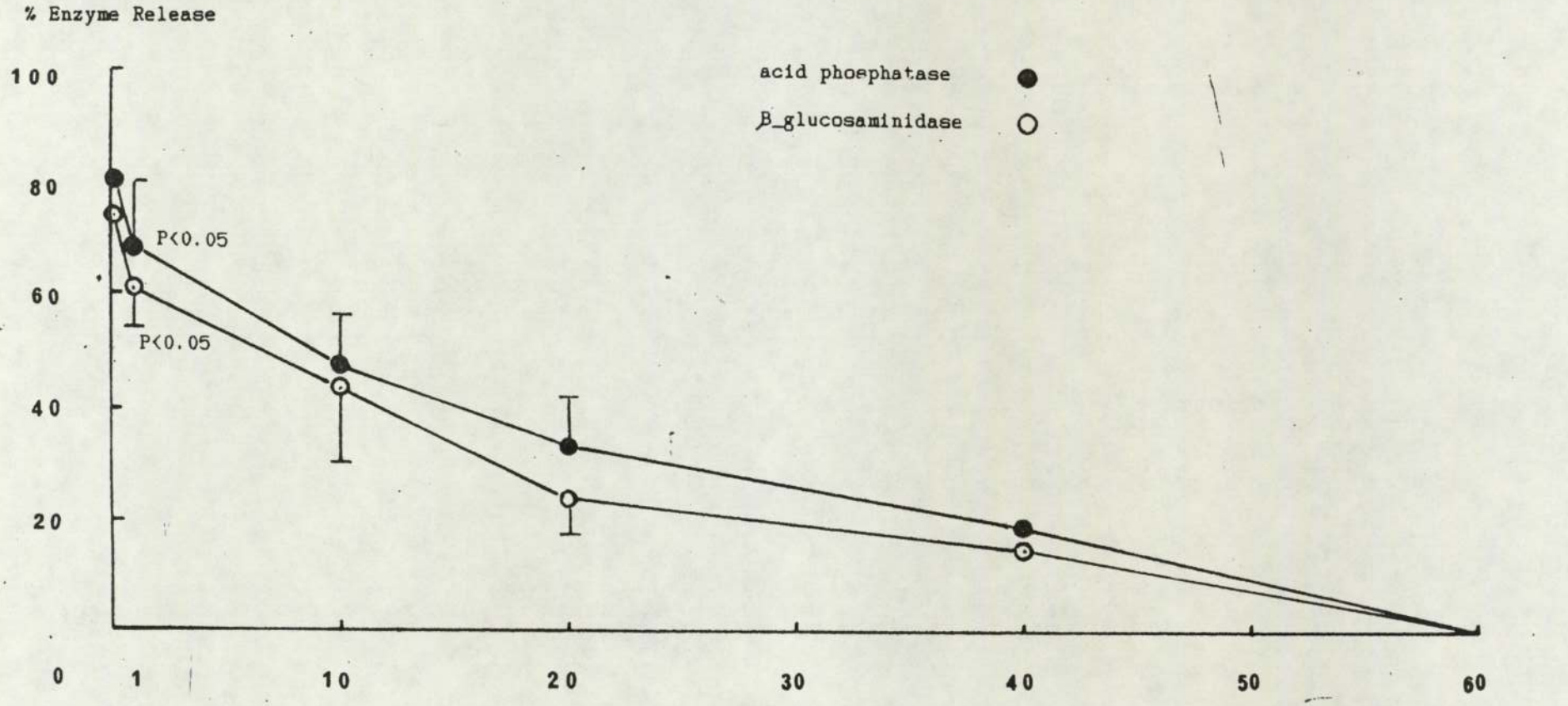


Figure 3.17 The effect of putrescine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with hypoxanthine/xanthine oxidase reacton. Each result represents the mean of four determinations ± SEM.

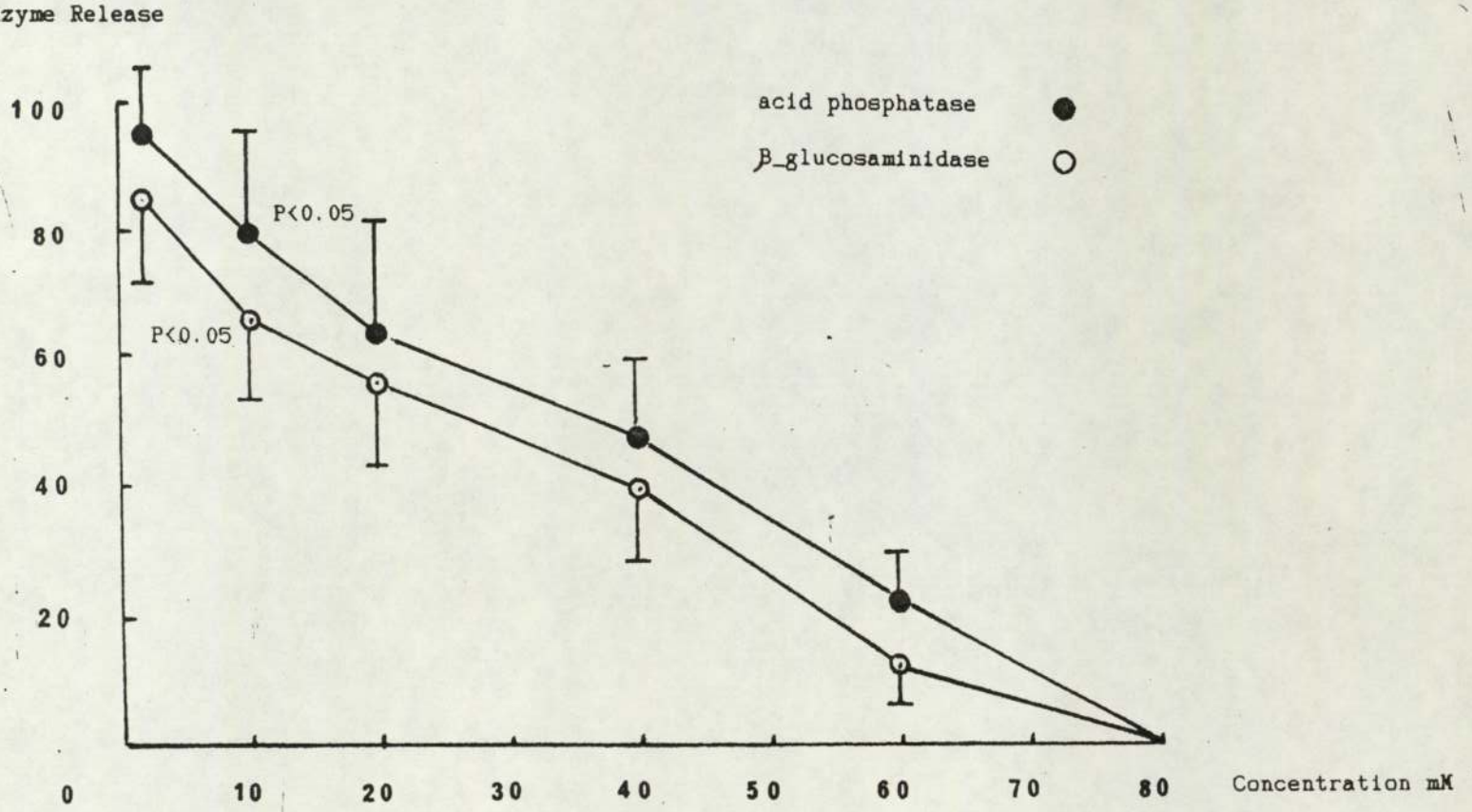


Figure 3.18 the effect of spermidine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with hypoxanthine/xanthine oxidase reaction. Each result represents the mean of four determinations ± SEM.

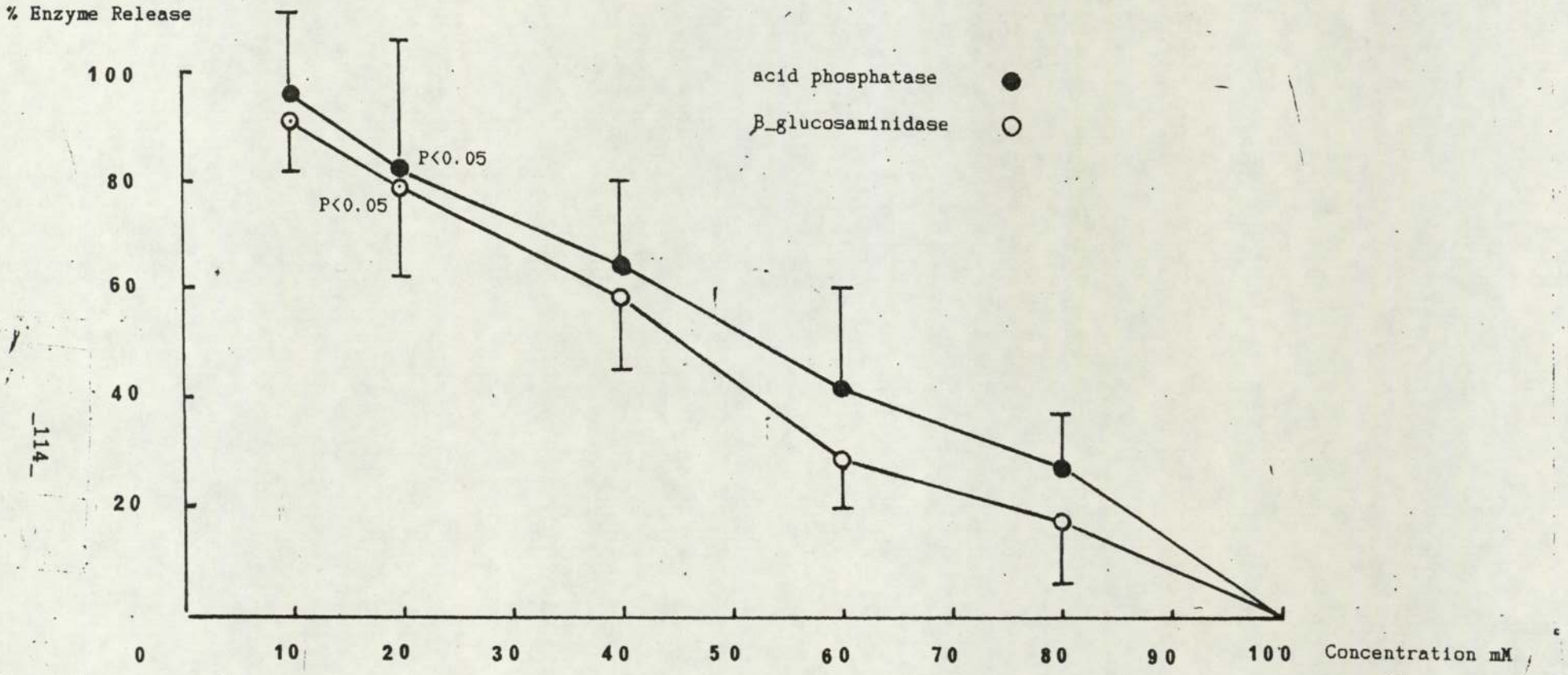


Figure 3.19 The effect of spermine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with hypoxanthine/xanthine oxidase reactin.
Each result represents the mean of four determinations ± SEM.

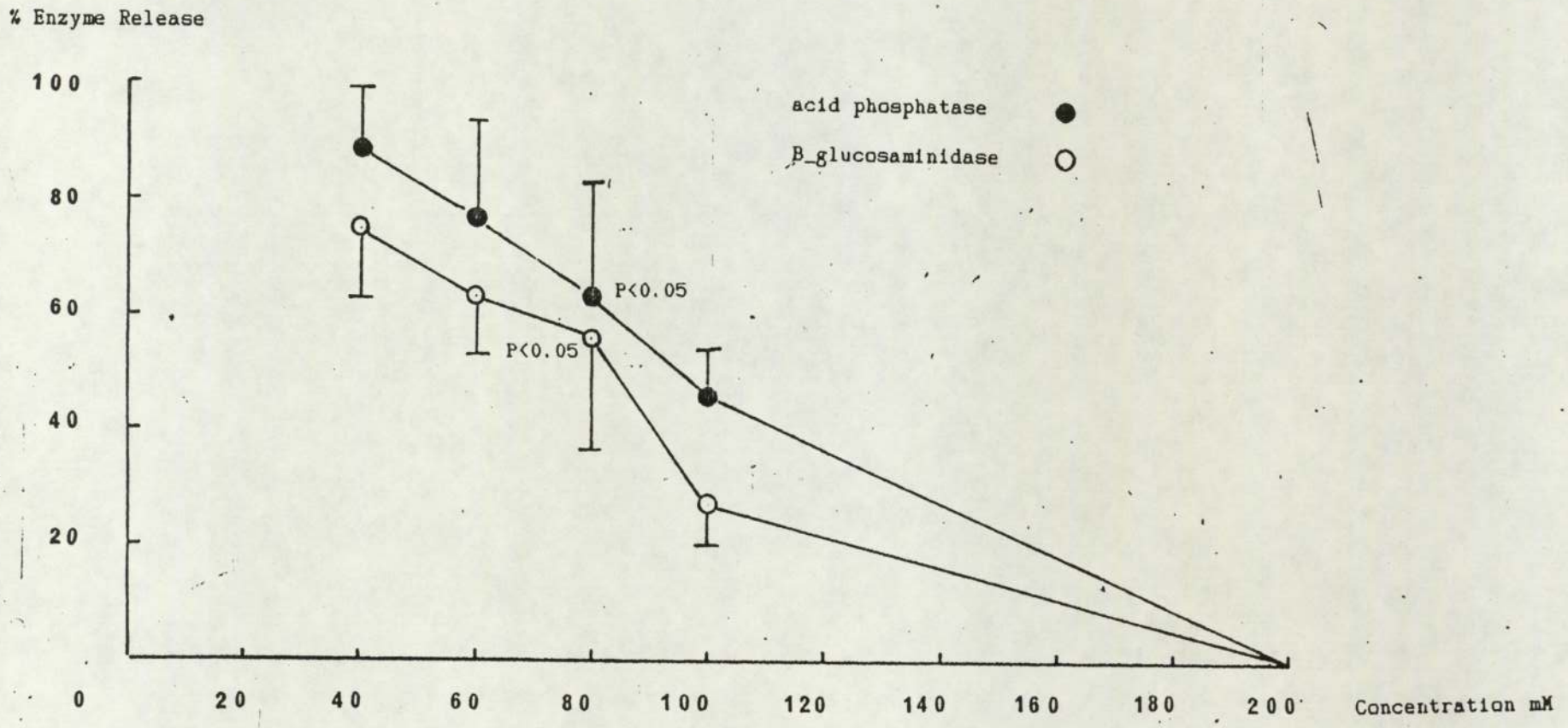


Figure 3.20 The effect of cadaverine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with hypoxanthine/xanthine oxidase reaction. Each result represents the mean of four determinations ± SEM.

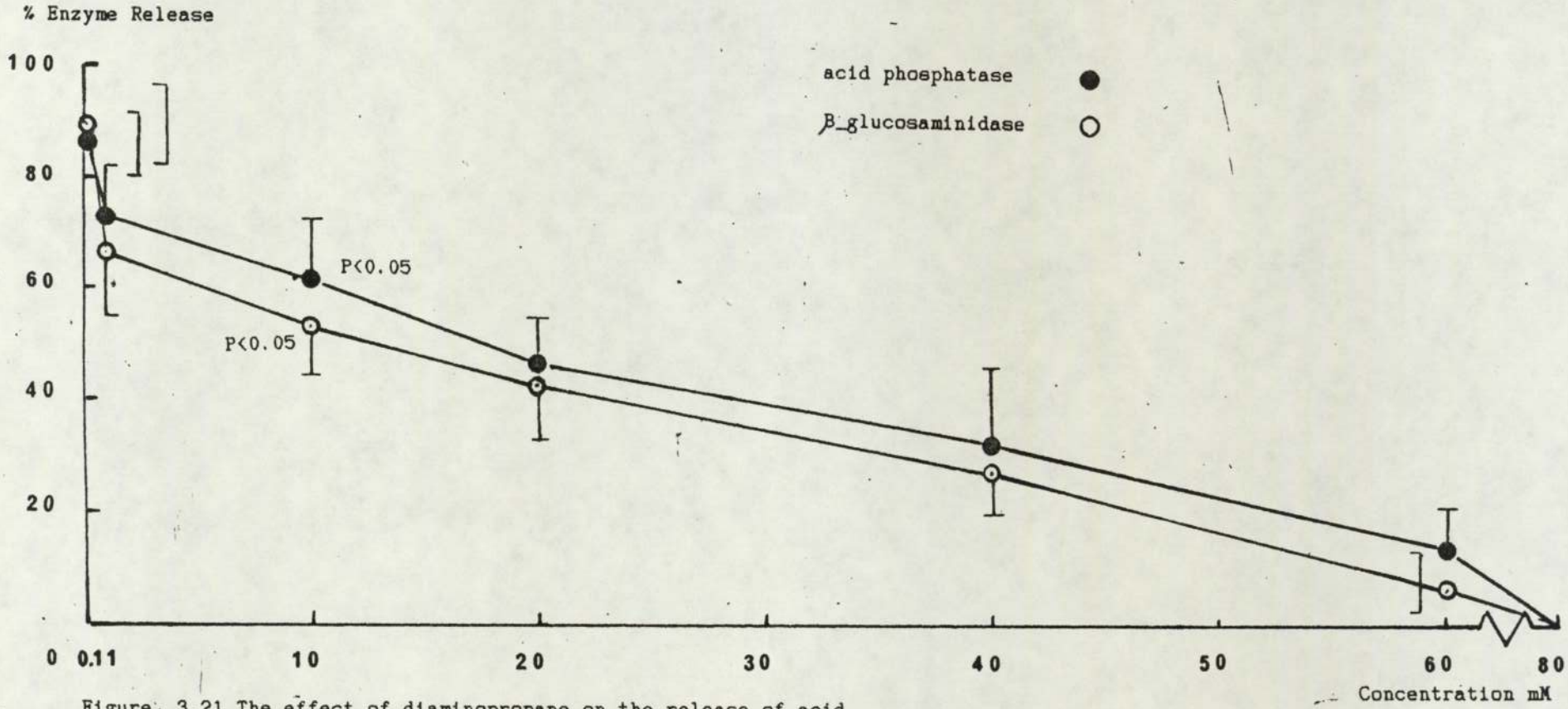


Figure 3.21 The effect of diaminopropane on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with hypoxanthine/xanthine oxidase reactionEach result represents the mean of four determinations ± SE

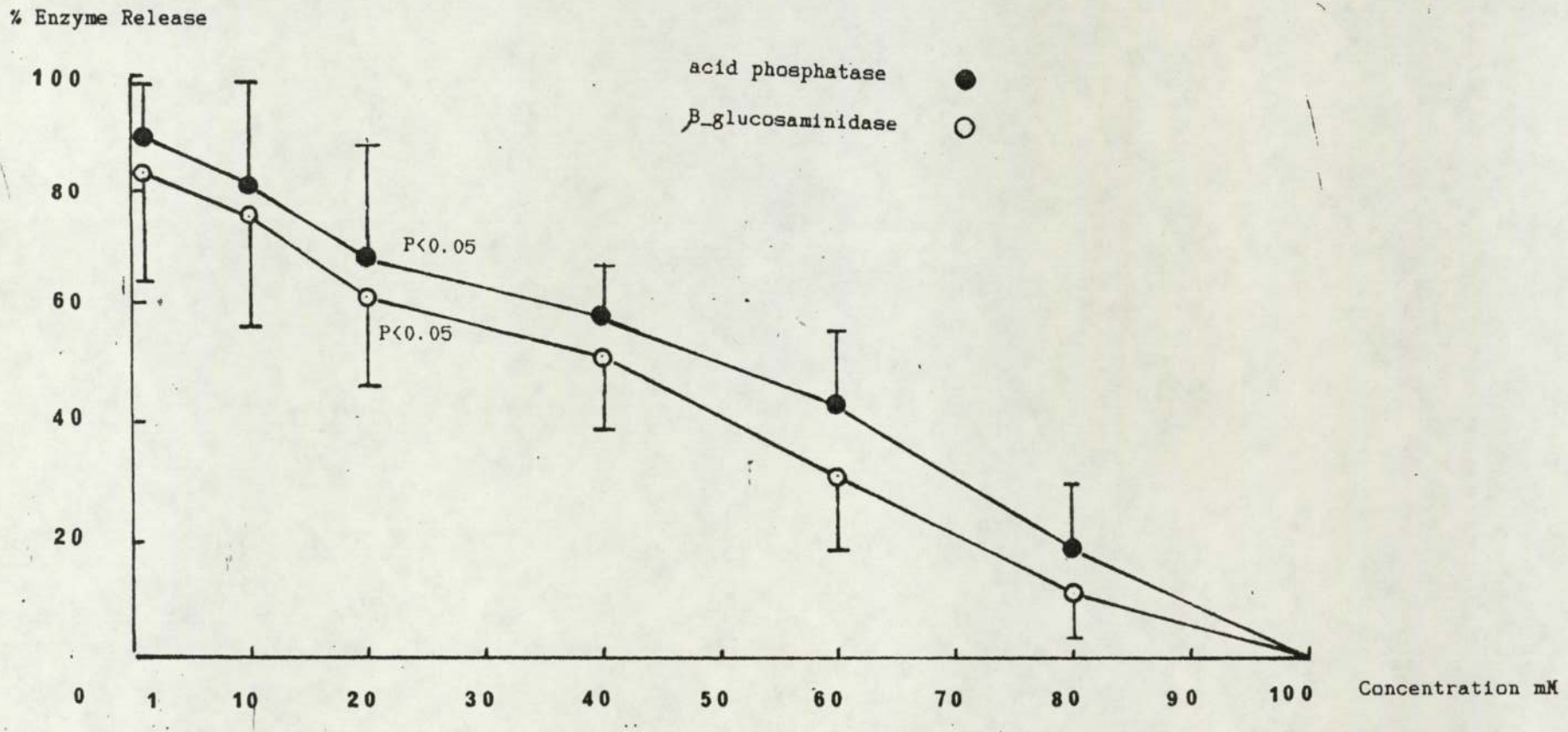


Figure 3.22 The effect of EDTA on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with hypoxanthine/xanthine oxidase reaction.

Each result represents the mean of four determinations ± SEM.

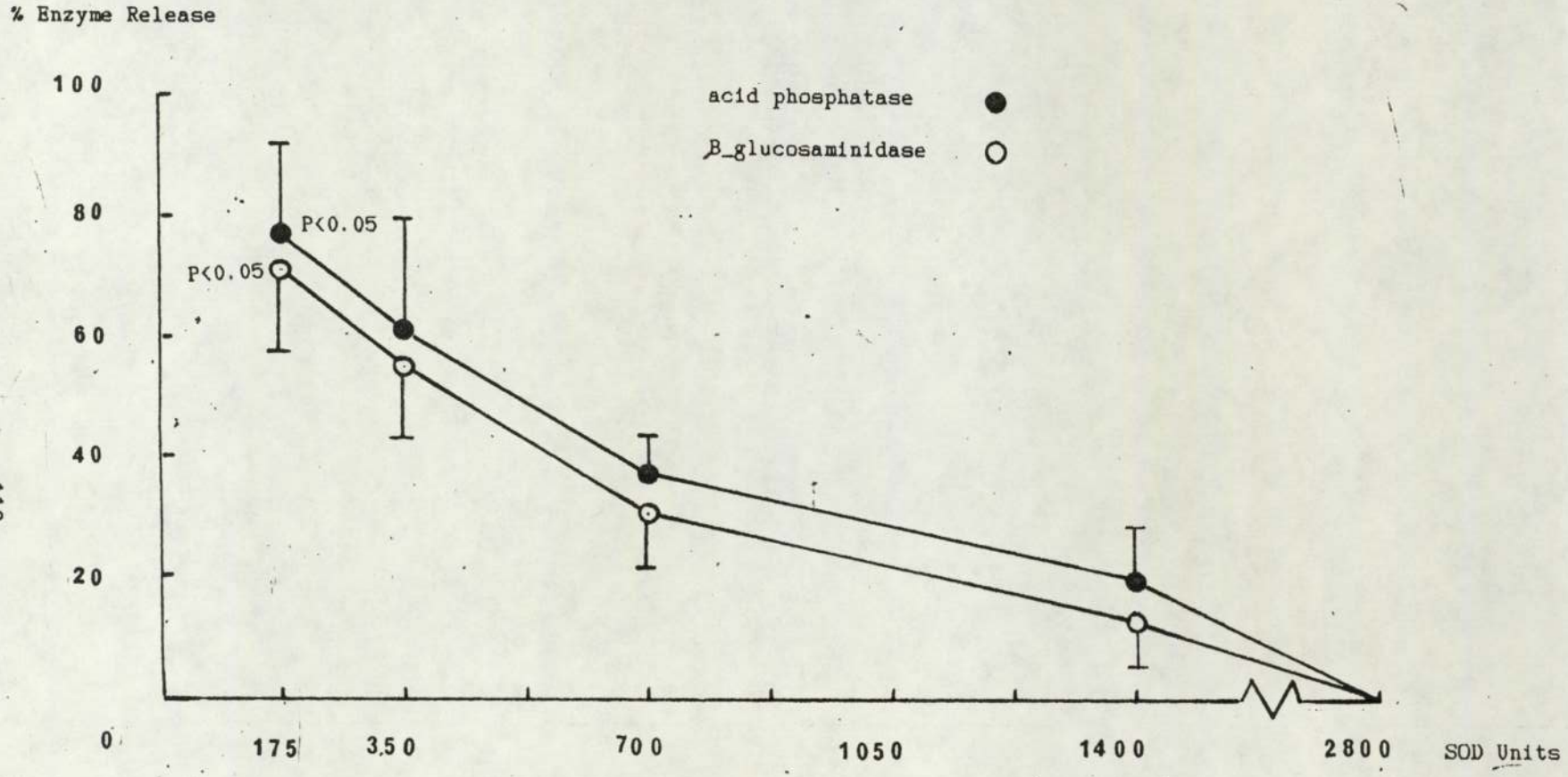


Figure 3.23 The effect of SOD on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes treated with with hypoxanthine/xanthine oxidase reaction.

Each result represents the mean of four determinations ± SEM.

3.11 The Effect of Oligoamines and EDTA on the Stability of Isolated Intact Guinea Pig Liver Lysosomes.

Figures 3.24, 3.25, 3.26, 3.27, 3.28, and 3.29 show that putrescine, spermidine, spermine, cadaverine, diaminopropane and EDTA respectively have no stabilizing effect on isolated lysosomes.

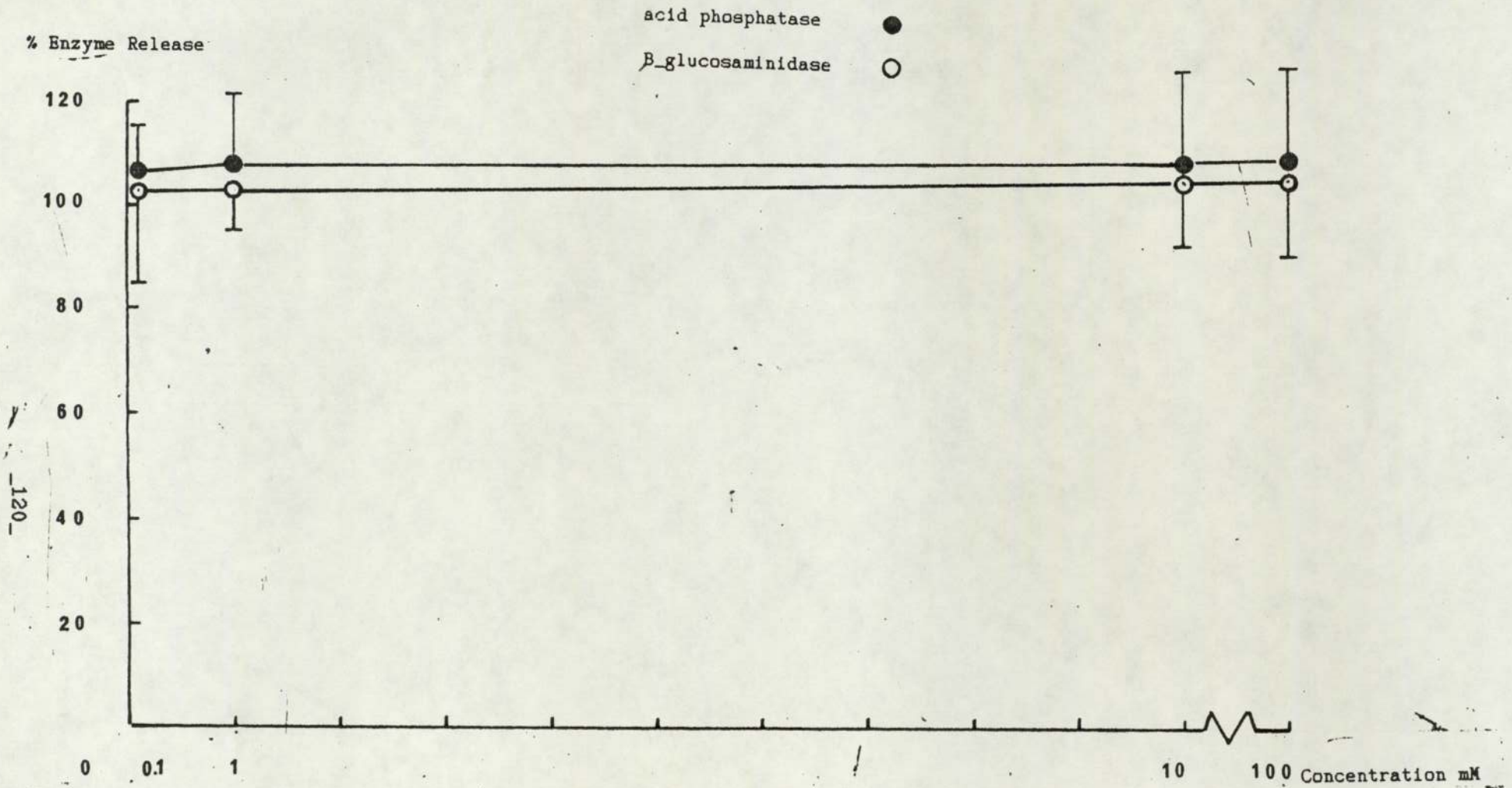


Figure 3.24 The effect of putrescine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

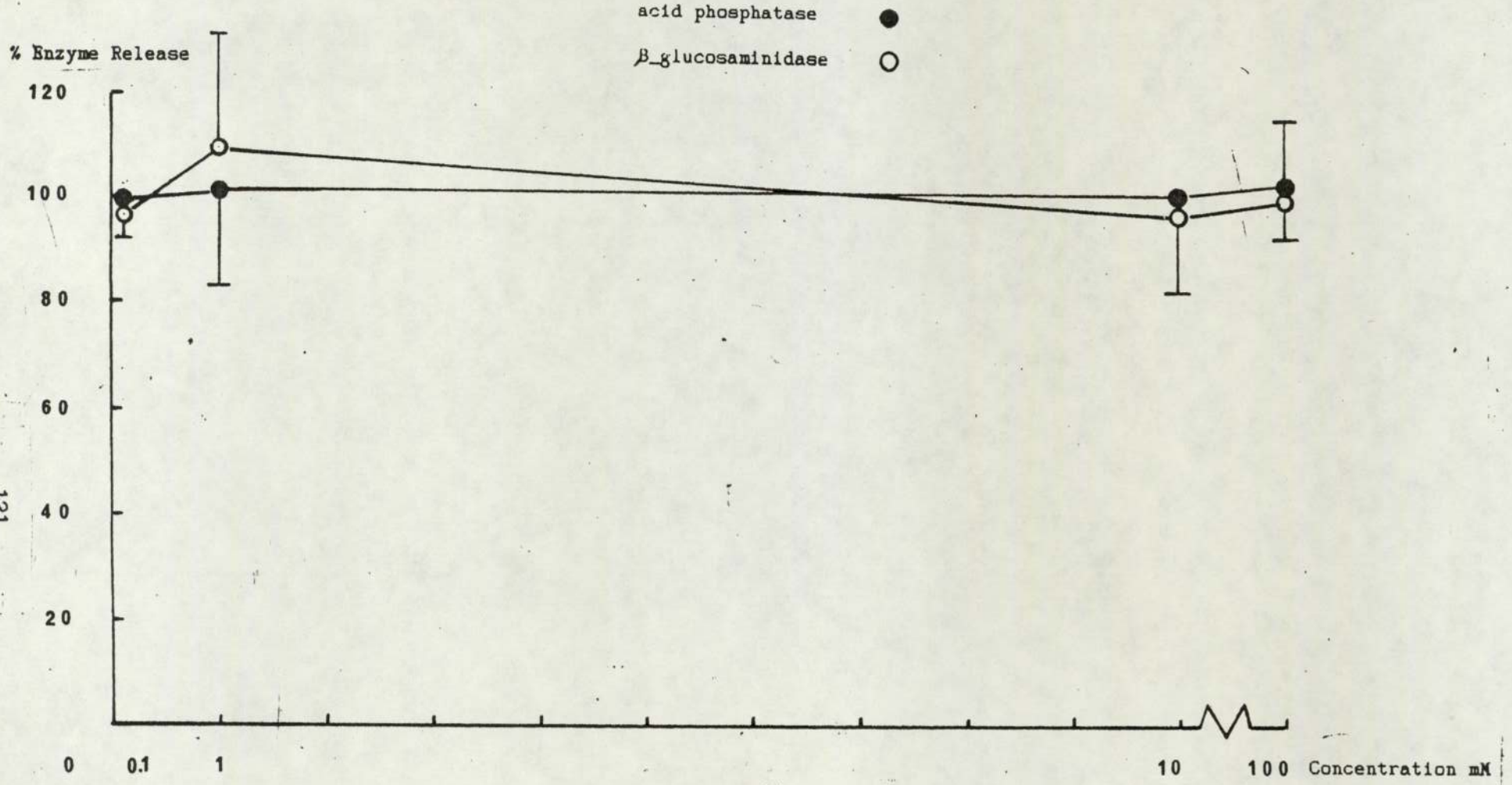


Figure 3.25 the effect of spermidine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

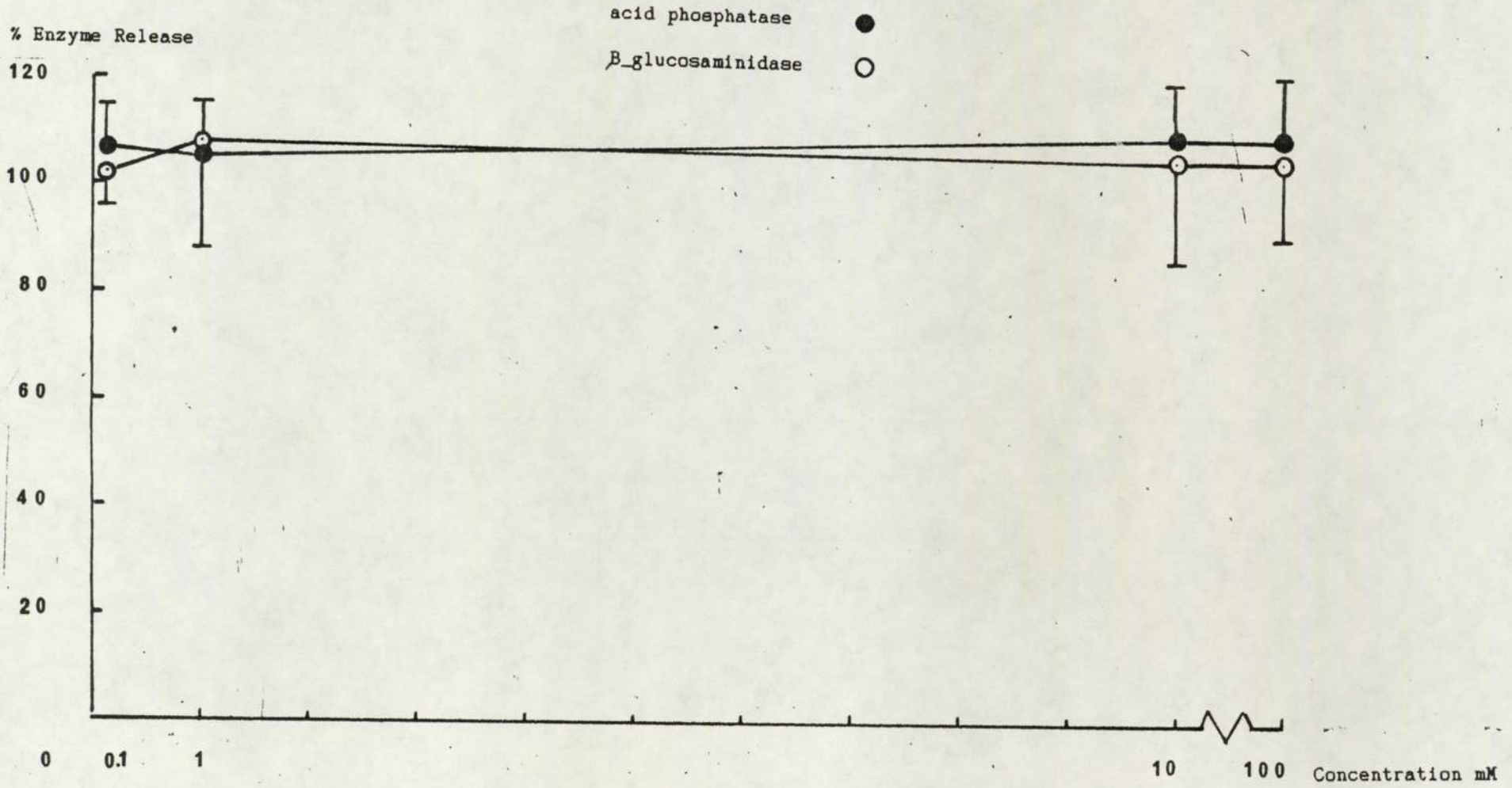


Figure 3.26 The effect of spermine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes. Each result represents the mean of four determinations ± SEM.

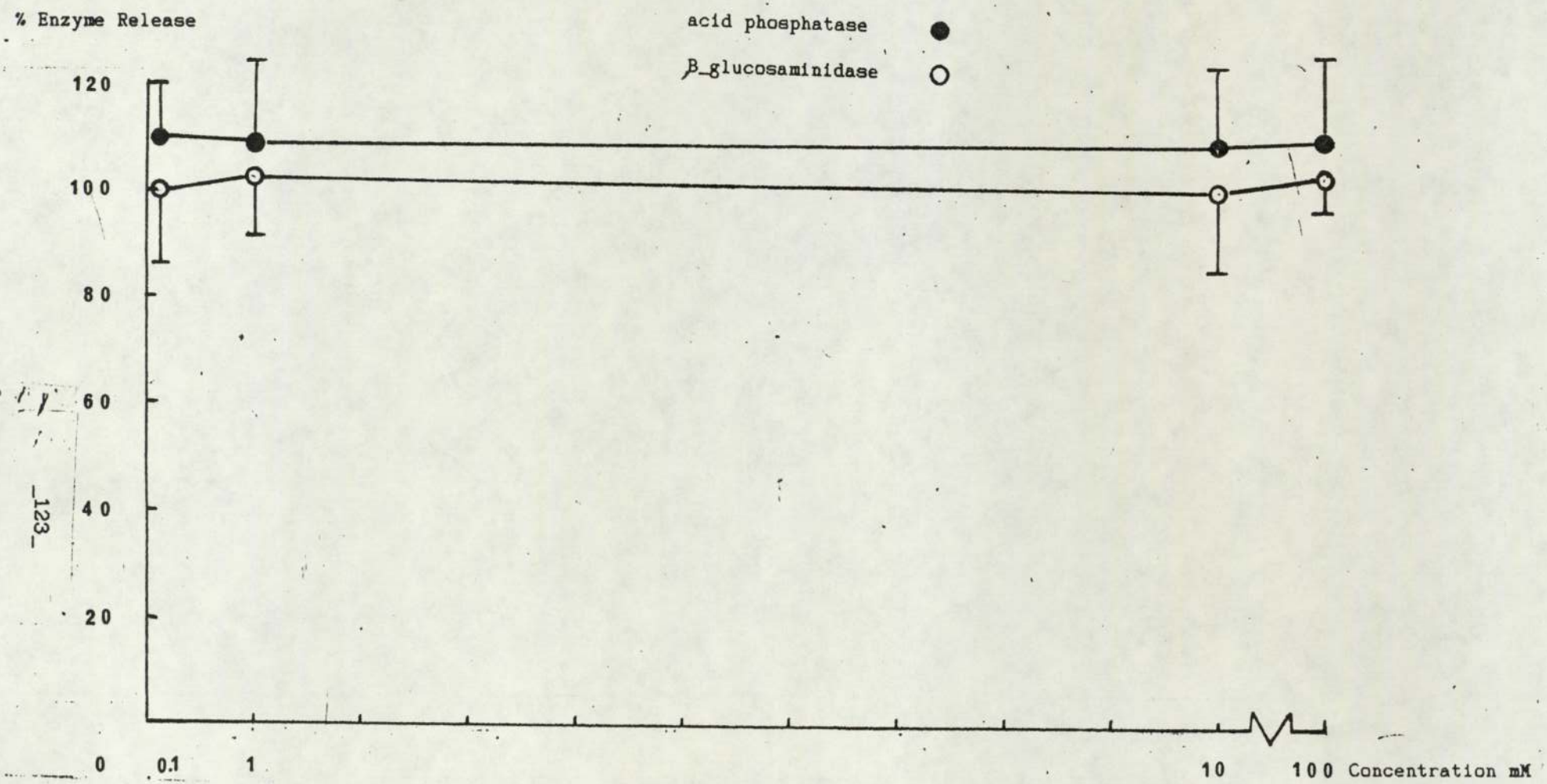


Figure 3.27 The effect of cadaverine on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

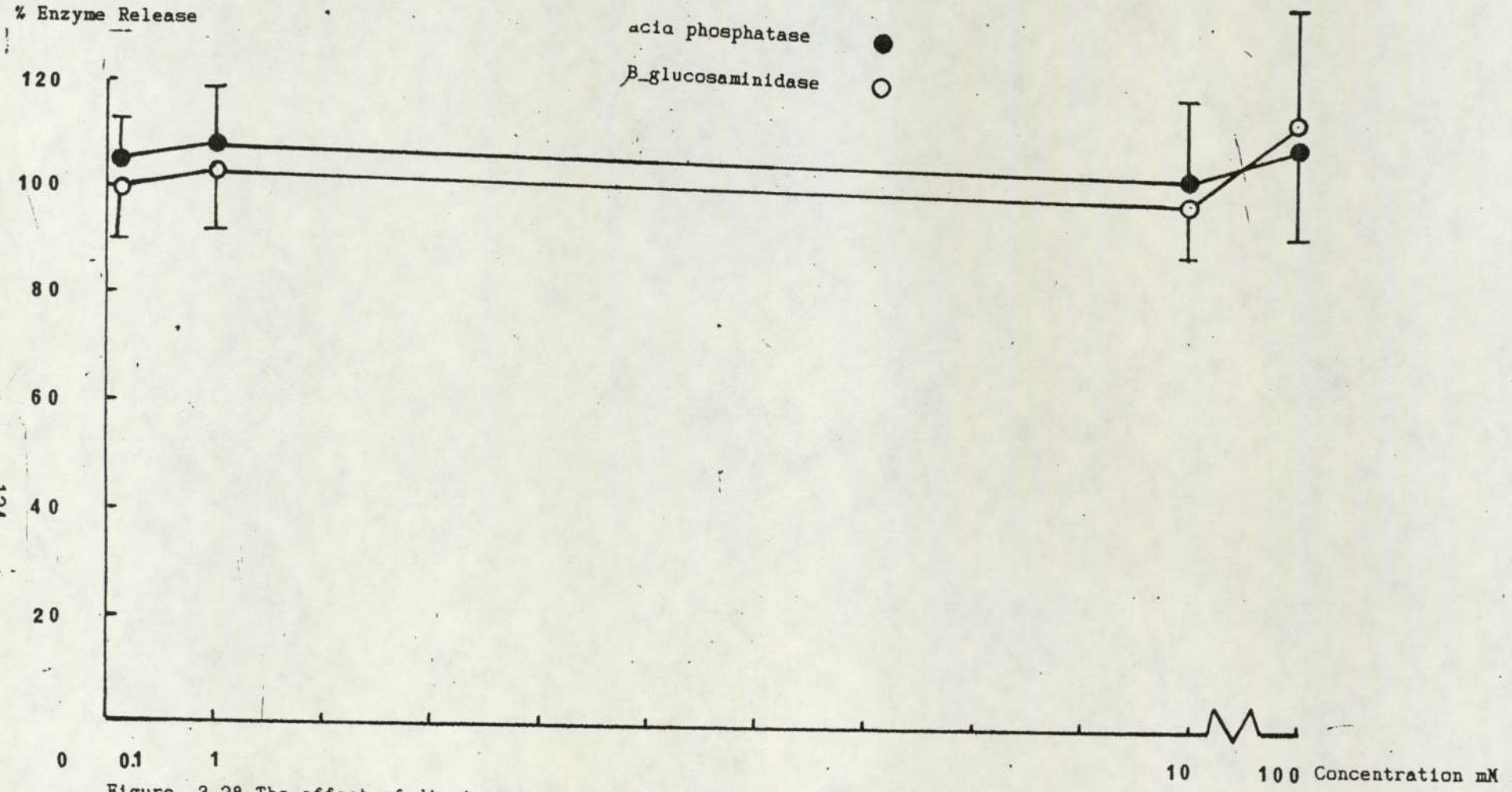


Figure 3.28 The effect of diaminopropane on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

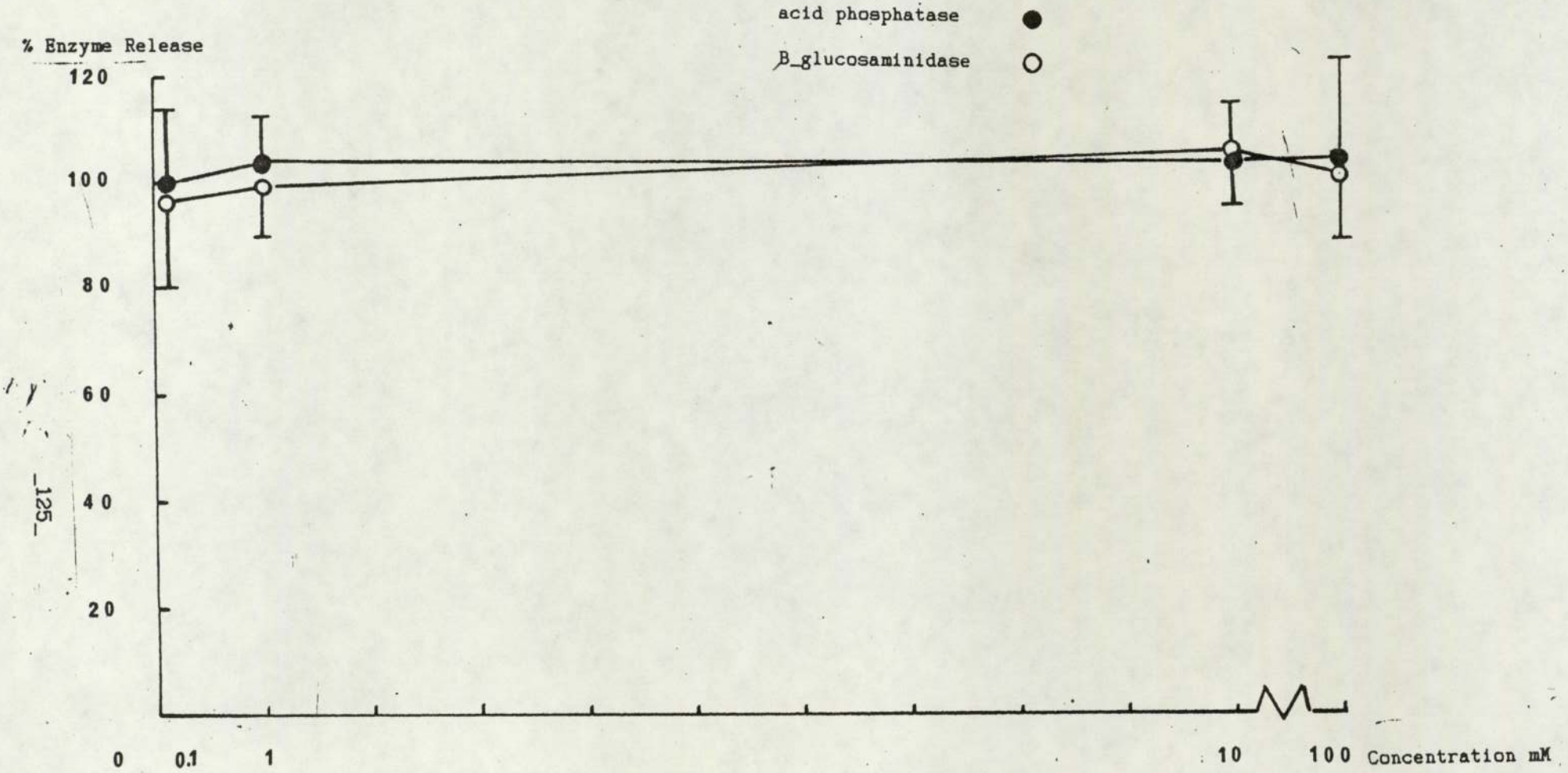


Figure 3.29 The effect of EDTA on the release of acid phosphatase and β-glucosaminidase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

3.12 The Uptake of Oligoamines By isolated Intact Guinea Pig Liver Lysosomes.

Figure 3.30 shows that (^{14}C) radioactive oligoamines studied were taken up by isolated intact lysosomes. When 5 n moles of radioactive oligoamines were added to the lysosome suspensions the uptake found was putrescine 1.18 ± 0.12 p moles; spermidine 1.09 ± 0.19 p moles and spermine 1.05 ± 0.15 p moles per mg protein. When 10 n moles were added the corresponding figures were putrescine 1.13 ± 0.05; spermidine 1.2 ± 0.12 and spermine 1.12 ± 0.09 p moles per mg protein. When 20 n moles were added the uptake was putrescine 2.4 ± 1; spermidine 1.9 ± 0.8 and spermine 1.7 ± 1.6 P moles per mg protein.

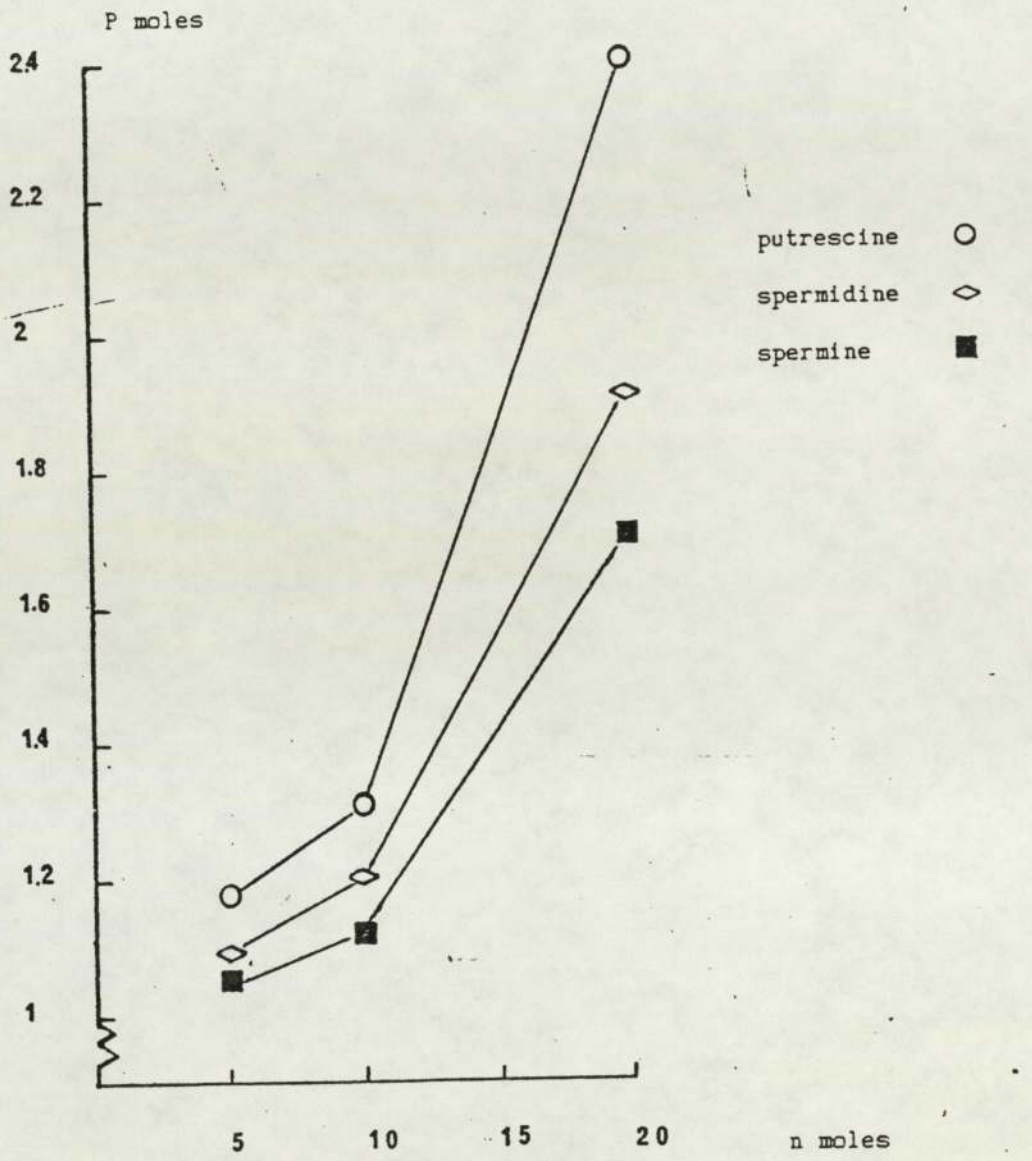


Figure3.30 The uptake of oligoamines by isolated intact guinea pig liver lysosomes .

Each result represents the mean of four determinations ± SEM.

3.13 The Effect of PMA Stimulated Peritoneal Guinea Pig PMNL's on the Stability of Isolated Intact Guinea Pig Liver Lysosomes .

The effect of increasing the number of stimulated PMNL's (to increase the fluxes of superoxide generated) on the lysis of lysosomes was that the lytic action significantly increased.

Figure 3.31 shows that when 5×10^5 stimulated PMNL's were incubated with the lysosome suspensions the amount of acid phosphatase released was 460% that of controls. When 8×10^6 stimulated PMNL's incubated with the lysosome suspensions the value of acid phosphatase released was 940%.

The results are expressed as a percentage of the control value where stimulated PMNL's were omitted.

3.14 The Effect of Oligoamines and EDTA on PMA Stimulated Peritoneal Guinea Pig PMNL's Induced Breakdown of Isolated Intact Guinea Pig Liver Lysosomes.

The oligoamines studied and EDTA protected lysosomes from breakdown by stimulated PMNL's.

Figure 3.32 shows that the oligoamines studied and EDTA inhibited the release of acid phosphatase from lysosomes in the presence of stimulated PMNL's. The results are expressed as a percentage of the values when the oligoamines or EDTA were omitted (adjusted to 100). Significance taken as $P<0.05$ (student t test) was at 10 mM for putrescine and diaminopropane, 20 mM for spermidine and EDTA, 40 mM for spermine and 60 mM cadaverine.

SOD used as a positive control significantly inhibited the release of acid phosphatase from lysosomes in the presence of stimulated PMNL's as Figure 3.33 shows.

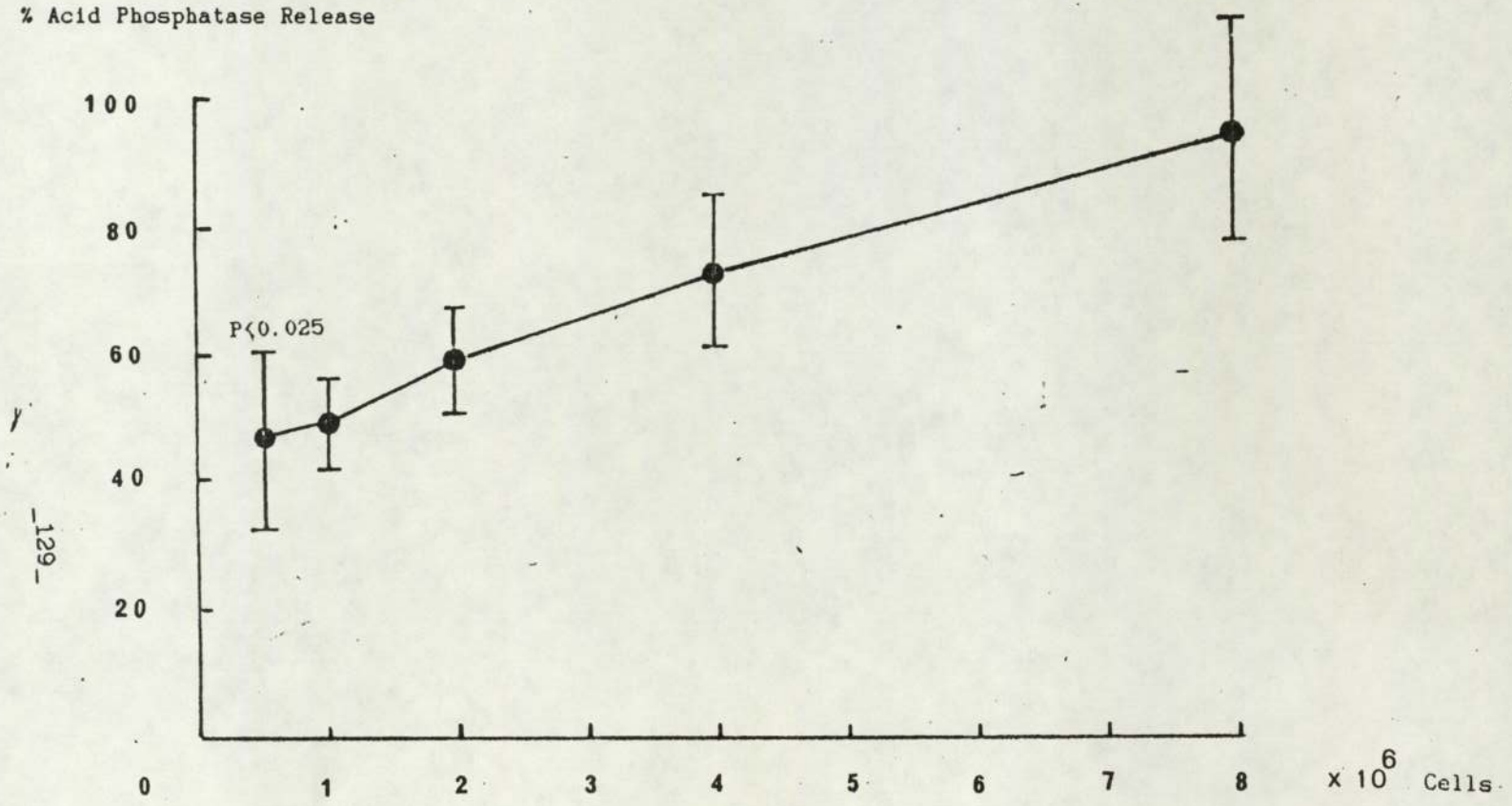


Figure 3.31 The effect of PMA stimulated PMNL's treated with cytochalasin-B on the release of acid phosphatase from isolated intact guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

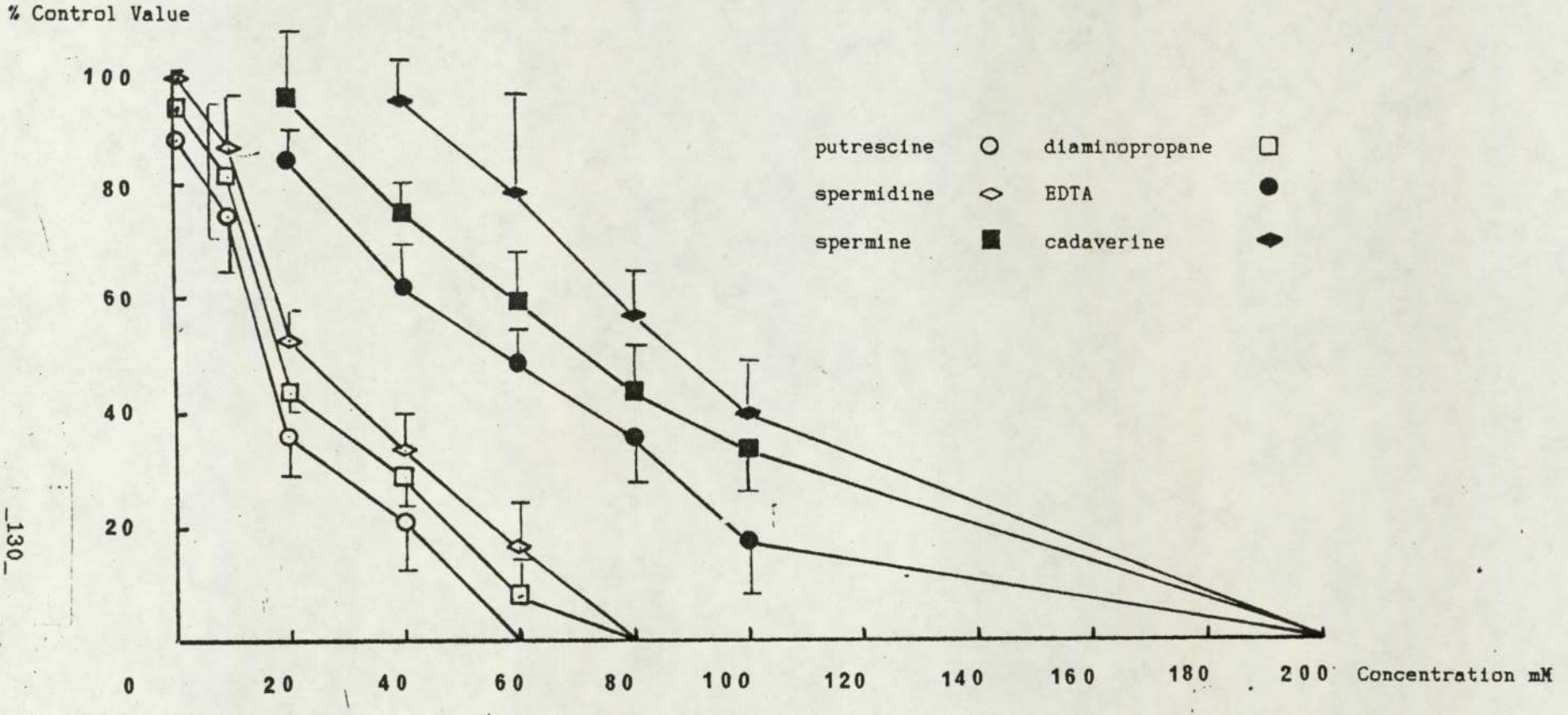


Figure 3.32 The effect of oligoamines and EDTA on the release of acid phosphatase from isolated intact guinea pig liver lysosomes treated with PMA stimulated PMNL's.

Each result represents the mean of four determinations ± SEM.

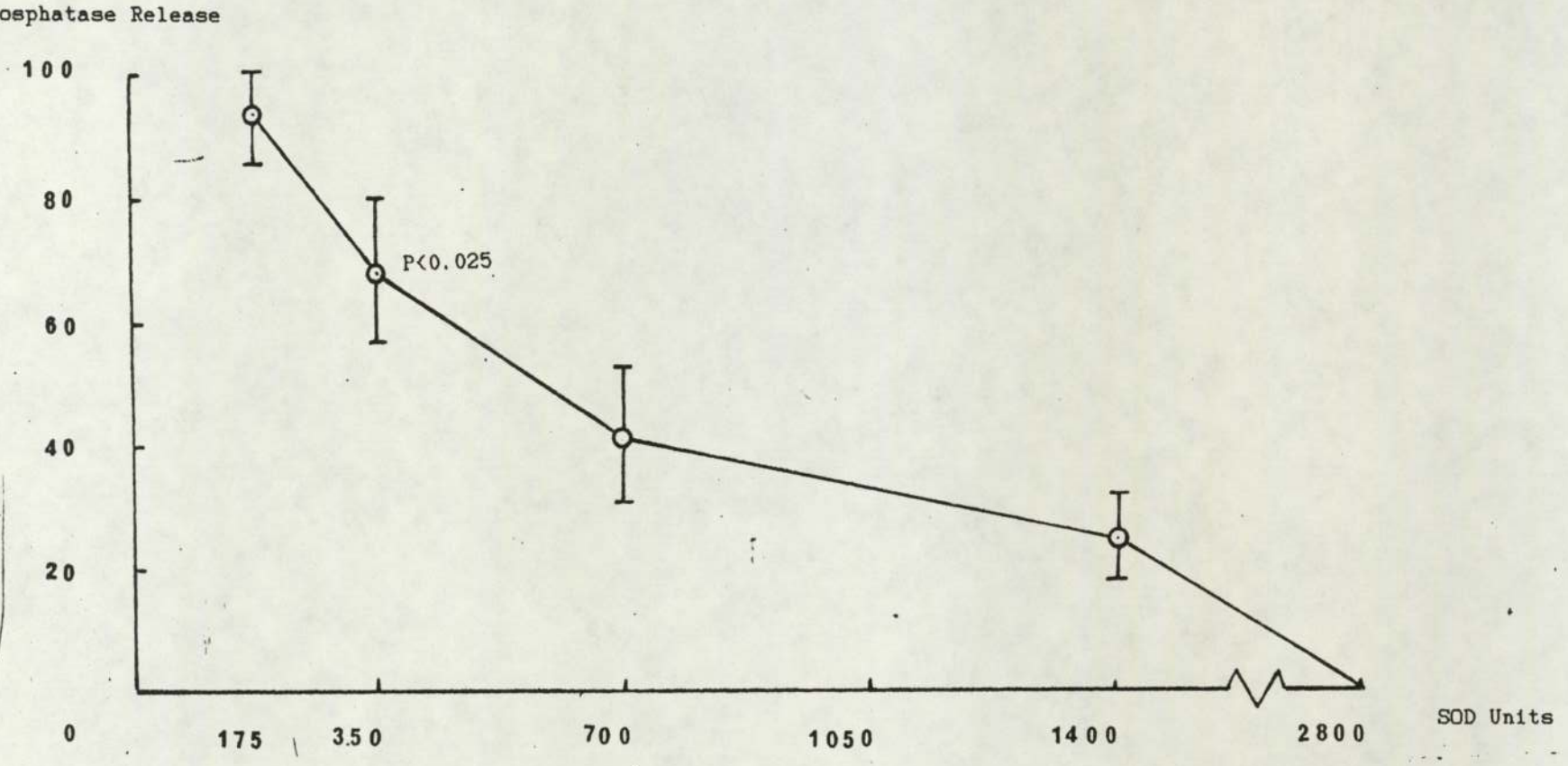


Figure 3.33 The effect of SOD on the release of acid phosphatase from isolated intact guinea pig liver lysosomes treated with PMA stimulated PMNL's.

Each result represents the mean of four determinations ± SEM.

3.15 Metabolism of Oligoamines by PMA Stimulated Peritoneal Guinea Pig PMNL's and the Hypoxanthine/Xanthine Oxidase System .

3.15.1 Metabolism of (^{14}C) Putrescine by Stimulated PMNL's .

Putrescine was found to be metabolised to metabolites tentatively identified as ɣ-aminobutyraldehyde, Δ-pyrolline, ɣ-aminobutyric acid and unchanged putrescine by stimulated PMNL's. ɣ_Aminobutyraldehyde is known to be unstable and rearranges non enzymatically to the more stable product Δ-pyrolline. Putrescine and ɣ-aminobutyric acid were identified by the use of their respective (^{14}C) standards ɣ-Aminobutyr-aldehyde and Δ-Pyrroline were tentatively identified from their chromatographic behaviour which was compared with that of the products of the reaction where putrescine was incubated with diamine oxidase. The spots identified as aldehyde derivatives also gave a positive reaction with Schiff's reagent.

Table 3.3 and 3.4 show the percentage of metabolites of putrescine by stimulated PMNL's and diamineoxidase respectively.

Table 3.3 % of Metabolites of (^{14}C) Putrescine by PMA Stimulated Peritoneal Guinea Pig PMNL's.

Metabolies	% of Total Count on TLC Plate
ɣ-Aminobutyraldehyde	5.6 ± 1.6
ɣ-Aminobutyric acid	8 ± 4.3
Δ-Pyrroline	20.7 ± 1.7
Unchanged (^{14}C) putrescine	65.7 ± 3.2

Each result represents the mean of four determinations ± SEM .

Table 3.4 % of Metabolites of (^{14}C) Putrescine by Diamine oxidase.

Metabolites	% of Total Count on TLC Plate
ɣ-Aminobutyraldehyde	29.9 ± 1.1
Δ-Pyrroline	15.2 ± 4
Unchanged (^{14}C) Putrescine	54.9 ± 2.7

Each result represents the mean of four determinations ± SEM.

Table 3.5 and 3.6 show Rf values for metabolites of (^{14}C) putrescine by stimulated PMNL's and diamine oxidase respectively.

Table 3.5 Rf Values for Metabolites of (^{14}C) Putrescine by PMA Stimulated PMNL's.

Metabolites	Rf Values			
	1	2	3	4
ɣ-Aminobutyraldehyde	0.38	0.22	0.16	0.09
ɣ-Aminobutyric acid	0.58	0.53	0.38	0.19
Δ-Pyrroline	0.82	0.74	0.73	0.50
Unchanged (^{14}C) putrescine	0.53	0.33	0.26	0.23
Standard (^{14}C) putrescine	0.51	0.30	0.28	0.25
Standard (^{14}C) ɣ-aminobutyric acid	0.60	0.49	0.41	0.20

The solvents used were:

1_1, propanol-water-conc. HCl

2_1, propanol-water-triethylamine

3_butanol-water-glacial acetic acid-pyridine

4_butanol-water-glacial acetic acid

Table 3.6 Rf Values for Metabolites of (^{14}C) Putrescine by Diamine oxidase.

Metabolites	Rf Values			
	1	2	3	4
ɣ-Aminobutyraldehyde	0.35	0.20	0.11	0.08
Δ-Pyrroline	0.80	0.74	0.68	0.48
Unchanged (^{14}C) putrescine	0.48	0.36.	0.27	0.28
Standard (^{14}C) putrescine	0.51	0.30	0.28	0.25

The solvents used were :

1_1, propanol-water-conc. Hcl

2_1, propano-wate-triethylamine

3_ butanol-water-glacial acetic acid-pyridine

4_ butanol-wate-glacial acetic acid

3.15.2 Metabolism of (^{14}C) Putrescine by the Hypoxanthine/Xanthine Oxidase Superoxide Generating System .

Putrescine was found to be metabolised to γ-aminobutyraldehyde, Δ-Pyrroline, and unchanged putrescine by hypoxanthine/xanthine oxidase system. Table 3.7 shows the percentage of the metabolites of (^{14}C) putrescine by hypoxanthine/xanthine oxidase system.

Table 3.7 % of Metabolites of (^{14}C) Putrescine by Hypoxanthine/Xanthine Oxidase System .

Metabolites	% of Total Count on TLC Plate
γ-Aminobutyraldehyde	8 ± 2.8
Δ- Pyrroline	19 ± 1.9
Unchanged (^{14}C) putrescine	73 ± 6.2

Each result represents the mean of four determinations ± SEM .

Table 3.8 shows Rf values of the metabolites of (^{14}C) putrescine by the hypoxanthine/xanthine oxidase system.

Table 3.8 Rf Values for Metabolites of (^{14}C) Putrescine by Hypoxanthine/Xanthine oxidase System.

Metabolites	Rf Values			
	1	2	3	4
γ_Aminobutyraldehyde	0.33	0.19	0.14	0.11
Δ- Pyrroline	0.90	0.82	0.65	0.53
Unchanged (^{14}C) putrescine	0.43	0.34	0.30	0.27
Standard (^{14}C) putrescine	0.51	0.31	0.28	0.25

The solvents used were :

1_ 1, Propanol-water-conc. Hcl 2_ 1, propanol-water-triethylamine

3_ butanol-water-glacial acetic acid-pyridine

4_ butanol-water-glacial acetic acid

3.15.3 Metabolism of (^{14}C) Spermidine by Stimulated PMNL's and Hypoxanthine/Xanthine Oxidase System.

Spermidine was found to be metabolised to an aldehyde derivative , minor unknown metabolite, and unchanged spermidine, when incubated with either stimulated PMNL's or hypoxanthine/xanthine oxidase system. Aldehyde derivative was detected by spraying TLC with Schiff's reagent and scanning the plates for radioactive substances.

Table 3.9 and 3.10 show the percentage of the metabolites of (^{14}C) spermidine by stimulated PMNL's and hypoxanthine/xanthine oxidase respectively.

Table 3.9 % of Metabolites of (^{14}C) Spermidine by Stimulated PMNL's.

Metabolites	% of Total Count on TLC Plate
Aldehyde derivative	29 ± 5.6
Unknown metabolite	3 ± 1.9
Unchanged (^{14}C) spermidine	68 ± 2.4

Each result represents the mean of four determinations ± SEM .

Table 3.10 % of Metabolites of (^{14}C) Spermidine by Hypoxanthin/ Xanthine Oxidase System.

Metabolites	% of Total Count on TLC Plate
Aldehyde derivative	17 ± 3.7
Unknown metabolite	5 ± 1.8
Unchanged (^{14}C) spermidine	78 ± 2.1

Each result represents the mean of four determinations ± SEM.

spermidine by stimulated PMNL's and hypoxanthine/xanthine oxidase system respectively.

Table 3.11 Rf Values for Metabolites of (^{14}C) Spermidine by Stimulated PMNL's.

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.59	0.49	0.21	o.11
Unknown metabolite	0.37	0.23	0.17	0.15
Unchanged (^{14}C) spermidine	0.48	0.33	0.25	0.09
Standard (^{14}C) spermidine	0.42	0.30	0.25	0.12

Table 3.12 Rf Values for Metabolites of (^{14}C) Spermidine by Hypoxanthine/Xanthine Oxidase System.

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.63	0.51	0.22	0.13
Unknown metabolite	o.36	0.20	0.14	0.14
Unchanged (^{14}C)spermidine	0.46	0.31	0.27	0.11
Standard (^{14}C) spermidine	0.42	0.30	0.25	0.12

The solvents used were :

1_1, propano-_water-conc. Hcl 2_ 1, propanol-water-triethylamine

3_butanol-water-glacial acetic acid-pyridine

4_butanol-water-glacial acetic acid

3.15.4 Metabolism of (^{14}C) Spermine by Stimulated PMNL's and Hypoxanthine/Xanthine Oxidase System.

Spermine was found to be metabolised to an aldehyde derivative, a minor unknown metabolite, and unchanged spermine, when incubated with either stimulated PMNL's or hypoxanthine/xanthine oxidase system. The Aldehyde derivative was detected by spraying the TLC plates with Schiff's reagent and scanning the plate for radioactive substances . Table 3.13 and 3.14 show the percentage of the metabolites of (^{14}C) spermine by stimulated PMNL's and hypoxanthine/xanthine oxidase system respectively.

Table 3.13 % of Metabolites of (^{14}C) Spermine by Stimulated PMNL's.

Metabolites	% of Total Count on TLC Plate
Aldehyde derivative	15 ± 3.6
Unknown metabolite	8 ± 4.1
Unchanged (^{14}C) spermine	77 ± 1.3

Each result represents the mean of four determinations ± SEM.

Table 3.14 % of Metabolites of (^{14}C) Spermine by Hypoxanthine/ Xanthine Oxidase System.

Metabolites	% of Total Count on TLC Plate
Aldehyde derivative	9.7 ± 2
Unknown metabolite	4 ± 3.7
Unchanged (^{14}C) spermine	86.3 ± 0.9

Each result represents the mean of four determinations ± SEM.

3.15 and 3.16 show the Rf values for metabolites (^{14}C) spermine by stimulated PMNL's and the hypoxanthine/xanthine oxidase system respectively.

Table 3.15 Rf Values for Metabolites of (^{14}C) Spermine by Stimulated PMNL's

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.63	0.50	0.28	0.19
Unknown metabolite	0.45	0.34	0.28	0.22
Unchanged (^{14}C) spermine	0.39	0.19	0.16	0.08
Standard (^{14}C) spermine	0.43	0.23	0.11	0.06

Table 3.16 Rf Values for Metabolites of (^{14}C) Spermine by Hypoxanthine/Xanthine Oxidase System.

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.68	0.59	0.30	0.23
Unknown metabolite	0.46	0.31	0.26	0.24
Unchanged (^{14}C) spermine	0.41	0.25	0.13	0.11
Standard (^{14}C) spermine	0.43	0.23	0.11	0.06

The solvents used were :

1_1, propano-_water-conc. Hcl 2_ 1, propanol-water-triethylamine

3_butanol-water-glacial acetic acid-pyridine

4_butanol-water-glacial acetic acid

3.15.5 Metabolism of Cadaverine by Stimulated PMNL's and Hypoxanthine/xanthine Oxidase System.

Cadaverine was found to be metabolised to an aldehyde derivative, an unknown metabolite, and unchanged cadaverine by either stimulated PMNL's or hypoxanthine/xanthine oxidase system. The aldehyde derivative was tentatively identified from its chromatographic behaviour which was compared with that of the products of the reaction where cadaverine was incubated with diamine oxidase. The spots identified as aldehyde derivative gave positive reactions with Schiff's reagent. Unchanged cadaverine was identified by the use of standard cadaverine.

Table 3.17, 3.18 and 3.19 show Rf values for metabolites of cadaverine by stimulated PMNL's, diamine oxidase and hypoxanthine/xanthine oxidase respectively.

Table 3.17 Rf Values for Metabolites of cadaverine by Stimulated PMNL's.

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.58	0.31	0.20	0.14
Unknown metabolite	0.37	0.35	0.29	0.20
Unchanged cadaverine	0.71	0.56	0.45	0.30
Standard cadaverine	0.80	0.60	0.51	0.28

The solvents used were :

1_1, propano-_water-conc. Hcl 2_ 1, propanol-water-triethylamine

3_butanol-water-glacial acetic acid-pyridine

4_butanol-water-glacial acetic acid

Table 3.18 Rf Values for Metabolites of Cadaverine by Diamine Oxidase

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.63	0.29	0.23	0.09
Unknown metabolite	0.43	0.38	0.30	0.23
Unchanged cadaverine	0.74	0.58	0.48	0.33
Standard cadaverine	0.80	0.60	0.51	0.28

Table 3.19 Rf Values for Metabolites of Cadaverine by Hypoxanthine/Xanthine Oxidase System.

Metabolites	Rf Values			
	1	2	3	4
Aldehyde derivative	0.65	0.30	0.25	0.10
Unknown metabolite	0.45	0.40	0.20	0.18
Unchanged cadaverine	0.78	0.51	0.40	0.25
Standard cadaverine	0.80	0.60	0.51	0.28

The solvents used were :

1_1, propano-_water-conc. Hcl 2_ 1, propanol-water-triethylamine

3_butanol-water-glacial acetic acid-pyridine

4_butanol-water-glacial acetic acid

3.16 Qualitative Determination of a Aldehyde Derivative as One of the Metabolites of Oligoamines by PMA Stimulated PMNL's and the Hypoxanthine/Xanthine Oxidase System.

The formation of an aldehyde derivative as one of the metabolites of the oligogamines produced by stimulated PMNL's or the hypoxanthine/ xanthine oxidase system was shown qualitatively by the observation of a red colour with dinitrophenyl hydrazine reagent. 1 ml of the incubation mixture, (in section 2.15) was treated with 0.5 ml of dinitrophenyl hydrazine in 6 N Hcl for 10 min at 37 °C, and then mixed with 2 ml of 3 N NaoH (Friedemann, and Haugen, 1943). A red colour indicated a positive reaction for aldehydes.

3.17 The Effect of Oligoamines and EDTA on the Activities of Free Acid Phosphatase and β Glucosaminidase of Guinea Pig Liver Lysosomes.

Figures 3.34, 3.35, 3.36, 3.37, 3.38, and 3.39 show that putrescine, spermidine, spermine, cadavereine, diaminopropane, and EDTA respectively have no significant effect on the activities of acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes.

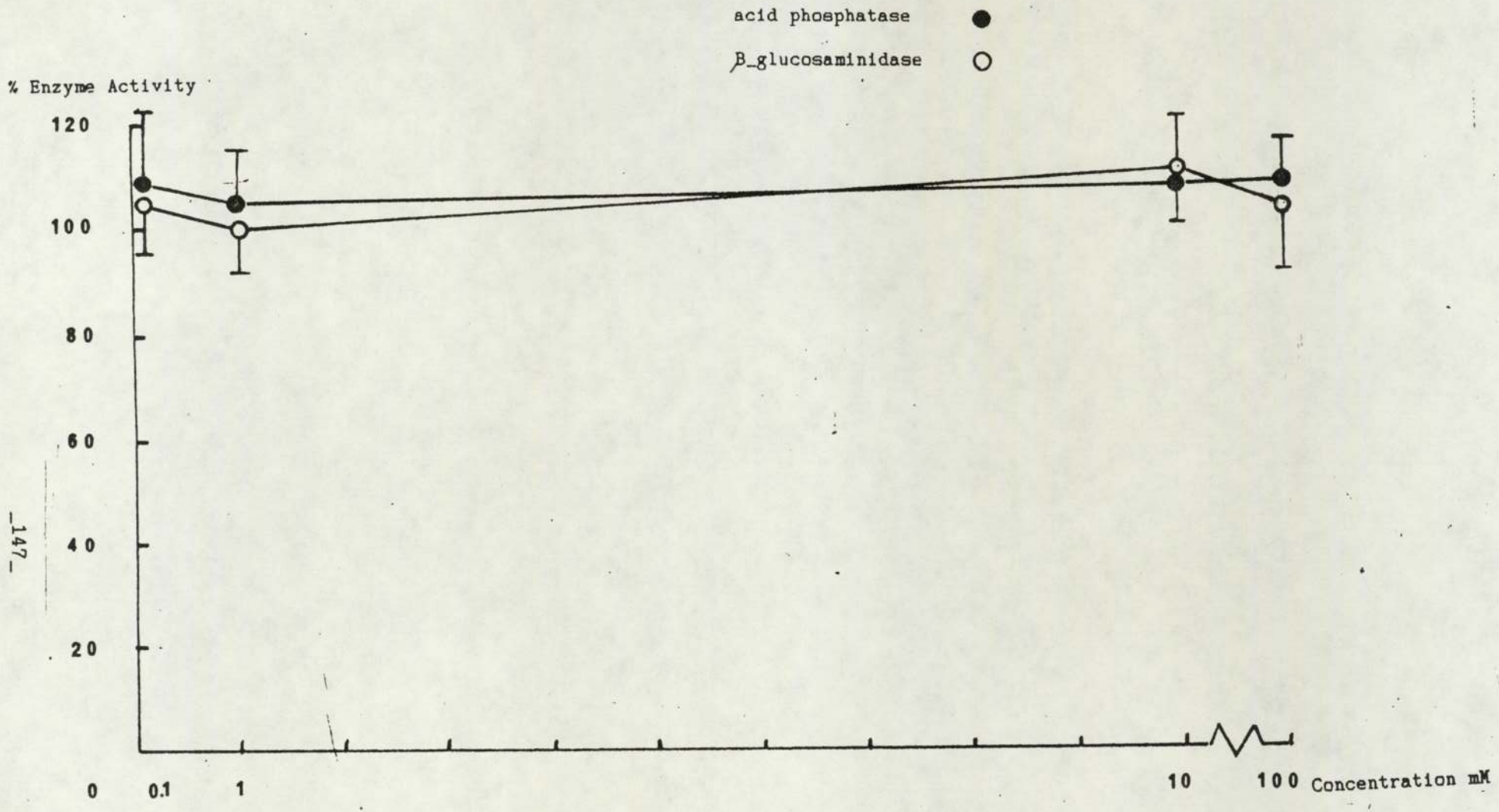


Figure 3.34 The effect of putrescine on the activities of free acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

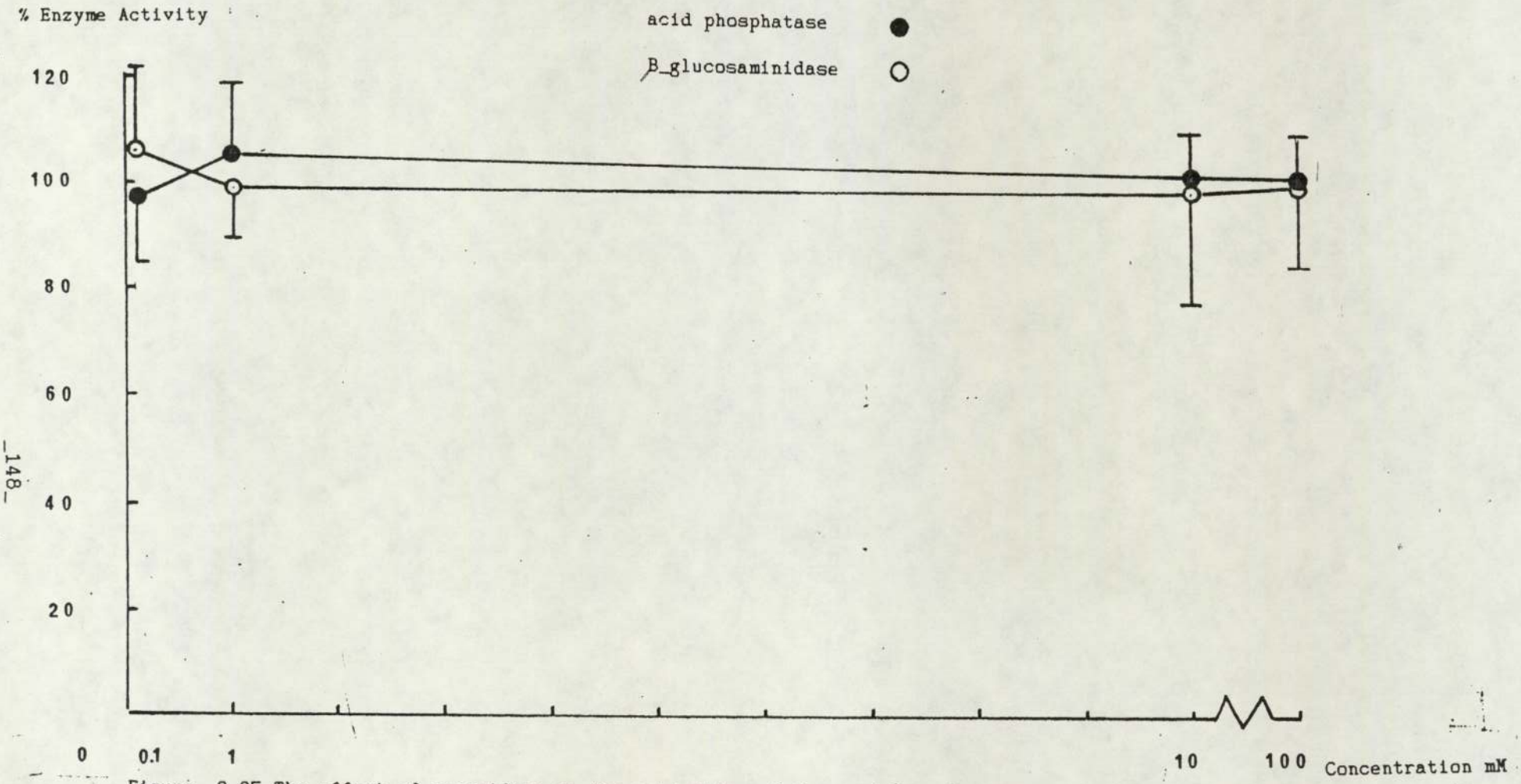


Figure 3.35 The effect of spermidine on the activities of free acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes.

Each result represents the mean of four determinations ± SEM.

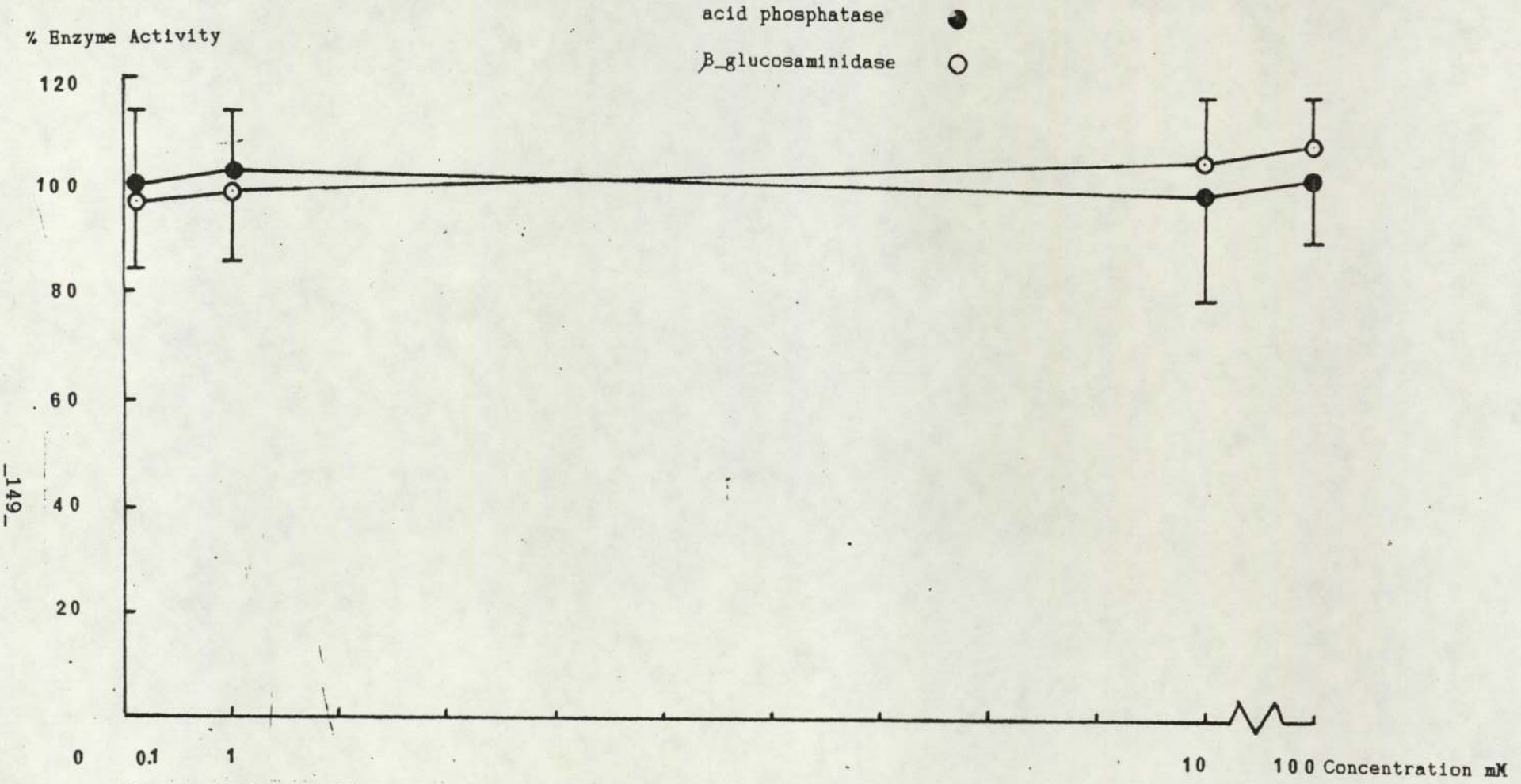


Figure 3.36 The effect of spermine on the activities of free acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes. Each result represents the mean of four determinations ± SEM.

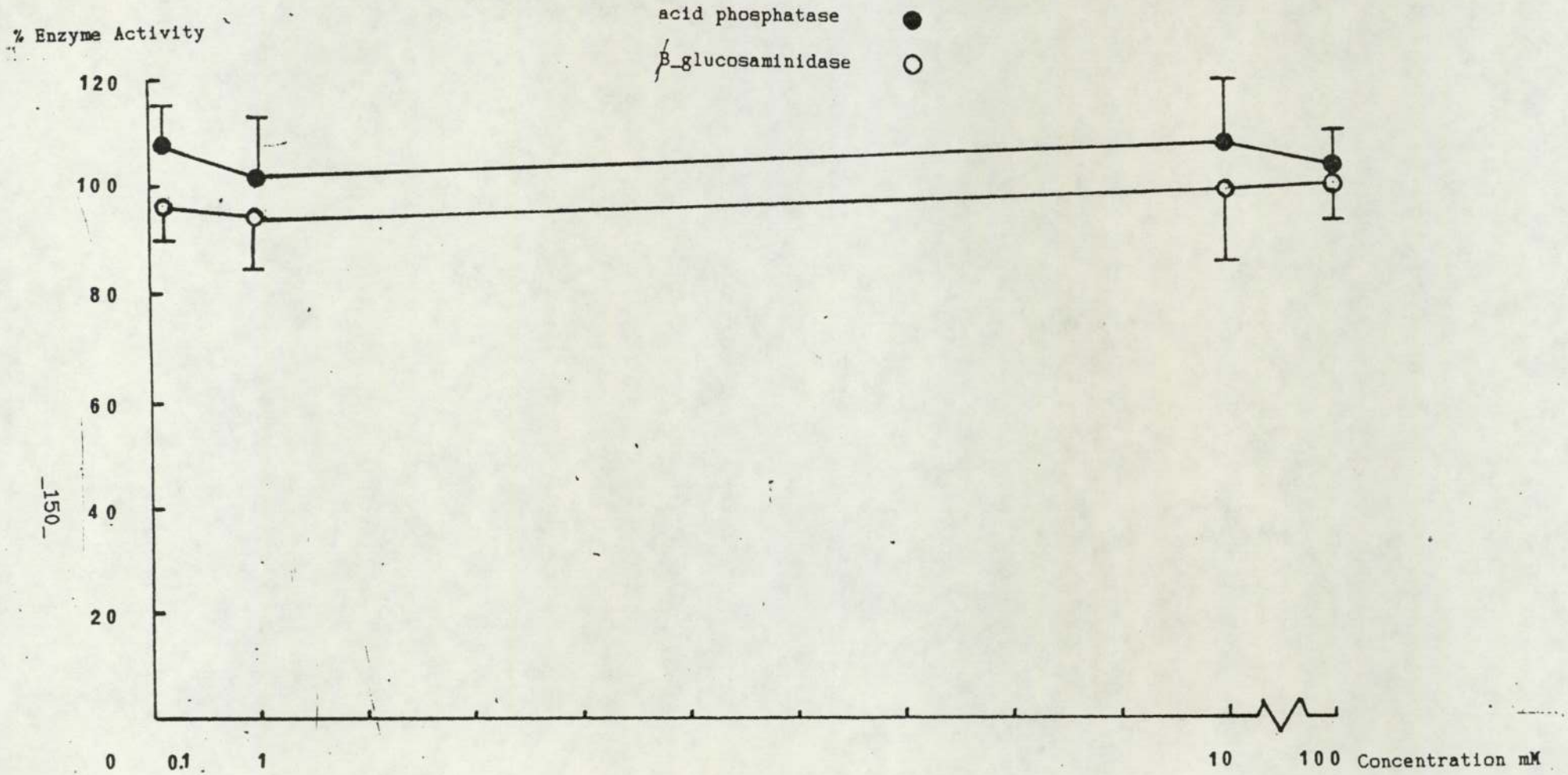
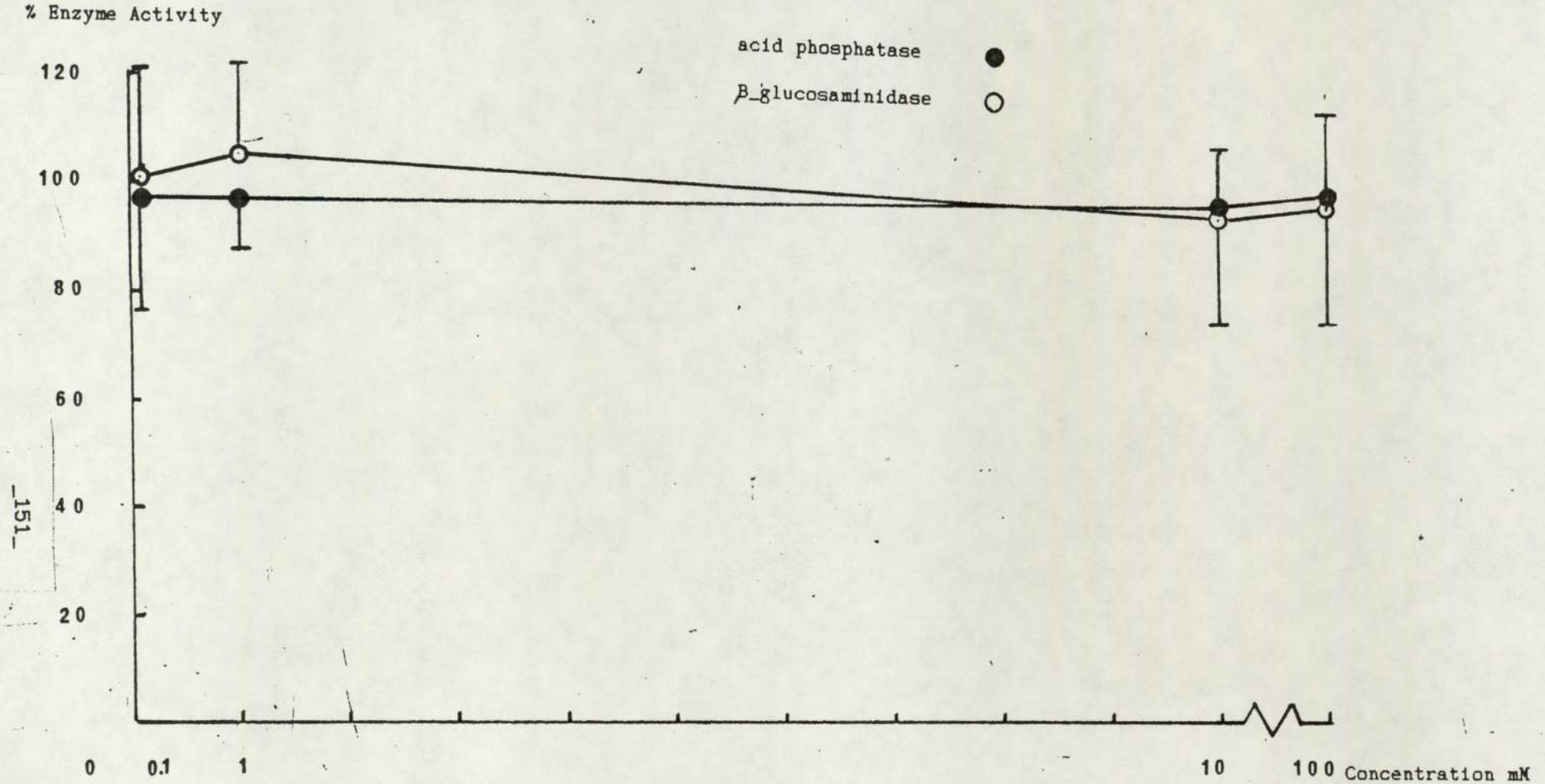


Figure 3.37 The effect of cadaverine on the activities of free acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes. Each result represents the mean of four determinations ± SEM.



Figuer 3.38 The effect of diaminopropane on the activities of free acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes. Each result represents the mean of four determinations ± SEM.

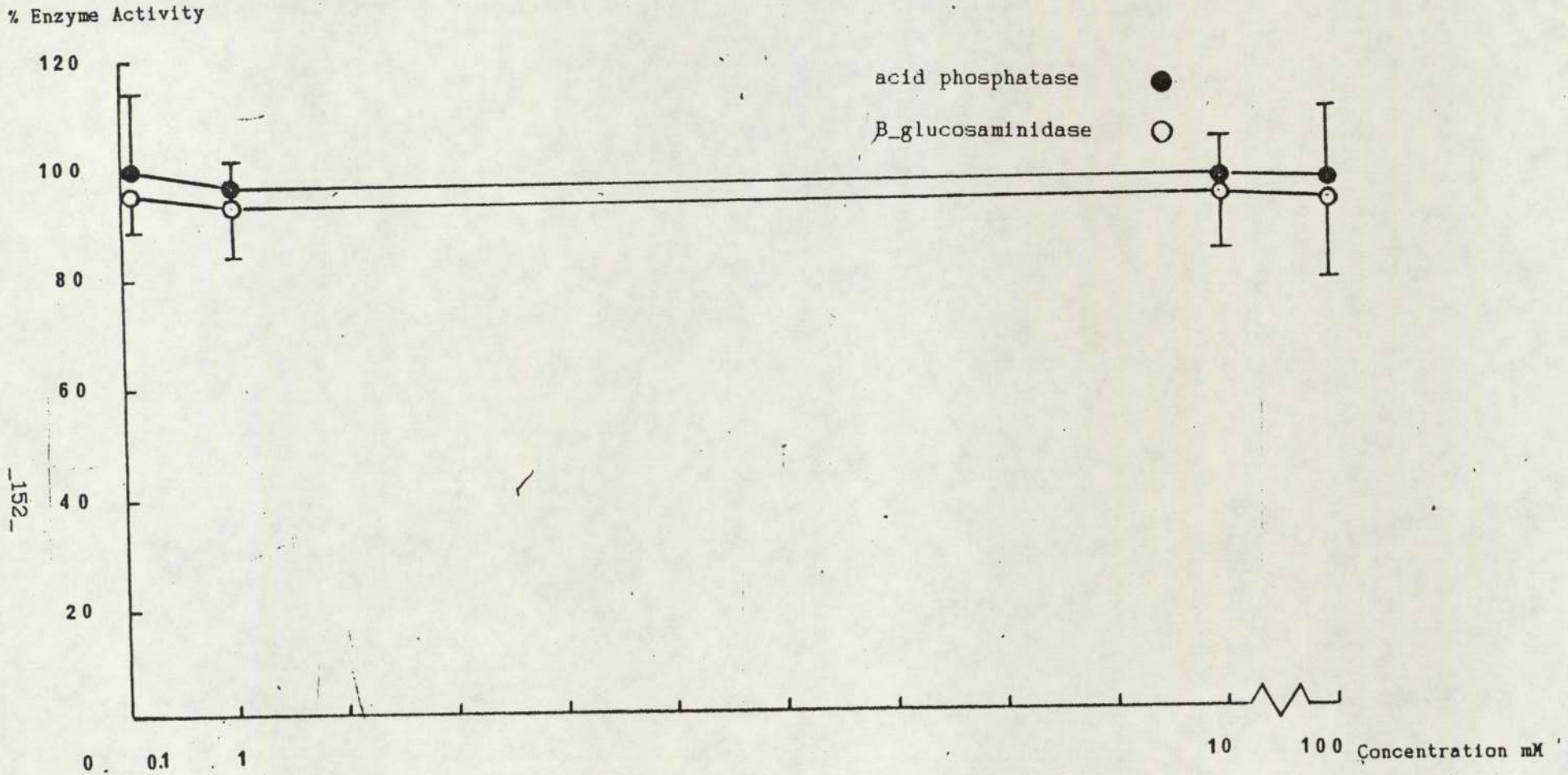


Figure 3.39 The effect of EDTA on the activities of free acid phosphatase and β-glucosaminidase of guinea pig liver lysosomes. Each result represents the mean of four determinations ± SEM.

3.18 The Effect of Oligoamines and EDTA on Lipid Peroxidation in Guinea Pig Liver Lysosomes.

3.18.1 The Effect of Oligoamines and EDTA on Lipid Peroxidation in Guinea Pig Liver Lysosomes Induced by Hypoxanthine/Xanthine Oxidase System.

Figure 3.40 shows that the oligoamines studied and EDTA inhibited lipid peroxidation in guinea pig liver lysosomes induced by hypoxanthine/xanthine oxidase system. The results are expressed as percentage of the absorbance values of 532 nm when oligoamines and EDTA were omitted.

Putrescine significantly ($p < 0.05$) inhibited lipid peroxidation, in guinea pig liver lysosomes, at 5 mM (17%). At 10 mM the significant ($p < 0.05$) inhibitory effect of diaminopropane was 19%. At 20 mM spermidine and EDTA significantly ($p < 0.05$) inhibited lipid peroxidation (28% and 23% respectively). The significant ($p < 0.05$) inhibitory effect of spermine and cadaverine was at 40 mM (54% and 33%).

3.18.2 The Effect of Oligoamines and EDTA on Lipid Peroxidation in Guinea Pig Liver Lysosomes Induced by Ascorbic Acid.Ferrous Sulphate.

Figure 3.41 shows that the oligoamines studied and EDTA inhibited lipid peroxidation, in guinea pig liver lysosomes, induced by ascorbic acid_ferrous sulphate. The significant ($p < 0.05$) inhibitory effect of putrescine, diaminopropane, spermidine, and EDTA was at 40 mM 61% , 59%, 55%, and 40% respectively). Spermine and cadaverine significantly ($p < 0.05$) inhibited lipid peroxidation at 80 mM (34% , and 28% respectively).

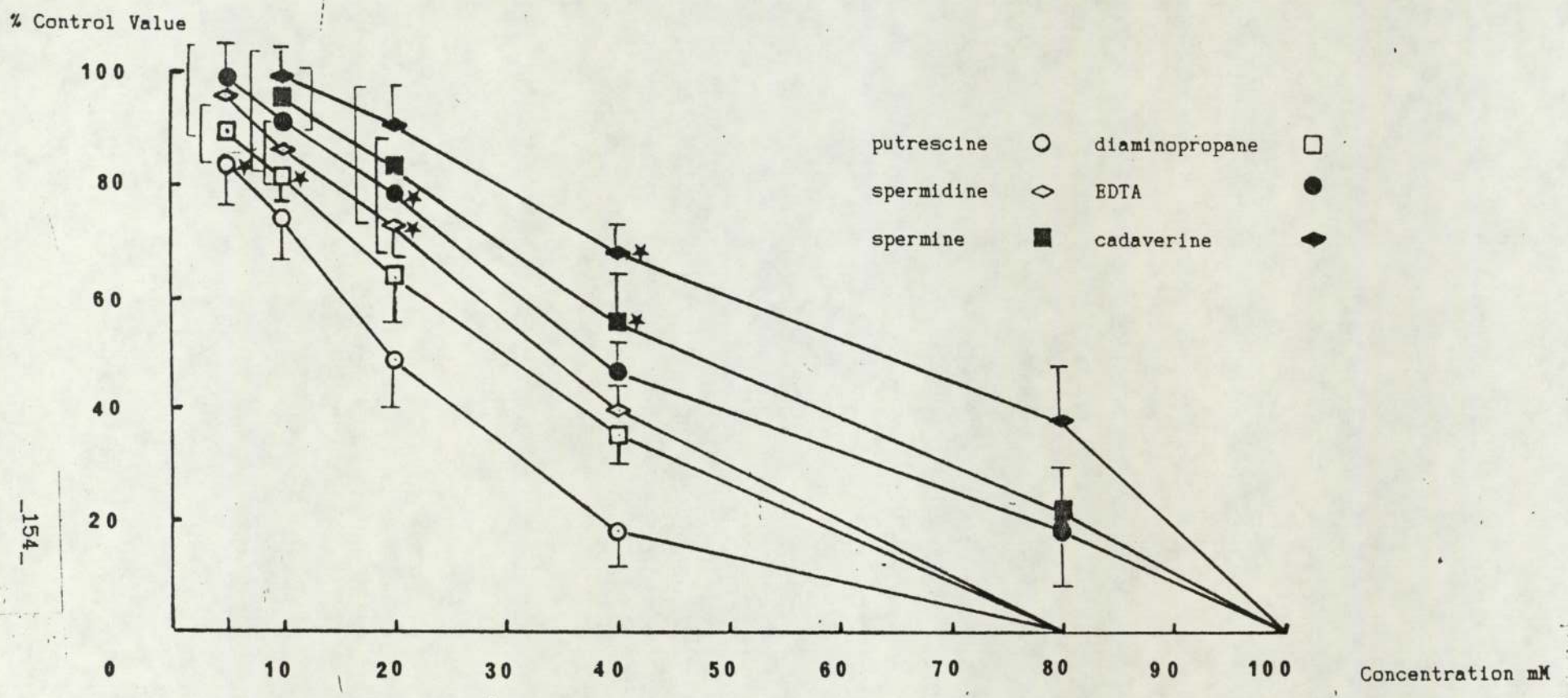


Figure 3.40 The effect of oligoamines and EDTA on hypoxanthine/xanthine oxidase induced lipid peroxidation in guinea pig liver lysosomes.
Each result represents the mean of four determinations ± SEM.
Significance was taken at $P < 0.05$ (★) (student t test) at the lowest effective concentration.

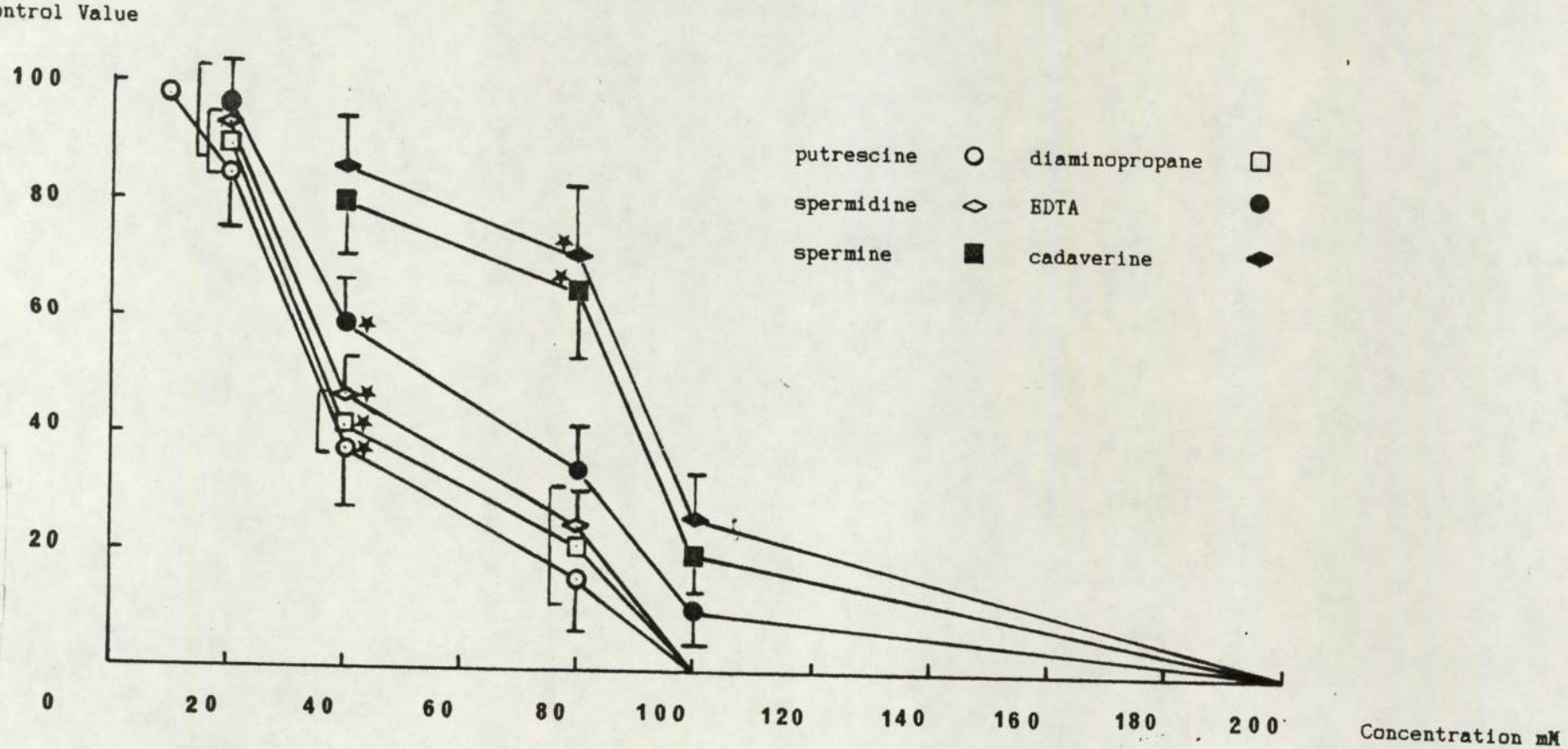


Figure 3.41 The effect of oligoamines and EDTA on ascorbic acid_ferrous sulphate induced lipid peroxidation in guinea pig liver lysosomes

Each result represents the mean of four determinations ± SEM.

Sinificance was taken at $P < 0.05$ (★) (student t test) at the lowest effective concentration.

3.19 The Effect of Oligoamine and EDTA on the Release of PGE_2 From PMA Stimulated Peritoneal Guinea Pig PMNL's .

Figures 3.42, 3.43, 3.44, 3.45, 3.46, and 3.47 show that putrescine, spermidine, spermine, cadaverine, diaminopropane, and EDTA respectively have no significant effect on the release of PGE_2 from PMA stimulated PMNL's.

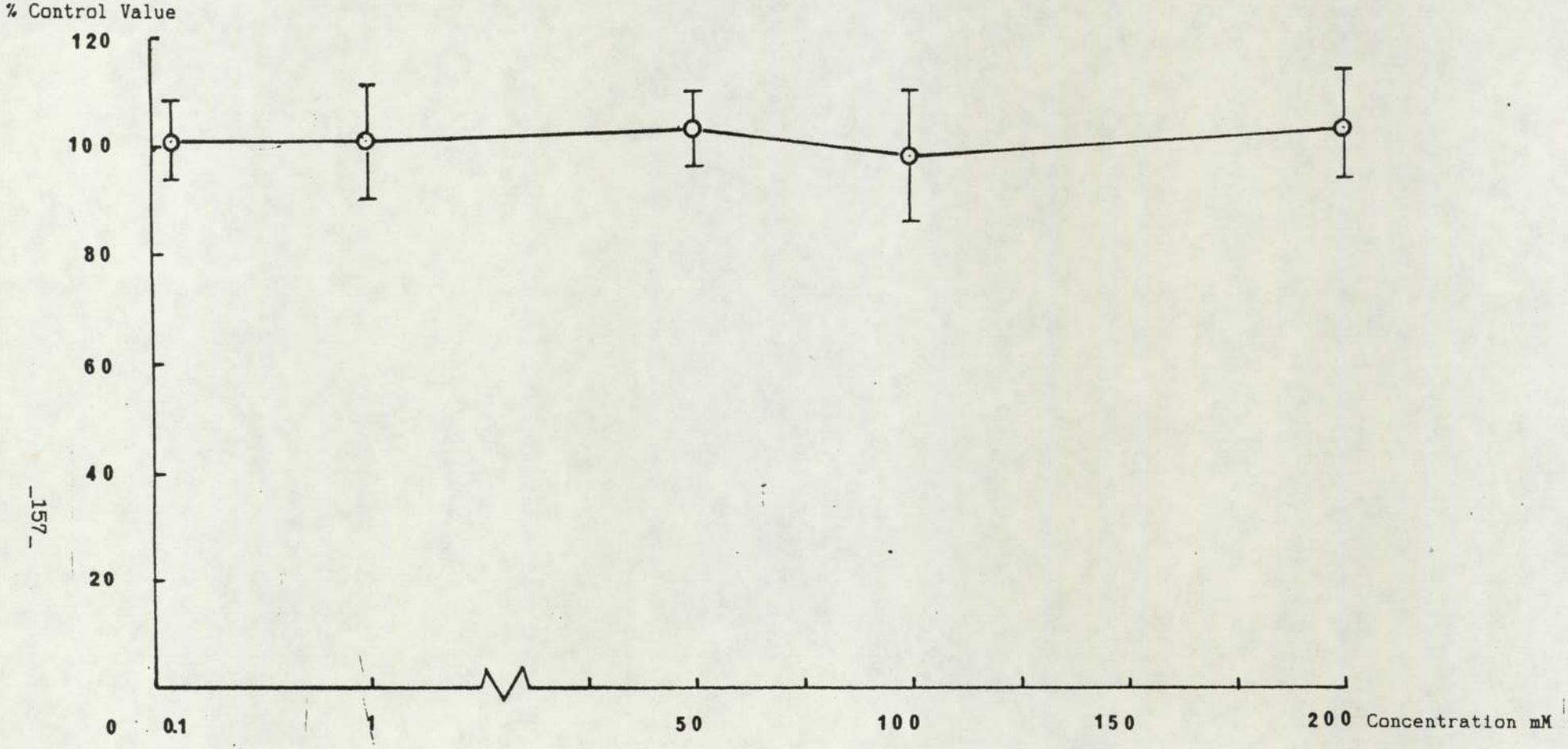


Figure 3.42 The effect of putrescine on the release of PGE_2 from PMA stimulated peritoneal guinea pig PMNL's.

Each result represents the mean of four determinations ± SEM.

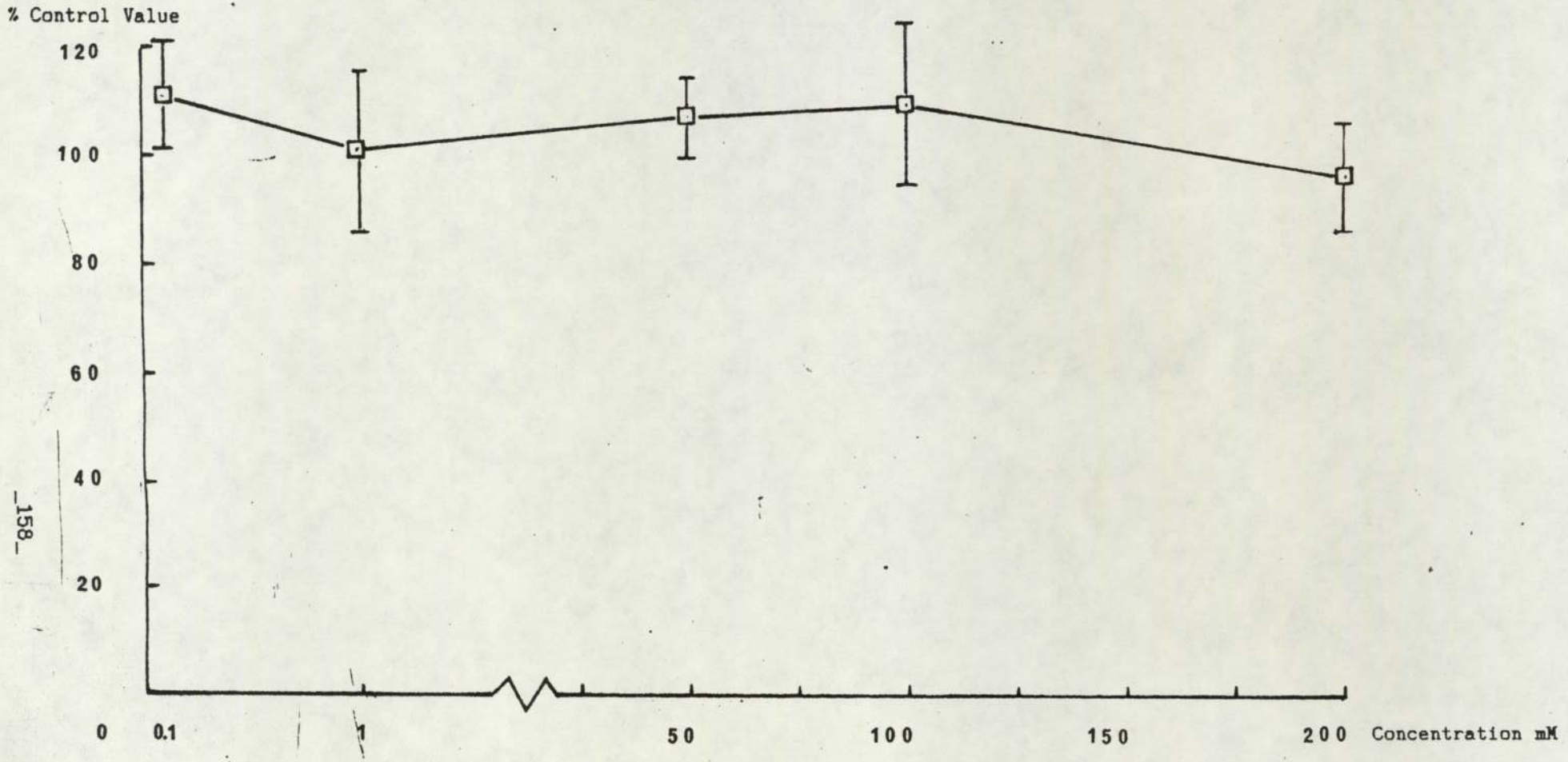


Figure 3.43 The effect of spermidine on the release of PGE_2 from PMA stimulated peritoneal guinea pig PMNL's.

Each result represents the mean of four determinations ± SEM.

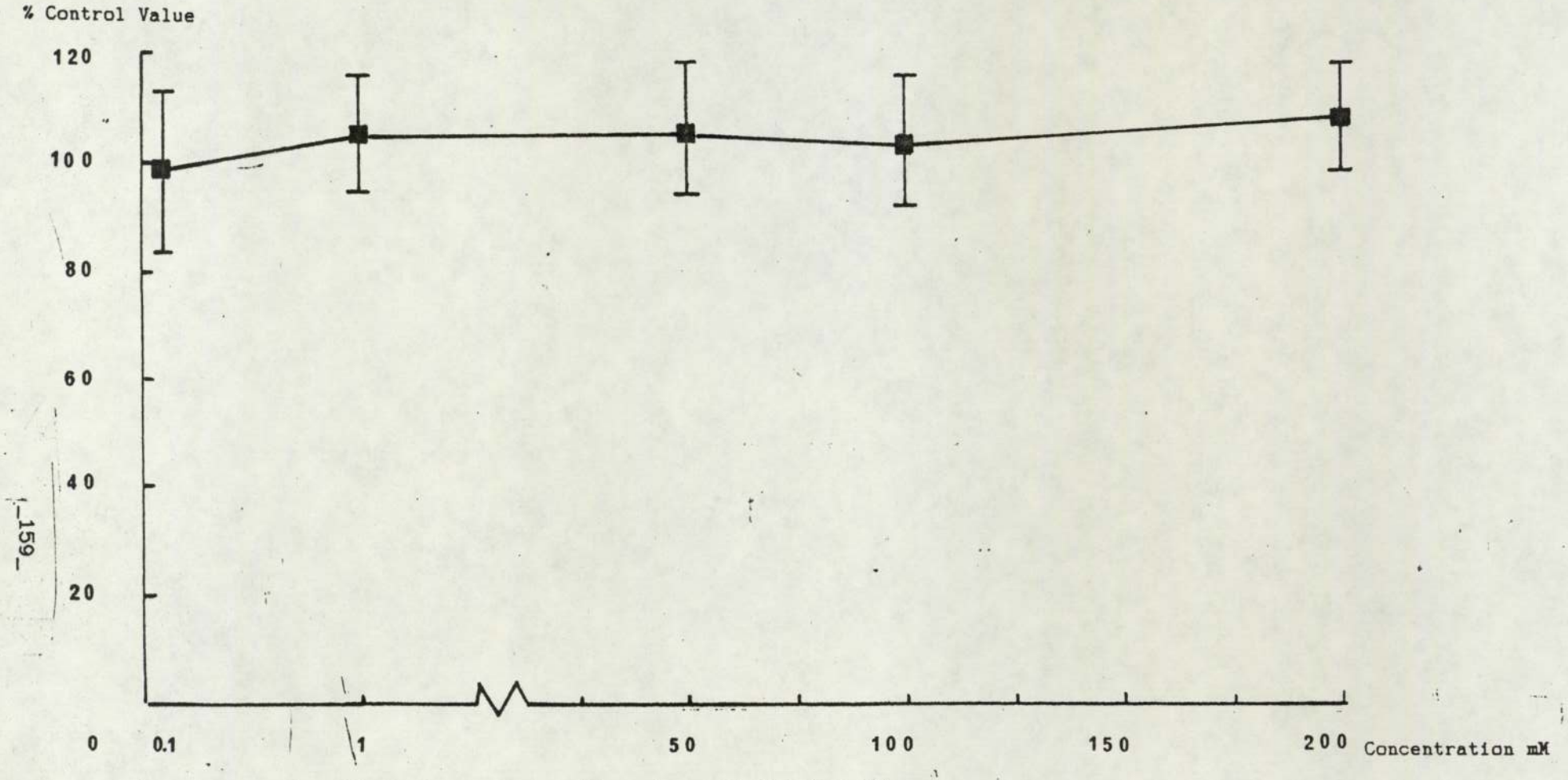


Figure 3.44 The effect of spermine on the release of PGE_2 from PMA stimulated peritoneal guinea pig PMNL's.

Each result represents the mean of four determinations + SEM.

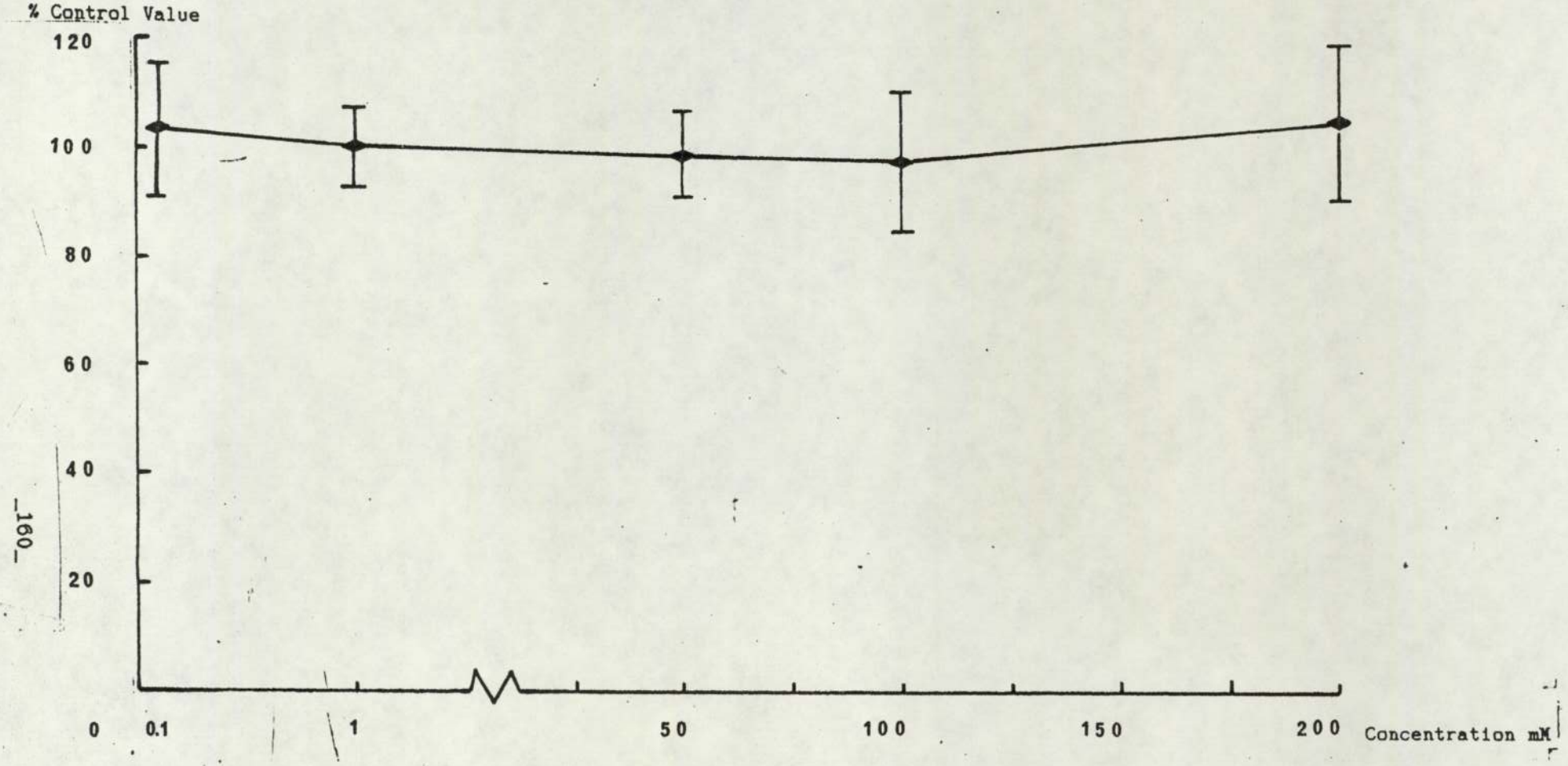


Figure 3.45 The effect of cadaverine on the release of PGE_2 from PMA stimulated peritoneal guinea pig PMNL's.

Each result represents the mean of four determinations ± SEM.

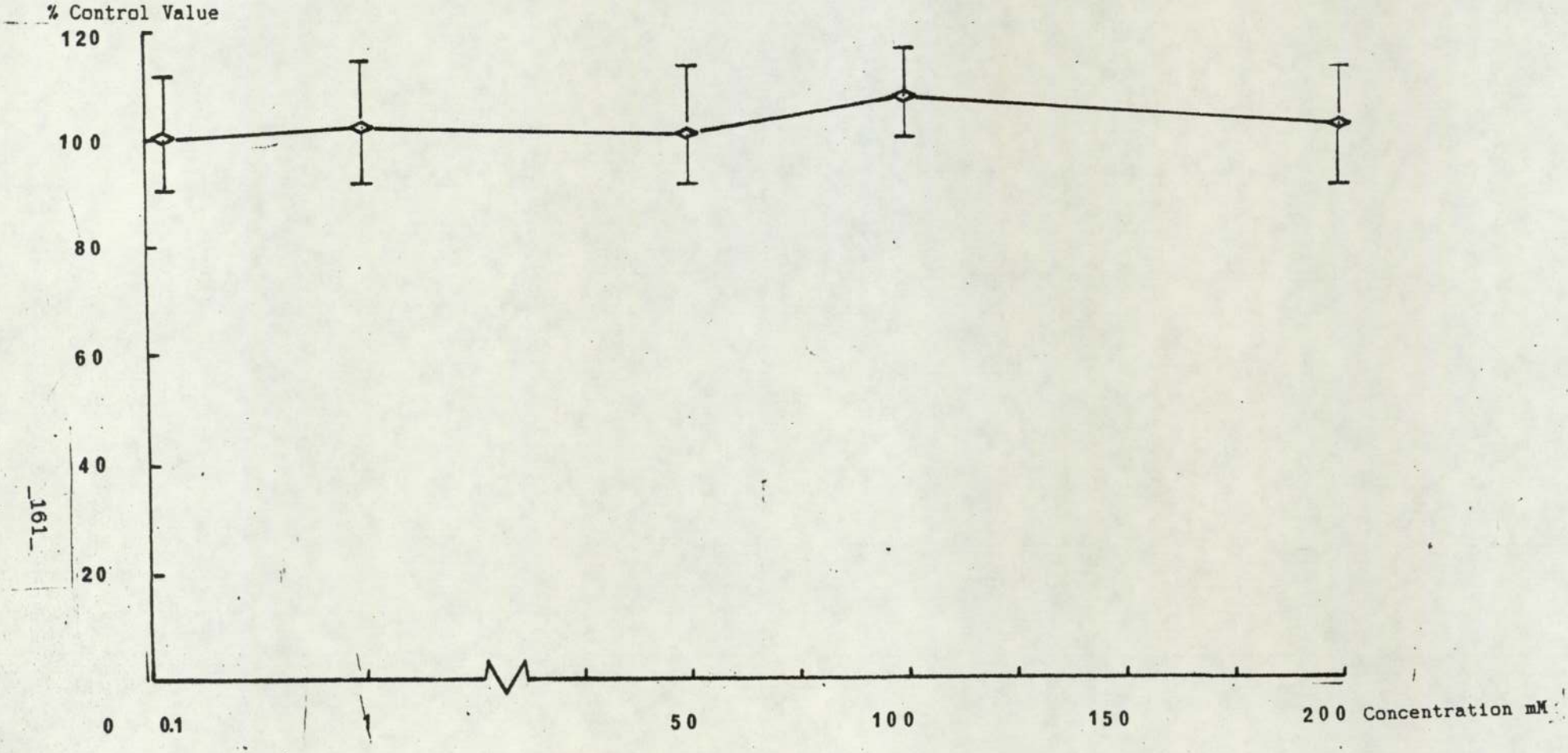


Figure 3.46 The effect of diaminopropane on the release of PGE_2 from PMA stimulated peritoneal guinea pig PMNL's.

Eachresult represents the mean of four determinations ± SEM.

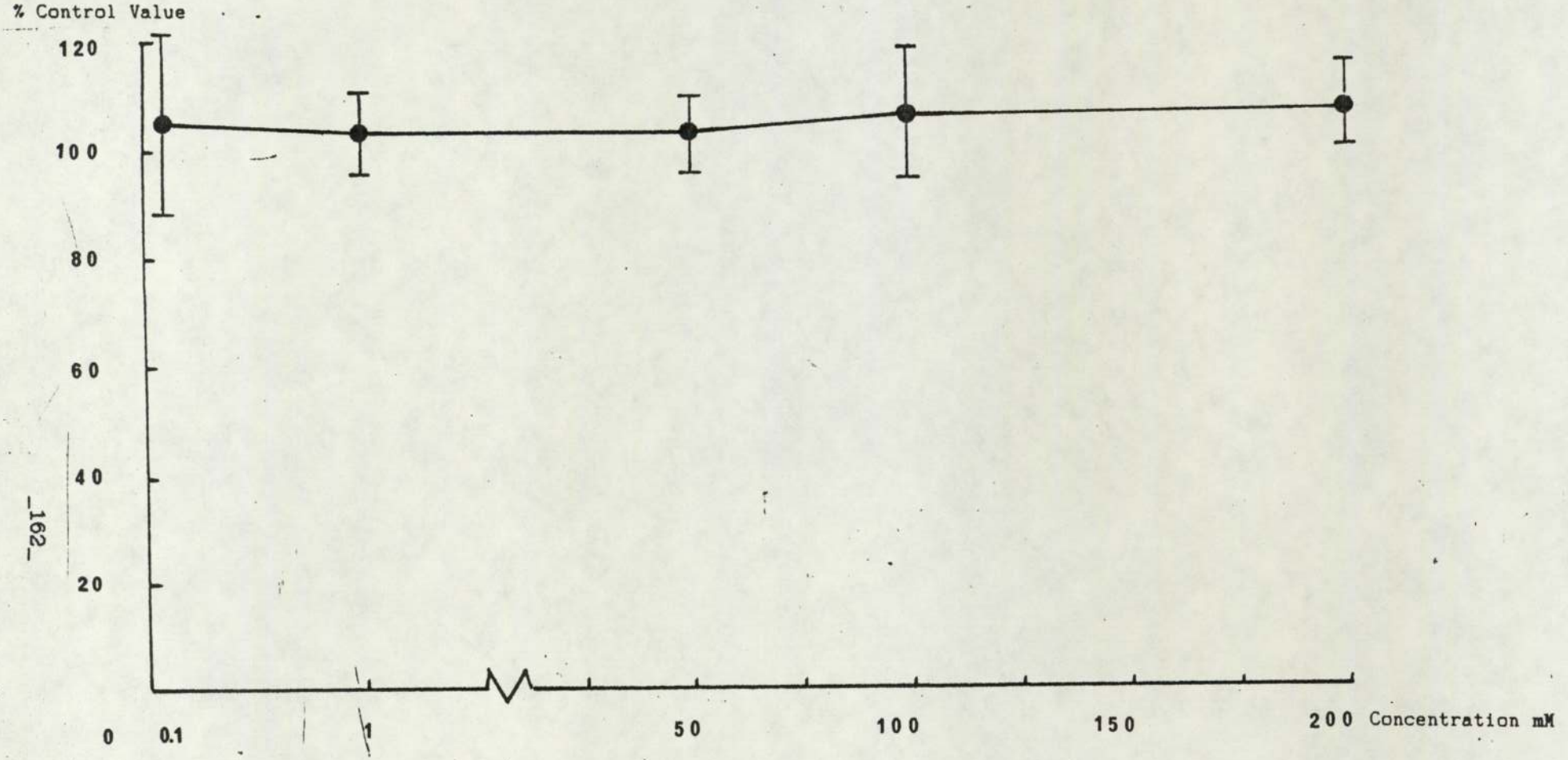


Figure 3.47 The effect of EDTA on the release of PGE_2 from PMA stimulated peritoneal guinea pig PMNL's.

Each result represents the mean of four determinations ± SEM.

3.20 The Effect of Oligoamines on the Uptake of (^{3}H) Leucine by PMA Stimulated Peritoneal Guinea pig Macrophages .

Figures 3.48, 3.49, 3.50, and 3.51 show that putrescine, spermidine, spermine, and cadaverine respectively increased the incorporation of (^{3}H) leucine into total cellular protein (acid insoluble radioactivity) and into intracellular precursors (acid soluble radioactivity).

At 1 mM spermine and spermidine significantly ($p< 0.05$) increased the uptake of (^{3}H) leucine by stimulated macrophages into total cellular protein (acid insoluble radioactivity was 145% and 127% respectively), and into intracellular precursors (acid soluble radiactivity was 135% and 122% respectively).

At 10 mM putrescine and cadaverine significantly ($p< 0.05$) increased the incorporation of (^{3}H) leucine into total cellular protein (159% and 138% respectively) and into intracellular precursors (131% and 123% respectively).

3.21 The Effect of Oligoamines on the Uptake of (^{3}H) Thymidine by PMA Stimulated Peritoneal Guinea pig Macrophages .

Figures 3.52, 3.53, 3.54, and 3.55 show that putrescine, spermidine, spermine, and cadaverine respectively increased the incorporation of (^{3}H) thymidine into total cellular DNA (acid insoluble radioactivity) and intracellular precursors (acid soluble radioactivity).

At 1 mM spermine and spermidine significantly ($P< 0.02$) increased the uptake of (^{3}H) thymidine by stimulated macrophages into total cellular DNA (acid insoluble radioactivity was 159% and 139% respectively) and into intracellular precursors (acid soluble radioactivity was 143% and 128% respectively).

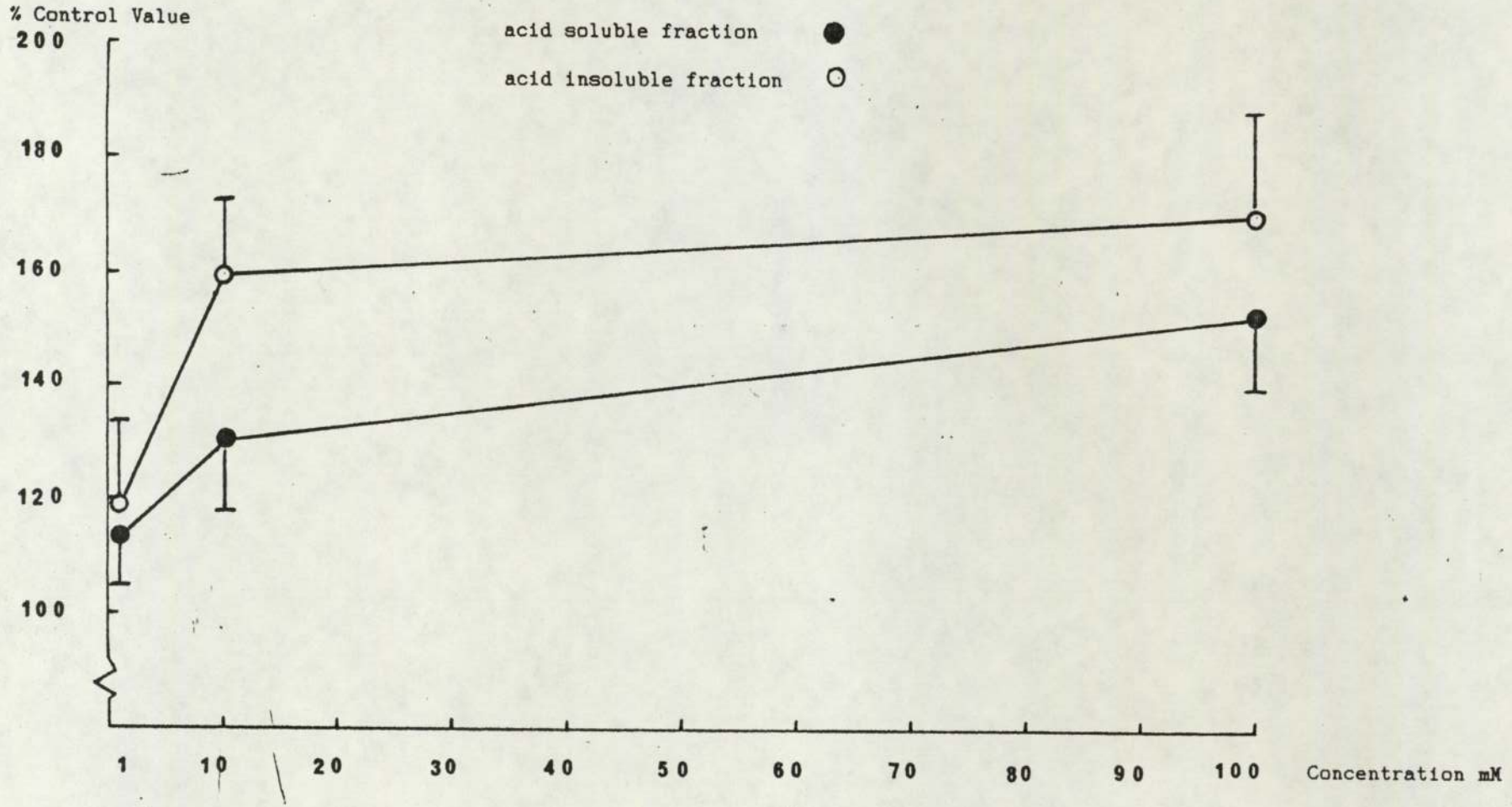


Figure 3.48 The effect of putrescine on the uptake of (^{3}H) leucine by PMA stimulated peritoneal guinea pig macrophages.

Each result represents the mean of four determinations ± SEM.

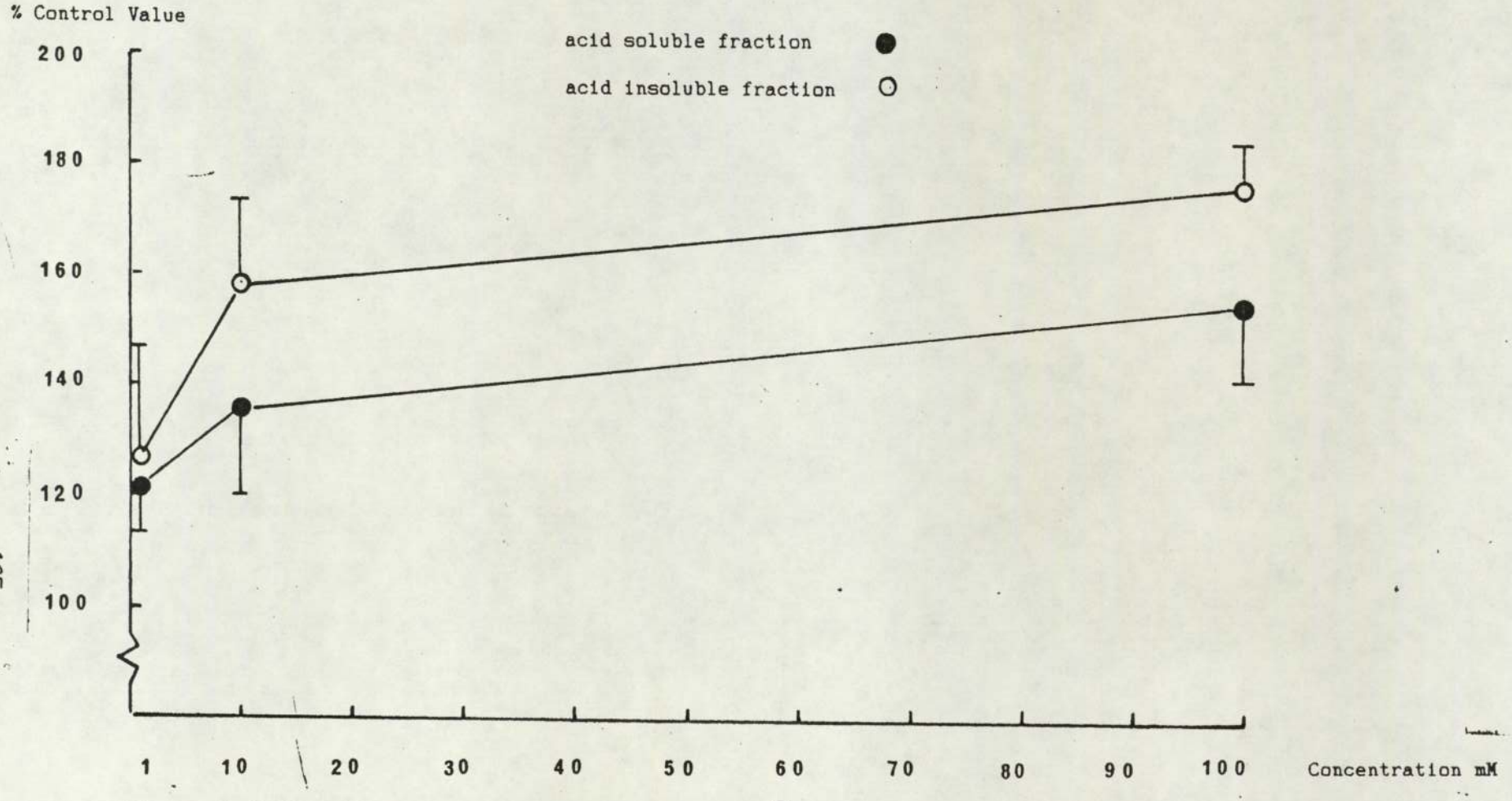


Figure 3.49 The effect of spermidine on the uptake of (^{3}H) leucine by PMA stimulated peritoneal guinea pig macrophages.

Each result represents the mean of four determinations ± SEM.

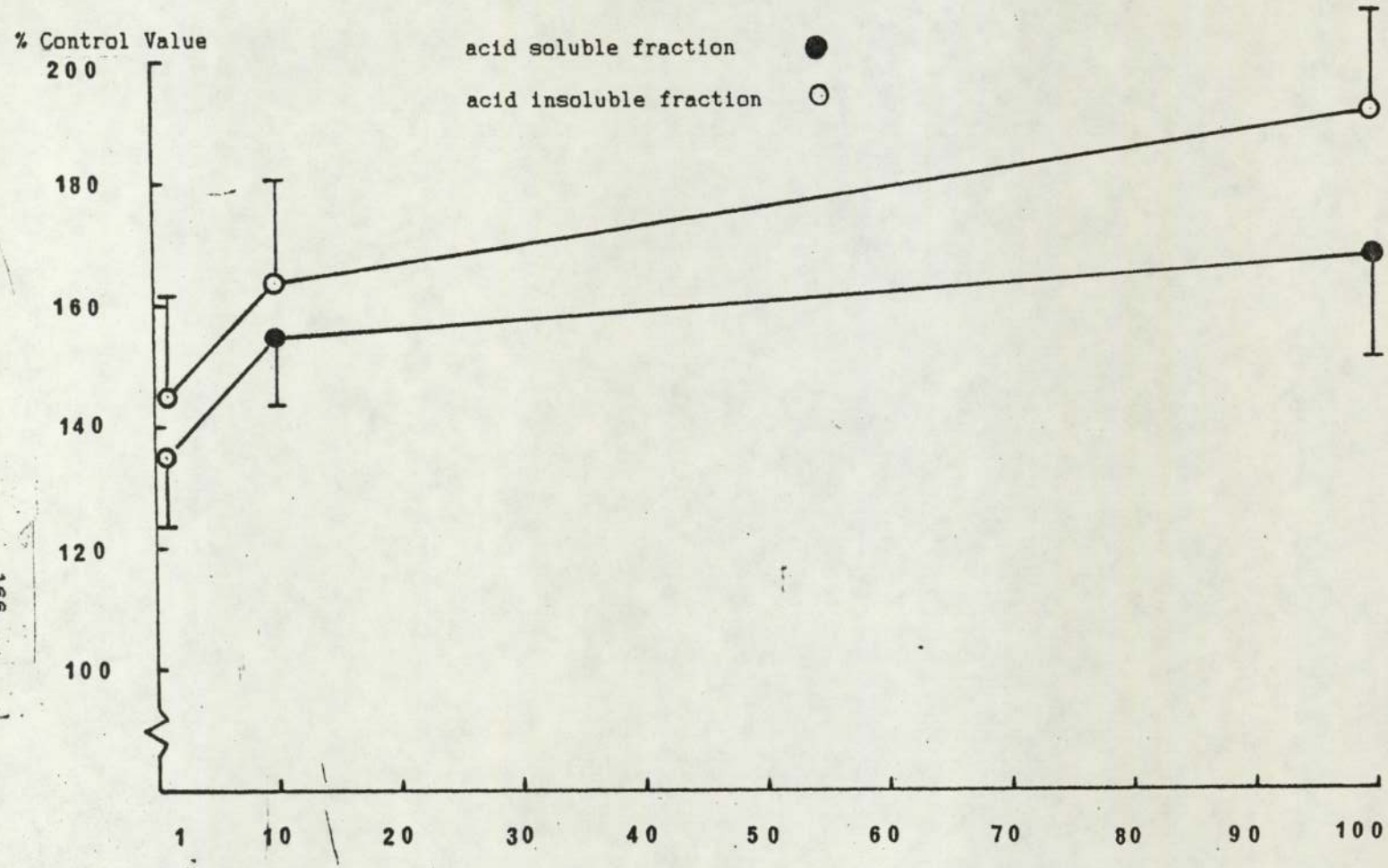


Figure 3.50 The effect of spermine on the uptake of (^{3}H) leucine by PMA stimulated peritoneal guinea pig macrophages.

Each result represents the mean of four determinations ± SEM.

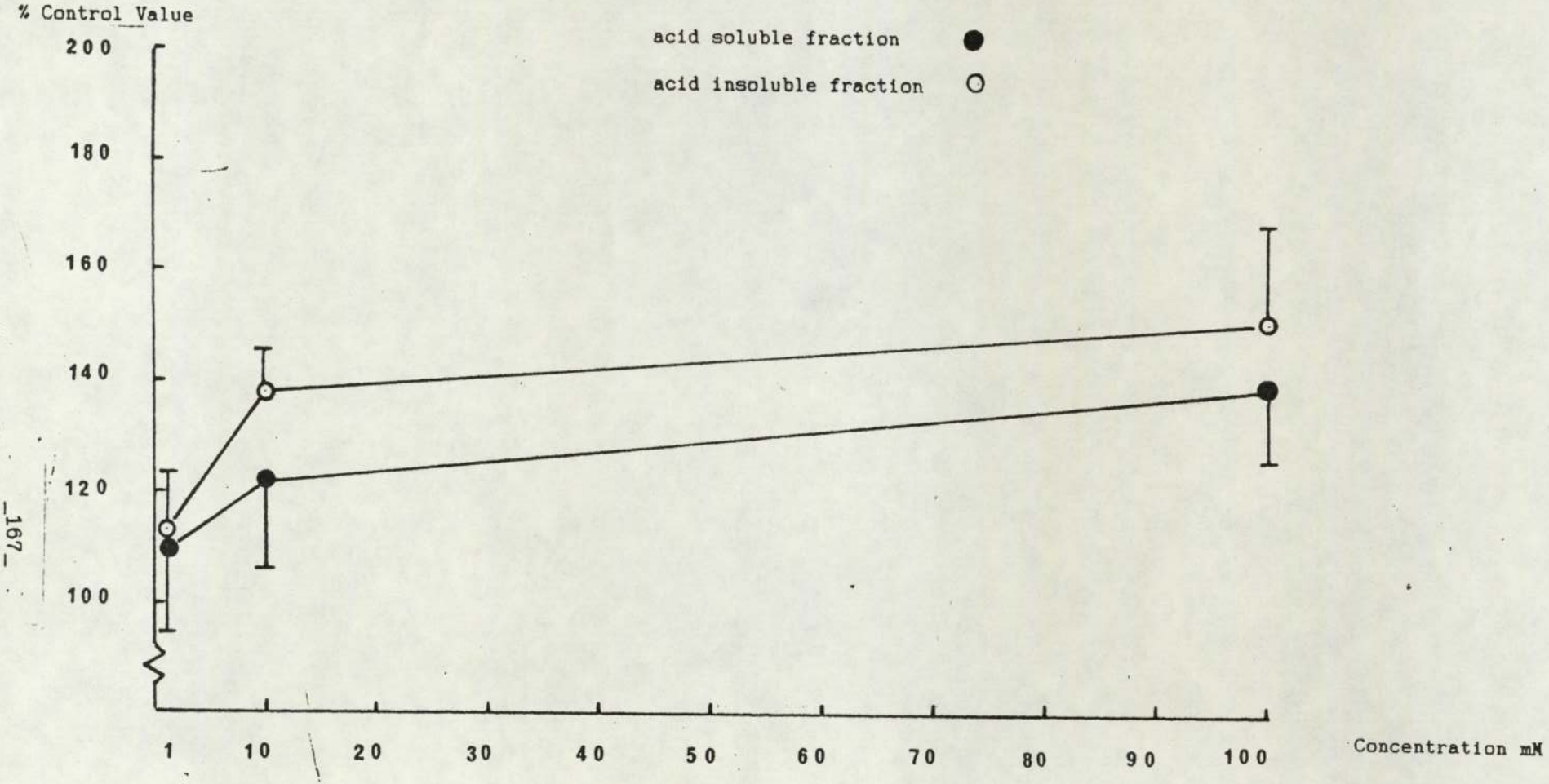


Figure 3.51 The effect of cadaverine on the uptake of (^{3}H) leucine by PMA stimulated peritoneal guinea pig macrophages.

Each result represents the mean of four determinations ± SEM.

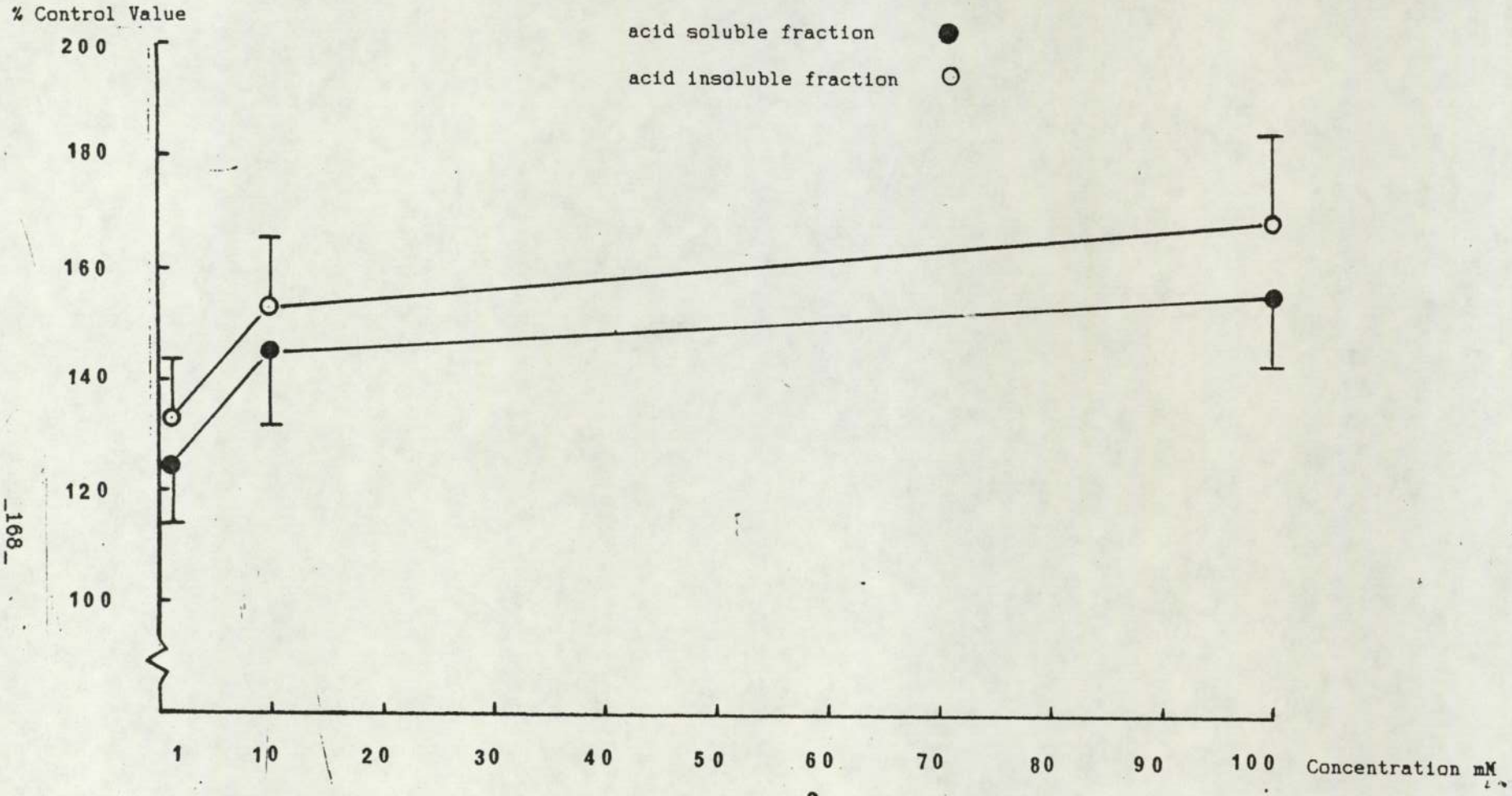


Figure 3.52 The effect of putrescine on the uptake of (^{3}H) thymidine by PMA stimulated peritoneal guinea pig macrophages.

Each result rpresents the mean of four determinations ± SEM.

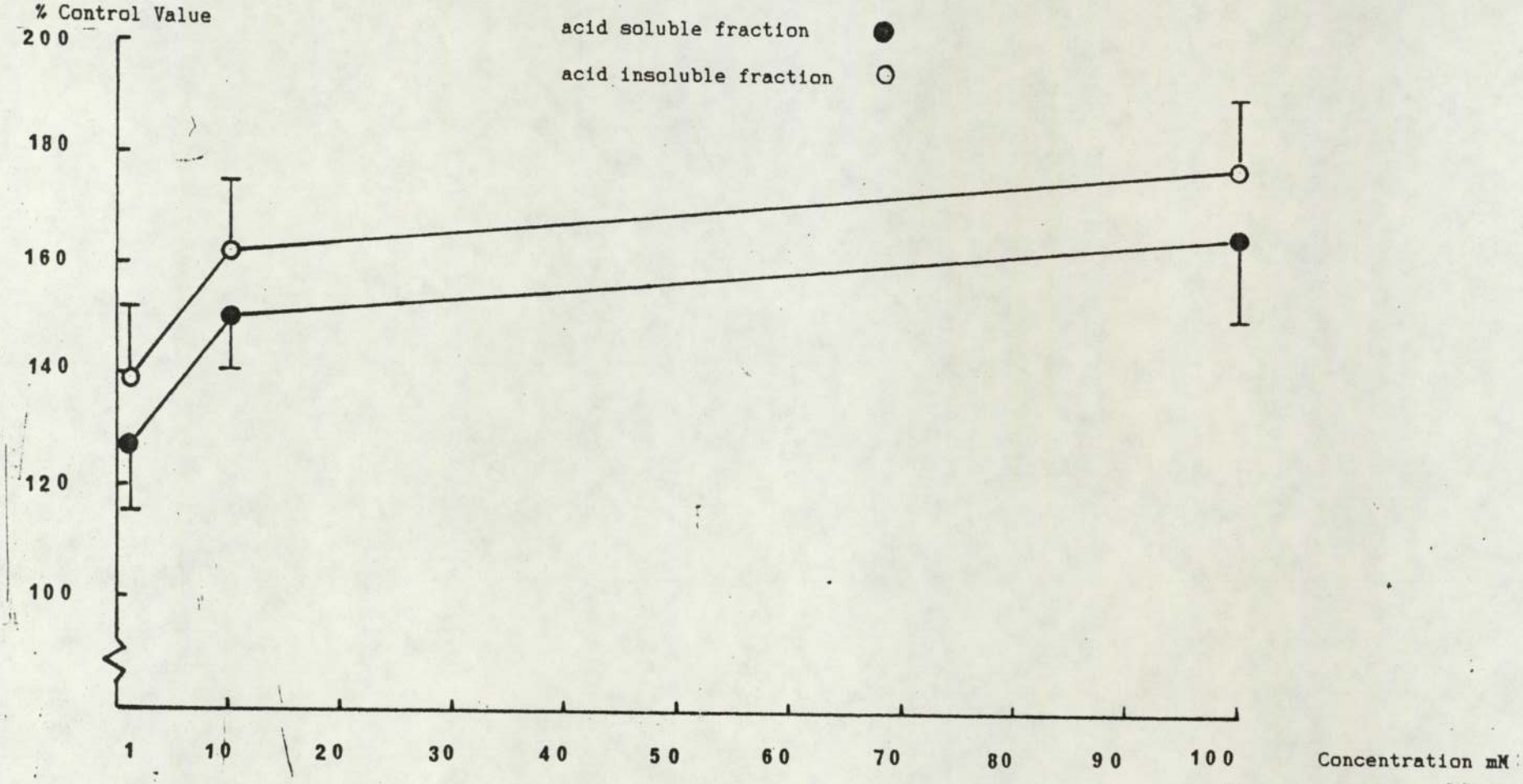


Figure 3.53 The effect of spermidine on the uptake of (^{3}H) thymidine by PMA stimulated peritoneal guinea pig macrophages.

Each result represents the mean of four determinations ± SEM.

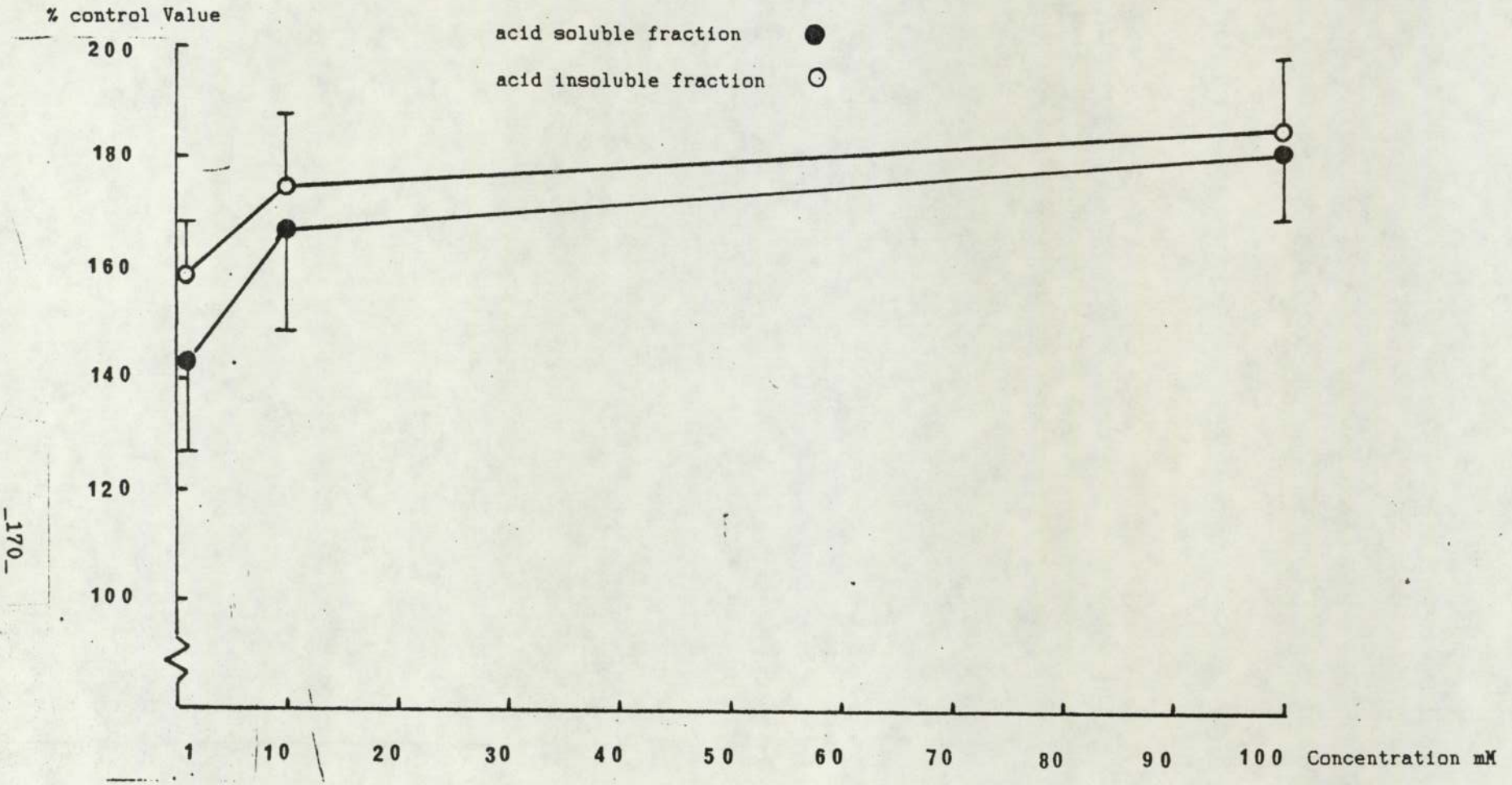


Figure 3.54 The effect of spermine on the uptake of (^{3}H) thymidine by PMA stimulated peritoneal guinea pig macrophages .

Each result represents the mean of four determinations ± SEM.

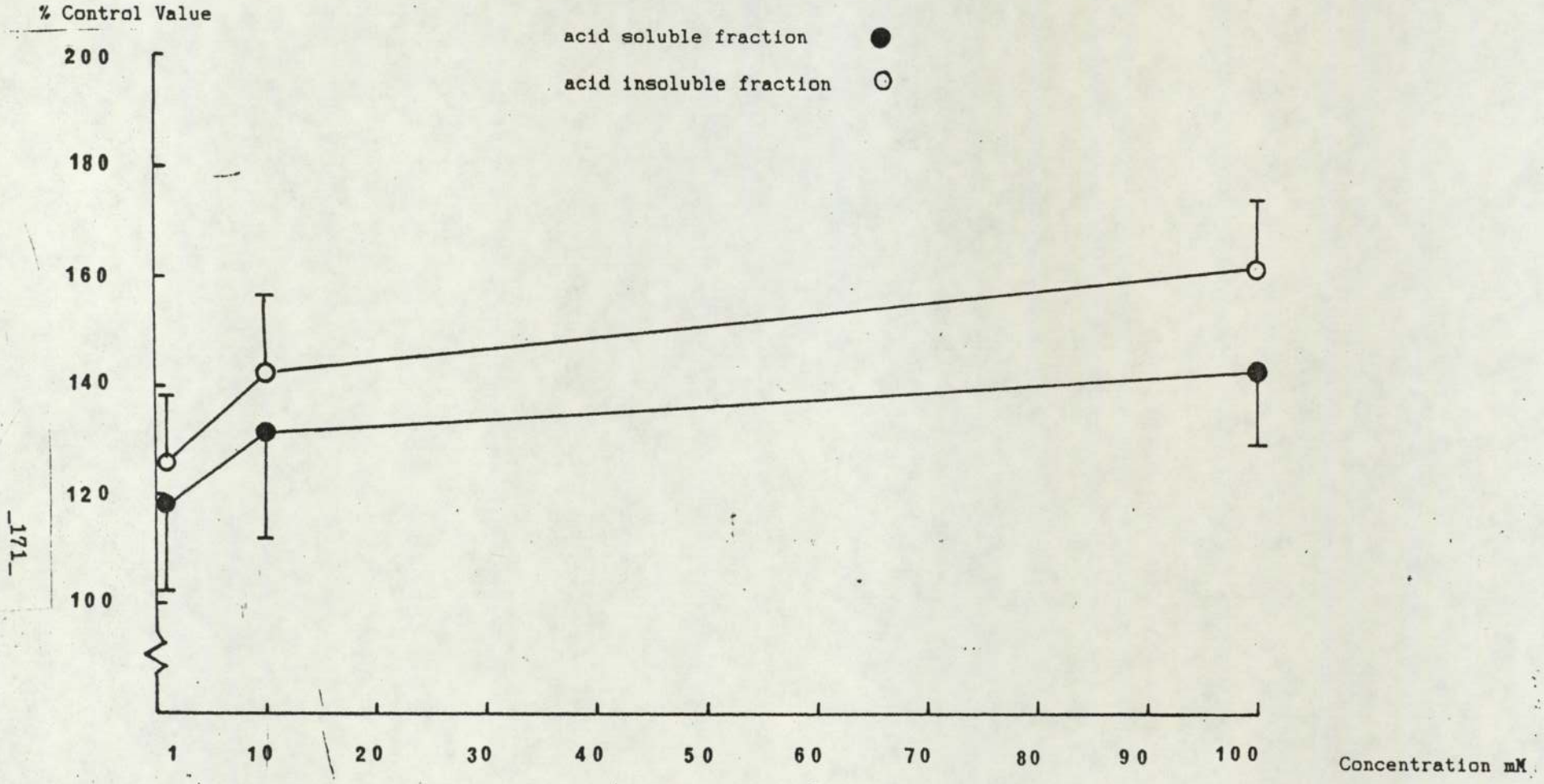


Figure 3.55 The effect of cadaverine on the uptake of (^{3}H) thymidine by PMA stimulated peritoneal guinea pig macrophages .

Each result represents the mean of four determinations ± SEM.

At 10 mM putrescine and cadaverine significantly ($p < 0.05$) increased the incorporation of (^3H) thymidine into total cellular DNA (acid insoluble radioactivity was 156% and 142% respectively) and into intracellular precursors (acid soluble radioactivity was 145% and 131% respectively).

3.22 The Effect of Dibutyryl Cyclic AMP, Theophylline and PGE_2 on LUMINOL Dependent PMA Stimulated Peritoneal Guinea Pig PMNL's Chemiluminescence .

Table 3.20 shows that dibutyryl cyclic AMP, theophylline, and PGE_2 significantly inhibited PMNL's chemiluminescence.

Table 3.20 The Effect of Dibutyryl Cyclic AMP, Theophylline, and PGE_2 on Luminol Dependent PMA Stimulated Peritoneal Guinea Pig PMNL's Chemiluminescence.

Treatment	Concentration	% of Control
Dibutyryl Cyclic AMP	100 µM	103 ± 2.1
	500 µM	61 ± 0.9
	1000 µM	39 ± 1.4
Theophylline	100 µM	99 ± 2.2
	500 µM	78 ± 3.1
	1000 µM	31 ± 1.1
PGE_2	10 nM	105 ± 3.3
	100 nM	82 ± 2.5
	200 nM	62 ± 1.7

Each result represents the mean of four determinations ± SEM .

4. DISCUSSION

Bird and Lewis, (1981) have reported that putrescine and spermidine possessed anti-inflammatory activity in animal models. The finding that oligoamines were anti-inflammatory was confirmed independently by Oyanagui, (1984). The results of the studies cited in this report suggest a possible mode of action for oligoamines and EDTA i.e. as scavengers of oxygen radicals in an order of effectiveness : putrescine >diaminopropane >spermidine >EDTA >spermine >cadaverine.

By employing the assay system originally used by McCord and Fridovich, (1969) to demonstrate the SOD activity of erythrocuprien, oligoamines putrescine, spermidine, spermine, cadaverine, diaminopropane and EDTA were found to be capable of inhibiting the reduction of cytochrome_C by active oxygen species produced by the action of hypoxanthine/ xanthine oxidase (an superoxide generating system). This action of oligoamines and EDTA could not be ascribed to the inhibition of the enzymatic activity of xanthine oxidase (measured as urate production) as the results showed.

Confirmatory results showed that the oligoamines and EDTA also inhibited the reduction of nitroblue tetrazolium (NBT) by the hypoxanthine/xanthine oxidase system. In order to assign a biological relevance to the above it was also demonstrated that the oligoamines studied and EDTA inhibited the reduction of cytochrome_C induced by stimulated PMNL's with a similar order of effectiveness.

The oligoamines studied and EDTA were also found to be capable of inhibiting stimulated PMNL's chemiluminescence. Evidence that chemiluminescence was produced by superoxide, or its metabolite

hydrogen peroxide, was obtained when SOD was substituted for oligoamines in experiments which showed a dose response suppression of chemiluminescence. The possibility that the oligoamines studied and EDTA may be toxic to the cells was excluded by incubating the cells with 200 mM of the oligoamines studied and EDTA for 1 h and the viability was confirmed by the trypan blue exclusion test. The viability (91 %) was the same as with cells incubated for 1 h without oligoamines and EDTA. Finally the oligoamines studied and EDTA, inhibited the aerobic photoreduction of NBT. This test has the virtue of producing superoxide independent of enzymes, proteins such as xanthine oxidase and cytochrome_C.

The possible protective nature towards tissues of the scavenging action of oligoamines and EDTA was shown by the protection of intact lysosomes to the lytic action of reactive oxygen species generated by the hypoxanthine/xanthine oxidase system or stimulated PMNL's. The oligoamines and EDTA have no direct stabilizing effect on intact lysosomes as the results showed. Chromatographic detection of an aldehyde metabolite from the products of interaction of the oligoamines studied with either the hypoxanthine/xanthine oxidase system or stimulated PMNL's suggests that chemically based oligoamine-superoxide interaction may be the basis of the scavenging actions of the oligoamines studied and EDTA. This obviously needs further study.

The scavenging action of the oligoamines reported in this study is in agreement with Vanella et al, (1979) who have showed that oligoamines, putrescine, spermidine and spermine inhibited the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase system and

also with Vanella et al, (1980 a) who have demonstrated that spermine can react directly with superoxide anion. Recently radical scavenging action of oligoamines has been reported by Drolet et al, (1986) which is consistent with the work reported in this thesis.

PMNL's are involved in acute inflammation (Weiss, 1983) and the results showed that oligoamines and EDTA are scavengers of reactive oxygen species. Putrescine has been reported to be about 10 times as anti-inflammatory as spermidine against carrageenan induced oedema in rats (Bird et al, 1983), however spermidine has been demonstrated to be a more potent anti-inflammatory agent than putrescine against carrageenan induced oedema in rats (Oyanagui, 1984). Bird and Lewis, (1981) have shown that spermine has no effect on carrageenan induced oedema in rats.

Oyanagui, (1984) has demonstrated the anti-inflammatory action of putrescine, spermidine and spermine against serotonin and carrageenan paw oedemata. The contradictory results for putrescine and spermine may be due to the different strains of animals being used for the experiments. Clearly the oligoamines are anti-inflammatory but the relative activity of individual oligoamines need further investigations.

Diaminopropane has been shown to inhibit carrageenan induced oedema in rats (Mohd_Hidir, 1985). EDTA has been demonstrated to inhibit lipid peroxidation (Kamataki and Kitagawa, 1974). Cadaverine was not found to have anti-inflammatory activity against carrageenan oedema in rats (Oyanagui, 1984; Bird, 1981).

The plasma levels of endogenous oligoamines are very low. Normal human serum determinations (Brossat et al, 1983) give values for

putrescine as 155, spermine 17, and spermidine 45 n moles/l. Clearly these values are far lower than the ones used in this study,. However, in human granulocytes reported levels are far higher (Cooper et al, 1976) putrescine 468 ± 244/10^9 cells, spermine 548 ± 183/10^9 cells and spermidine 241 ± 156 n moles/10^9 cells. An additional factor which may be relevant is that during inflammation oligoamine levels are increased in inflammatory exudate (Bird et al, 1983; Bird and Lewis, 1981) and it is known that stimulated macrophages and T_lymphocytes show sharply induced ornithine decarboxylase activity (Klimpel et al, 1970; Kay and Lindsay, 1974)

Oxygen induced lung injury in rats significantly increased ODC, putrescine, spermidine and spermine levels and inhibition of this elevation by difluoromethyl ornithine, an irreversible inhibitor of ODC adversely affects repair (Thet et al, 1984). If oligoamines do scavenge oxygen species in vivo it is likely that this effect is a local one either within the cells or in the immediate environment surrounding the cells and that the effect will be localised to site of inflammatory foci (regions of high ODC activity) where they may contribute to general pool of anti-oxidants such as tocopherol (Burton et al, 1983) and caeruloplasmin (Goldstein et al, 1979). These anti-oxidants supplement established inracellular defences such as SOD, catalase and glutathione peroxidase/reductase which protects cells from the toxic effect of oxygen species.

Copper ions are intimately involved with the dismutation of superoxide anion. Wesser and Shubotz, (1982) have suggested a relationship between the anti-inflammatory and the scavenging activities of copper containing compounds. These include cupric ions, copper complexes

with aminoacids and copper anti-inflammatory complexes which display SOD activity some greater in magnitude in vitro than endogenous SOD. This appears to be consistent with the results reported in this work which showed that copper sulphate inhibited the reduction of cytochrome_C induced by the hypoxanthine/xanthine oxidase system and also with the results which showed that copper-oligoamine complexes inhibited the reduction of cytochrome_C, induced by the hypoxanthine/ xanthine oxidase system, more efficiently than copper sulphate or the oligoamines individually. The SOD like activity of copper-oligoamine complexes reported in this study is in agreement with Kimura et al, (1981) who have showed SOD activity of some macrocyclic oligoamine complexes.

The results showed that the products of the reaction of hypoxanthine /xanthine oxidase system and from stimulated PMNL's have lytic effect on isolated intact lysosomes. Superoxide is in part either directly or indirectly responsible for the lytic action since SOD is protective as the results showed. Hypoxanthine/xanthine oxidase system induced lysis of lysosomes is in agreement with (Fong et al, 1973) who have reported that this system induced lysis of intact lysosomes. Superoxide is also lytic to erythrocytes (Kellog and Fridovich, 1977). It is also capable of producing a range of active oxygen species (Frodovich, 1983) capable of damaging membranes.

The hydroxyl radical is known to induce lipid peroxidation and lipid peroxidation was found to be associated with breakdown of lysosomes in this study. The hydroxyl radical is produced directly both in the hypoxanthine/xanthine oxidase system (Beauchamp and Fridovich, 1970) and by stimulated PMNL's (Tauber and Babior, 1977). However, it is also produced non enzymatically by the action of superoxide on iron

(Ambruso and Johonston, 1981), and liver lysosomes are rich in iron. Another possibility is that the action of iron with hydrogen peroxide produced in the hypoxanthine/xanthine oxidase system (Massey and Verger, 1963) or by stimulated PMNL's enhances levels of hydroxyl radical which may have contributed to the lysosomal damage (Ambruso and Johonston, 1981).

Both hydrogen peroxide and hydroxyl radical induced lysis of intact liver lysosomes in vitro. Oligoamines and EDTA which showed scavenging action were found to give some protection against the lytic action of oxygen species on lysosomes. As the results showed that SOD also protected the lysosomes, a scavenging effect by SOD on superoxide anion apparently formed part of this protection.

An inhibitory effect by oligoamines on lipid peroxide formation has been reported by Kitada et al, (1979). In these studies which used liver microsomes, spermine was found to be the most effective oligoamine with spermidine more effective than putrescine which was only slightly inhibitory. The mechanism suggested involved the binding of spermine to phospholipids in the microsomes. A similar result was reported by Tadolini et al, (1984) using phospholipid vesicles. In the same study Tadolini et al, (1984) did not find an inhibition by oligoamines on lipid peroxidation of egg yolk lecithin vesicles. EDTA also inhibited lipid peroxidation (Kamataki and Kitagawa, 1974) which was also found in this study. Interaction of oligoamines with erythrocyte membranes was also found to have stabilizing effect in another study (Ballas et al, 1983) where the stabilizing effect was in the following order spermine >spermidine >putrescine.

Although the results reported in this study showed that putrescine inhibited lipid peroxidation more effectively than the other

oligoamines studied and EDTA, the experimental target and conditions were different from those described elsewhere. It appears that the inhibition of lipid peroxidation by oligoamines is a property of group rather than specific compounds and may vary with phospholipid composition. Therefore oligoamines may well depend on the chemical nature and binding characteristics of the target. In this work putrescine was taken up by the intact lysosomes at a faster rate than spermidine or spermine. However the location of oligoamines in the lysosomes was not investigated so it was not possible to determine the membrane concentrations.

In rheumatoid arthritis, which is an inflammatory disease, iron salts are present in synovial fluid and tissue (Rowley et al, 1984; Guttridge et al, 1981) and superoxide or hydrogen peroxide can interact to form hydroxyl radical. Evidence of lipid peroxidation has been found in rheumatoid synovial fluid by the detection of fluorescent end products (Lunec and Dormandy, 1979) of lipid peroxidation and thiobarbituric acid (TBA) positive material detected in fluids from patients (Rowley et al, 1984). The TBA positive material correlated with the amounts of iron salts present (Rowley et al, 1984). Reactive oxygen species generated by stimulated PMNL's and hypoxanthine/xanthine oxidase induced lysis of intact lysosomes (reported in this study) may be relevant to the in vivo events occuring in rheumatoid arthritis where PMNL's constitute over 90 % of cells found in the synovial fluid of RA patients (20 x 10) (Palmer, 1969). Lysosomes and lysosomal enzymes have been implicated in the pathogenesis of RA (Hollander et al, 1964). Dingle (1962) has demonstrated that lysosomal enzymes degrade cartilage matrix. Luscombe,

(1963) has showed that rheumatoid synovia contained higher levels of acid phosphatase compared to normal synovia. Fallet, (1972) has reported that acid phosphatase and β_glucosaminidase may be responsible for articular degeneration. The results showed that oligoamines studied have no direct effect on lysosomal enzymes activity (acid phosphatase and β_glucosaminidase). However oligoamines have been shown to exhibit possible indirect anti-inflammatory effects through their ability to act as scavenging agents for active oxygen species. Also oligoamines have been shown to chelate ferrous ions (Kitada et al, 1982), chelation of ferrous ions may inhibit the formation of the more damaging hydroxyl radical.

This work showed that oligoamines are metabolised into aldehyde derivatives by products from either hypoxanthine/xanthine oxidase system or stimulated PMNL's. Putrescine appears to be metabolised to γ_aminobutyraldehyde, _pyrroline and γ_aminobutyric acid by stimulated PMNL's. Although the usual route for γ_aminobutyric acid formation in mammals is accepted as the enzymatic decarboxylation of glutamic acid, it has been reported to be formed from putrescine in rat liver and brain (Seiler et al, 1971). Although the pathway for the conversion of putrescine to γ_aminobutyric acid has not yet been determined, there is evidence from work with mice that diamine oxidase is involved in the first step of putrescine metabolism (Fogel et al, 1981). The results reported here appear to be consistent with these observations.

This study also demonstrated that spermidine and spermine are metabolised by hypoxanthine/xanthine oxidase and stimulated PMNL's into aldehydes which were detected by Schiff's reagents. Tabor et al,

(1964) has shown that spermine and spermidine are metabolised by beef plasma amine oxidase into N,N_bis (3_propionoaldehyde) 1,4 diaminobutane and N_(4_aminobutyl)_3_diaminopropionoaldehyde respectively. Buffoni and Blaschko, (1971) have shown that spermidine and spermine are not metabolised by pig plasma polyamine oxidase. This may be explained by the different enzymes or experimental conditions and that the metabolism of spemidine and spermine may depend on the nature and species of the metabolising agent.

Cadaverine in this report was metabolised into an aldehyde compound by either the hypoxanthine/xanthine oxidase system or stimulated PMNL's which is in agreement with Henningsson and Henningsson (1983) who have shown that an aminoaldehyde is one of the metabolites of cadaverine by diamine oxidase.

As first shown by Hirsch and Dubos, (1952) the products formed after oxidation of spermine and spermidine inhibited the growth of cell types including tumour cells, bacteria, trypanosomes, plant and viruses (Tabor and Tabor, 1972). Morgan et al, (1980) have shown that the oxidation products of spermidine and spermine inhibited cellular proliferation.

The metabolism of oligoamines into aldehyde compounds may be of interest since it may inhibit cellular proliferation which is a characteristic of chronic inflammation. Oligoamines were anti-inflammatory against adjuvant arthritis which is a chronic model of inflammation (Bird et al, 1983).

In chronic inflammation oligoamines may have a complex role. Oligoamines and the enzymes involved in their biosynthesis have been

shown to be vital participants in cell proliferation (Cohen, 1977; Fillingame et al, 1975). The progression of cell from a quiescent to a proliferating state is accompanied by changes in their oligoamine contents (Janne et al, 1978). Putrescine and its two oligoamine metabolites spermidine and spermine are involved in the induction of cell division (Rupniak and Paul, 1978; Cohen, 1977). Paradoxically it has also been shown that in in vitro interaction between spermidine or spermine and ruminant sera can inhibit cellular proliferation (Allen et al 1979; Alarcon et al, 1961). This phenomenon was initially regarded as being due to the formation of cytotoxic products of the interaction such as acrolein (Alarcon et al, 1961). Later evidence suggests that an aminoaldehyde, formed by the oxidative deamination of these oligoamines by serum polyamine oxidase may be the factor directly involved in the inhibition (Allen et al, 1977; Tabor et al, 1964). Allen et al, (1979) have shown that oligoamines inhibit lymphocyte proliferation, in the presence of foetal calf serum, and confirm that the polyamine oxidase of bovine serum is responsible for converting the oligoamines to inhibitory factors.

Zurier and Sayadoff, (1975) have shown that stimulated PMNL's released PGE series into the surrounding medium in vitro. Prostaglandins have a dual role, they are pro-inflammatory causing vasodilation and oedema, intensifying the effect of other inflammatory mediators, in the acute phase of inflammation, however pharmacological doses of PGE_1 and PGE_2 are anti-inflammatory during the chronic stage of inflammation (Bonta and Parnham, 1978). PGE_2 has been shown to inhibit adjuvant arthritis in rats, (Bonta et al, 1978), and inhibit superoxide productin from stimulated macrophages (Lim et al, 1983).

The results showed that oligoamines and EDTA did not effect PGE output from stimulated PMNL's. This is in agreement with previous work where oligoamines have no effect on arachidonate metabolism (Fryer et al, 1977).

The well documented role of oligoamines in promoting nucleic acid and protein synthesis, is consistent with the increased uptake of leucine and thymidine by stimulated macrophages by oligoamines (as the results showed) may result in the synthesis of anti-inflammatory protein (Oyanagui, 1984) although this awaits further study.

The exact mechanism of the anti-inflammatory action of putrescine and oligoamines is unkn own. However it is possible that either putrescine itself is an inflammatory mediator or it may be involved in the production of anti-inflammatory mediators. De novo synthesis of putrescine has been reproted to be an essential factor of new protein synthesis known to be involved in the anti-inflammatory action of the glucocorticoids dexamethasone (Bartholeyns et al, 1981). This anti-inflammatory protein is suggested to inhibit the enzyme phospholipase A_2 which releases arachidonic acid, the precursor of prostaglandins biosynthesis from membrane phospholipid (Flower and Blackwell, 1979).

The anti-inflammatory effects of dexamethasone on bradykinin induced oedema has been shown to be due to the synthesis of a protein (Tsurufuji et al, 1980). Paw swelling induced by serotonin or bradykinin is principally dependent on an increase of vascular permeability caused by the opening of intercellular junctions of vascular endothelium (Majno et al, 1969), and the prostaglandin is excluded (lack of inhibitory effect on oedema by indomethacin).

Oyanagui, (1984) has suggested that oligoamines may act to reduce the vascular permeability through the induction of the synthesis of a certain protein *in vivo* which is given the name'vascular permeability inhibitory protein' or 'vasoregulin'.

'Vasoregulin' was induced by dexamethasone and other glucocorticoids in Namalva cells (Oyanagui and Suzuki, 1985). Oligoamines and SOD also gave good yields of vasoregulin in the culture of these cells. In the same study it has been demonstrated that the precipitate produced by trichloroacetic acid (crude released vasoregulin) suppressed serotonin induced paw oedema of mice when dosed 30 min before serotonin. Carrageenan paw oedema of rats was also suppressed when the compound was injected at the same time as carrageenan. Vasoregulin was also partially purified and characterised in the same study. Vasoregulin was also inhibited by superoxide anion (Oyanagui and Suzuki, 1985). An aminoaldehyde oxidation product of polyamine oxidase-polyamine system has been shown to inhibit human neutrophil locomotion (Ferrante, 1985). Oxidized polyamines could limit an inflammatory reaction by inhibiting neutrophil locomotion. Oxidized polyamines may arise as a consequence of tissue damage (Gaugas, 1980); these agents could serve to limit further neutrophil accumulation and concomitantly trap cells already present at inflammatory sites. Thus, the products of the PAO-polyamine reaction may function as regulators of inflammatory responses. This must be considered of particular importance since macrophages contain PAO and the cells can secrete the enzyme following stimulation (Morgan *et al*, 1980). Possibly, this could be one means by which the monocytes/macrophages outphase the neutrophils at inflammatory sites. Obviously, further studies on anti-inflammatory properties of the PAO-polyamine system would be

interesting, and identification of products responsible could lead to the synthesis of a new class of compounds for the treatment of inflammatory diseases.

In conclusion, oligoamines and superoxide anion may be very important to our understanding of the basic mechanisms of inflammation.

Drugs that raise cyclic AMP levels either by stimulating its synthesis *via* adenyle cyclase e.g PGE_2 or beta receptor agonist (such as salbutamol or isoprenaline) or by preventing its breakdown by inhibiting phosphodiesterase (e.g theophylline) shows synergistic anti-inflammatory action against animal models of inflammation. This appears to be consistent with the results reported in this study which showed that PGE_2, cyclic AMP analogue (dibutyryl cyclic AMP) and theophylline, inhibited superoxide induced chemiluminescence of stimulated PMNL's. This is in agreement with Lim *et al*, (1983) who have showed that PGE_2, dibutyryl cyclic AMP and theophylline inhibited superoxide production by stimulated macrophages.

Further Work

1_Synthesis of aldehyde oxidation products of oligoamines.

2_The effect of synthesized aldehyde products of oligoamines on chronic inflammation in animal models.

3_The effect of copper-oligoamine complexes on different animal models of inflammation.

4_Estimation of oligoamine levels in rheumatoid arthritis (RA) patients especially in pregnant women and their relevance to disease activity.

5_The effect of oligoamines on depressed functions of RA lymphocytes.

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