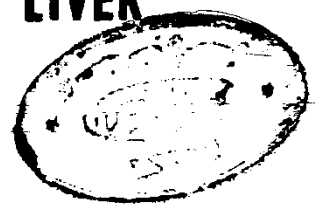
STUDY OF SOME METABOLIC EFFECTS OF DISODIUM ETHYLENE DIAMINE TETRA ACETATE (Na_2 EDTA) ON RAT'S LIVER



THESIS

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رسالة

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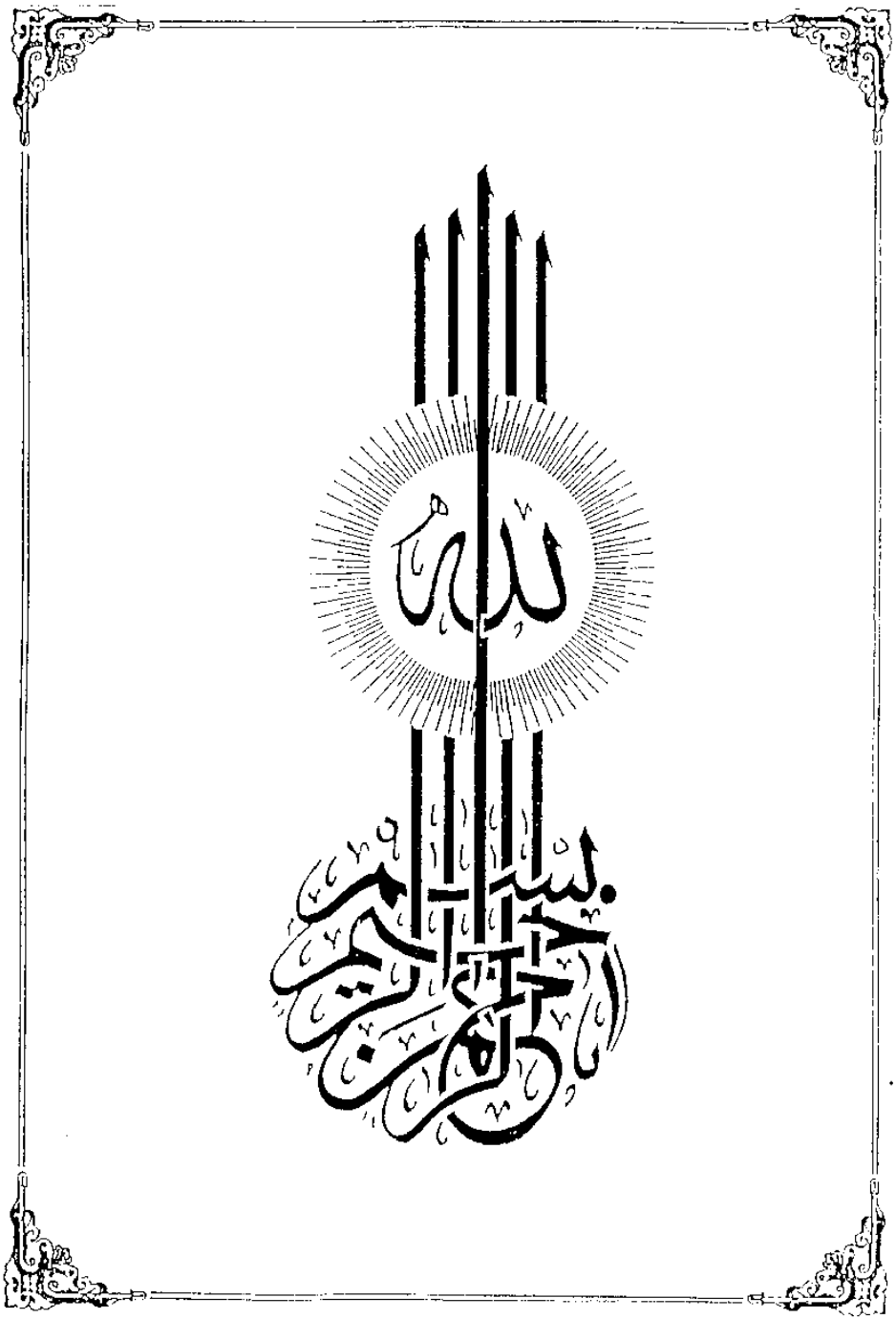

C O N T E N T S

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In a previous work, SABA et al. (1981)[1] found that Na_2 EDTA given as a single injection to rats, the latter being sacrificed after half an hour, resulted in a decrease in serum calcium, an increase in blood urea, glucose and cholesterol (total and free) as well as a decrease in activity of serum alkaline phosphatase enzyme.

The aim of the present work is to study the effects of the chelating agent Na_2 EDTA given to rats as one single injection and as repeated injections (five), on the total lipids of the rat liver, as well as phospholipids and cholesterol. Also the study will include estimations of the liver enzymes LDH and GOT. An additional part is to study the first three parameters in the serum of the experimental animals and to try to find any relation between the serum and liver changes.

INTRODUCTION

I N T R O D U C T I O N

EDTA (ETHYLENE DIAMINE TETRA ACETIC ACID)

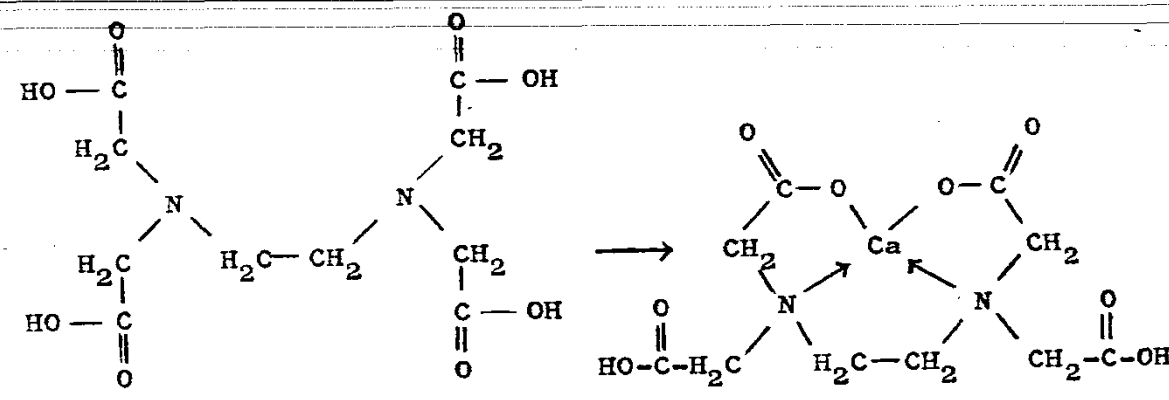
CHELATION AND THE DISCOVERY OF EDTA:

In the formation of covalent or ionic bond, each atom contributes one electron to the shared pair in the common molecular orbital. A coordinate covalent bond is formed when both shared electrons are contributed by one atom. Chelate formation involves formation of coordinate covalent bonds between an electrophilic electropositive atom of a metal and nucleophilic atoms or ligands (usually N, O or S) of an organic molecule so as to form a ring containing the metal atom and 2, 3 or 4 atoms from the organic molecule[2,3,4].

The bond is often indicated by an arrow with its head pointing away from the atoms (the ligands) which have donated the electron pair. The reaction is called chelation and the compound formed is a chelate. The words are peculiarly appropriate. They are derived from the Greek word "chele" which means the crab's claw and in a chelate compound the metal is surrounded by and held firmly within the rest of the molecule as a claw of a crab which surrounds and seizes its victim[5].

The compound ethylene diamin tetra-acetic acid (EDTA), discovered by MUNZ in 1935, is a typical example

of chelating agents. MUNZ showed that EDTA had a strong affinity for calcium ions with which, it formed a complex containing unionized calcium i.e. a chelate. The nature of the reaction is shown below:



EDTA Ca EDTA

Calcium has unfilled "d" orbitals that can accept electron pairs from the nitrogen. The calcium is then held firmly in the heterocyclic organometallic rings formed and can no longer provide calcium ions.

MUNZ applied his discovery to the textile industry. EDTA was employed to remove calcium ions from the water used to wash the fibres, because of its high affinity for calcium. The sodium salt of EDTA (disodium edetate "Na_2 EDTA"), and a number of closely related compounds have been applied as industrial and analytical reagents owing to their property of forming poorly dissociable chelate complexes with many divalent metals as lead, zinc, cobalt and nickel[5].

It was observed that some metal-EDTA chelates are more stable and are formed preferentially even in the presence of calcium ions e.g. lead-EDTA chelate. The stability of metal chelates depends upon properties of the metal and of the chelating agent and compounds can therefore be selected to bind certain metals with a degree of specificity sufficient to make them therapeutically useful in the treatment of intoxications.

The first real hint of the potentiality of chelating agents in therapy was observed in 1951. A young boy who was dying from lead poisoning was given repeated doses of calcium disodium EDTA ($CaNa_2$ EDTA). Three days after the start of treatment with EDTA, he had completely recovered and this compound was then recognized as being the drug of choice for the treatment of lead poisoning.

It will be appreciated that the calcium disodium EDTA used in this first clinical trial is itself a chelate, but the chelated calcium can be displaced by lead. If EDTA itself were given, there should be a possibility that, as the lead was cleared from the body, the calcium chelate would be produced and excreted and so cause calcium deficiency.

The value of EDTA in the treatment of lead poisoning lies in the fact that lead EDTA complex is relatively

non-toxic, soluble and rapidly excreted. This provides a more valuable combination of circumstances: the lead in the body is rendered harmless as soon as it comes into contact with the EDTA and then it is quickly disposed of. Furthermore, the greater affinity of EDTA for lead than for calcium, allows the former to replace the latter in calcium-disodium EDTA complex.

ROLE OF CHELATION IN DRUG ACTION:

All chelating agents remove or inactivate some of the trace metals present in the organism, and it has been suggested that some drugs exert their pharmacologic action through this mechanism(6).

Thus several antimicrobial, tuberculostatic, antipyretic, antidiabetic, cytostatic and fungicidal drugs are chelating agents(7).

For instance, 8-hydroxyquinoline (an amoebicidal and fungicidal agent) is utilized as a chemotherapeutic agent due to its ability to chelate iron, a metal essential for the metabolism of certain micro-organisms. The same mechanism of action explains partially the tuberculostatic activity of isoniazid(6).

Several other drugs have the property of forming chelates and owe their biological action partially or

totally to this property: tetracyclin, salicylic acid, thiouracil and thiosemicarbazone[3,7,8 & 9].

Some chelating agents are used as antidotes in poisoning by metallic ions e.g. dimercaprol, penicillamine, desferrioxamine and others[4].

It should be mentioned also, that many substances present in the biological systems are chelates, e.g. insulin, hemoglobin, myoglobin, chlorophyll, vitamin B_{12} and several enzymes[6].

PHARMACOLOGICAL ACTIONS OF EDTA SALTS:

The rapid intravenous administration of Na_2 EDTA results in hypocalcemic tetany. However, a slow infusion (less than 15 mg/min.) into a normocalcemic individual elicits no hypocalcemic symptoms, indicating the ready availability of extracirculatory calcium stores[10].

When Na_2 EDTA is administered slowly over a period of days calcium is mobilized from bone and excreted in the urine as the EDTA complex, although during this time there is little alteration of the plasma calcium level[11]. In contrast, $CaNa_2$ EDTA can be administered intravenously in relatively large quantities with no untoward effects and no change in the plasma or total body calcium levels. The administration of $CaNa_2$ EDTA will result in chelation

of any metals having greater affinity for EDTA than has calcium, depending of course on the availability of such metals. Next to calcium, zinc seems to be the most accessible metal to EDTA in the body. Intravenous administration of $CaNa_2$ EDTA increases enormously the urinary excretion of zinc[12].

ABSORPTION, FATE and EXCRETION:

Na_2 EDTA is rapidly absorbed from the gastro-intestinal tract while the therapeutically commonly used $CaNa_2$ EDTA is poorly absorbed[13,14], and when administered orally $CaNa_2$ EDTA does not increase the urinary excretion of calcium[11]. After intravenous administration, $CaNa_2$ EDTA disappears exponentially from the circulation with a half life of 20 to 60 minutes.

In blood, all the drug is found in plasma. About 50% is excreted in the urine in one hour and over 95% in 24 hours. For this reason adequate renal function is necessary for successful therapy. Renal clearance of the compound in dogs equals that of inulin i.e. glomerular filtration accounts entirely for urinary excretion[15]. Altering either pH or the flow of urine has no effect on the rate of excretion. Almost none of the compound is metabolized. The drug is distributed mainly in the extracellular fluid[16] and penetrates poorly into cells[17]

but very little gains access to the spinal fluid (5% of plasma concentration). The concentration of calcium in the spinal fluid remains unchanged after injection of Na_2 EDTA[18].

TOXICITY:

SEVEN (1960)[19] discussed extensively the toxic effects of EDTA. The hypocalcemic effects seen upon rapid administration of Na_2 EDTA have been mentioned.

In general $CaNa_2$ EDTA, when used properly, has a low incidence of untoward reactions. Renal damage with fatal nephrosis may occur if the dosage is excessive[20].

Experimentally in rats and also in human, severe hydropic degeneration of proximal tubules has been observed in some cases, with almost total destruction of the proximal tubular epithelium[21,22,23]. Both in the human and in experimental animals these effects are usually reversible and urinary abnormalities disappear rapidly upon cessation of treatment.

Intravenous infusion of solutions of a concentration more than 0.5% may cause thrombophlebitis. Prolonged administration in high doses may produce transient bone marrow depression[20].