بسم الله الرحمن الرحيم

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HISTOLOGICAL CYTOLOGICAL AND CYTOCHEMICAL STUDIES OF THE EFFECT OF SOME CYANINE DYES ON SOME ORGANS IN MICE

Thesis

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بسم الله الرحمن الرحيم

This work is dedicated to my husband, my parents and my children

CONTENTS

Chapter I

Introduction 1

Aim of the work. 22

Chapter II

Material and Methods 26

Chapter III

Observations 34

The liver 34

light Microscopy 34

Electron Microscopy 47

Abbreviations . . . . . i-ii

Light Micrographs (Figs.:1-126) 57

Electron Micrographs (Figs.:127-150) .

The Kidney 58

Light Microscopy 58

Electron Microscopy 70

Light Micrographs (Figs.:151-242) 83

Electron Micrographs (Figs.:243-263).

Chapter IV

Discussion 84

Chapter V

Summary 124

Conclusions 131

References 133

Arabic Summary

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Chapter I

INTRODUCTION

INTRODUCTION

Because of their special properties, the cyanines (methines) as a class of organic compounds are interested not only for the chemist, but also for numerous other scientists working in the fields of biology, medical science, technology and physics.

The cyanine structure contains two heterocyclic rings linked by a simple methine chain containing an odd number of carbon atoms. The nature of the terminal heterocyclic ring incorporated into cyanine dyes markedly affects the chromophoric character (i.e. colour) of the cyanine dye, as does the changing length or substituents in the polymethine chain.

Classification of cyanines (Hamer, 1964):

Cyanine dyes are conveniently classified according to the number of -CH=(methine, methylidine or methenyl) groups in the chain between the two ring systems and according to the ring system present. If one methine group is present, the dye is classified as monomethine or simple cyanine. If there are 3 methine groups, the dye is classified as trimethine or carbocyanine. Dyes with 5 methine groups are known as pentamethines or dicarbocyanines, and dyes with 7 methine groups are known as heptamethines or tricarbocyanines.

In the present investigation 3 types of monomethine (simple) cyanine dyes were used.

Cyanine dyes have many biological and biochemical effects. Attempts have been made to systematically relate the electro-chemical properties of cyanine dyes with their biological actions. Control of electrochemical potentials in biochemical reactions could provide new ways of limiting abnormal or infectious cell growth in disease. A strong relation was found between the reduction potential of a cyanine dye and its ability to inhibit cell division. Some of the cyanine dyes are growth inhibitors to bacteria, and to the mitosis of fertilized sea urchin eggs **(Zigmand et al., 1980, Gilman et al., 1981).**

Applications of cyanine dyes:

Cyanine dyes have various important applications **(Venkataraman, 1971)**. They have been tested for therapeutic uses and are used as anticancer agents **(Uchimi et al., 1980)**, and in the future time, cyanine dyes may find important applications in this field **(c.f. Fahmy, 1985)**.

Certain cyanine dyes are useful for treating parasitic infections **(Goodman and Gilman, 1975)**.

Other applications of cyanine dyes are their use as inhibitors of respiratory chain reactions involving electron transport **(Kinnally and Tedeschi, 1978)**.

With the increasing and widespread use of cyanine dyes, it is important to recognize any biological side effects **(Howard and Wilson, 1979)**.

Waggoner (1976) has noted that 3,3-dipropylthiocarbocyanine inhibits oxidation in rat liver mitochondria.

Cohen et al, (1974) and Howard and Wilson (1979), reported on the effect of the cyanine dye 3,3-dipropylthiocarbocyanine on the respiration of isolated mitochondria (i.e., mitochondrial energy conservation) from neurons and rat liver respectively.

Similarly, **Okimaus et al. (1979)** reported that cyanine dyes are inhibitors of electron transport reactions in isolated liver mitochondria. Aza analogues of cyanine dyes are potent mitodepressive and mutagenic agents. These cyanine dyes were found to display a structure activity relationship with regard to the cytological effects **(Abdou et al. 1982, 1983)**.

Cyanine dyes affected the peritoneal mast cells of rats **(Sugiyama and Utsumi, 1976)**.

In a number of systems, the fluorescence of a dye molecule has been useful in correlating membrane potential with biological properties of the system **(Waggoner, 1976)**. **Miller and Koshland (1978)** investigated the effects of cyanine dye-membrane probes on cellular properties. They reported that several cyanine dyes interfere with bacterial motility.

The use for chromorphoric and fluorescent probes or receptor molecules has become widespread. Changes in spectral and fluorescent properties are associated with changes in the environment or concentration of the dye. It has been generally

acknowledged that a danger of inhibitory or disruptive effects of the dyes on biological processes exists, especially if applied to the study of membrane function in mitochondria or intact cell plasma membranes. The application of these probes to the study of biological function, e.g. energization processes in mitochondria, requires an investigation of their effect on metabolism. Among the most extensively utilized have been the electrofluorometric cationic dyes, such as the various cyanine and acridine dyes. These have been used to monitor membrane potentials in: neurons **(Cohen et al., 1974)**; in liposomes **(Sims et al., 1974; Kimmisch et al., 1977)**; in mitochondria **(Laris et al., 1975; Kinnally and Tedeschi, 1978)**, micro-organisms-certain bacteria **(Brewer, 1976)**; red blood cell ghosts **(Sims et al., 1974; Simons, 1976; Kimmisch et al., 1977; Philo and Eddy, 1978)** and various ascites tumour cells **(Laris et al., 1976)**. The cyanines have also been employed to monitor membrane potentials in excitable cells **(Waggoner, 1976)**.

There have also been reports which have indicated an uncoupling or inhibition of phosphorylation in mitochondria **(Laris et al.1975, Kinnally and Tedeschi, 1978)**. Several accounts have been reported on inhibition of NAD-linked respiration in mitochondria **(Kinnally and Tedeschi 1978; Howard and Wilson, 1979)**.

In the course of the use of various cationic cyanine dyes, the selective inhibition of NAD-linked (energy-linked) respiration, at concentrations that has little effect on other mitochondrial functions, was observed by **Schneider and Conover (1980)**, during dye uptake in rat liver mitochondria, but no effect on succinate oxidation or coupled phosphorylation.

Estimation of membrane potential of mitochondria have ranged from a small potential, internally positive **(Tupper and Tedeschi, 1969** - *Drosophila* giant mitochondria) to high potential, internally negative values **(Bakeeva et al., 1970)**.

Uncoupling of oxidative phosphorylation in mitochondria by hydrophobic cations is generally thought to be due to dissipation of the membrane potential caused by electrophoretic transfer of these cations through the mitochondrial inner membrane **(Bakeeva et al., 1970)**.

Various organic, hydrophobic cations such as cyanine dyes **(Kinnally and Tedeschi, 1978; Terada and Nagamune, 1983; Terada et al., 1985)** have been

reported to inhibit respiratory chain reactions involving electron transport, i.e., they act as uncouplers (inhibitors) of oxidative phosphorylation in mitochondria.

The divalent cationic trinuclear cyanine dye tri-S-C_7(5) is so hydrophobic that it binds almost completely to mitochondria, irrespective of their energy state. It acts as an uncoupler only in the presence of P_i or arsenate **(Terada and Nagamune, 1983).**

Similarly the divalent cationic trinuclear cyanine dyes tri-S-C_4(5) and tri-S-C_7(5) uncouple oxidative phosphorylation in mitochondria, only when inorganic phosphatase (P_i) was present in the incubation medium **(Terada et al., 1985).**

Also, **Takeguchi et al. (1985)**, reported about the formation of a leakage-type ion pathway in lipid bilayer membranes by the divalent cationic cyanine dyes: tri-S-C_4(5) and tri-S-C_7(5) cooperation with inorganic phosphate (P_i), and the role of the cyanine dyes in uncoupling of oxidative phosphorylation.

Shinohara et al. (1987) studied the effect of the divalent cationic cyanine dye tri-S-C_4(5) on oxidative phosphorylation in rat liver mitochondria in relation to the transport of ADP into mitochondria.

Bushnell et al. (1987) tested selected fluorescent cyanine dyes for uptake by mitochondria in intact plant cells (barley, maize and onion).

Waggoner and Stryer (1970) synthesized a number of fluorescent analogues of membrane lipid which permitted an 0investigation of different transverse regions of the lipid bilayer.

Fluorescent dyes have been used in many biological and model systems in a variety of studies involving, e.g., protein conformational changes of mitochondrial transmembrane potential **(Birkett et al, 1971).**

Wiedmer and Sims (1985) used a fluorescent cyanine dye to measure membrane potential changes due to the assembly of complement proteins.

Shinohara and Terada (1987) found that the divalent cations (O-phenanthroline)$_2$ - Cu^{2+} complex is an uncoupler of oxidative phosphorylation in mitochondria.

Cyanine dyes are introduced as new fluorescent reagents for covalently labeling proteins and their biomolecules. **Waggoner et al. (1989a, b)** studied cyanine dye labeling reagent for sulfhydryl and isothiocyanate groups.

By reviewing the literature, it appears that the majority of these investigations were from the physiological point of view, since several of the various cationic dyes show adverse effects on mitochondrial function, i.e., on the respiratory and energy-linked activities of mitochondria, presumably as the result of swelling or uncoupling. Thus, numerous authors examined the effect of dyes on oxidative phosphorylation in relation to the transport of ADP into mitochondria.

From this review, it is evident that numerous investigators used different fluorescent cyanine dyes in their studies **(Sims et al., 1974; Laris et al., 1975, 1976; Simons, 1976; Kimmich et al., 1977; Kinnally and Tedeschi, 1978; Miller and Koshland, 1978; Philo and Eddy, 1978; Howard and Wilson, 1979; Schneider and Conover, 1980; Zigmand et al., 1980; Takeguchi et al., 1985; Wiedmer and Sims, 1985; Cabrini and Verkman, 1986, 1987; Bushnell et al., 1987; Girgis and Khalil, 1987; Shinohara and Terada,1987; Shinohara et al., 1987; Terada et al., 1985; Waggoner etal., 1989a, b).**

Some studies dealt with dyes, other than cyanines.

Bumett and Squire (1986) made a study on the effect of dietary administration of the dye disperse Blue 1 on the urinary system of the rats.

Sole and Chipman (1986) made a study on the mutagenic potency of chrysoidines and Bismark brown dyes.

Borzelleca et al. (1987) evaluated the potential teratogenecity of FD and C blue No.2 in rats and rabbits.

Kari et al. (1989) made a comparative study on carcinogenicity of two structurally similar phenylenediamine dyes (HC blue No.1 and HC blue No.2) in rats and mice.

Morgan et al. (1989 a) made toxicity studies of C.L. pigment red 3 in rats and mice. **Morgan et al. (1989 b)** made toxicity studies of the 3,3̄-dimethyoxybenzidene (DMOB) and C.L. direct blue 15 in the rats.

In spite of its non-inclusion in the prescribed list of food colours, orange II is extensively employed to colour a variety of food stuffs. Thus, **Singh et al. (1987)** made acute and short term toxicity studies on orange II.

Yamada **(1991)** studied the effects of the food additive coal tar dye, Rose Bengal (Food Red No.105), on isolated rat hepatocytes.

Das and Giri (1988) made a study on chromosomal aberrations induced by secondary and tertiary amine-containing dyes and in combination with nitrite *in vivo* in mice.

Similar to cyanine dyes, the hydrophobic cationic dye rhodamine 6G (Rh 6G) has been reported to act as an uncoupler of oxidative phosphorylation in mitochondria **(Gear, 1974).**

Evenson et al. (1985) reported that rhodamine 123 (Rh 123) induces ultrastructural alterations of mitochondria in cultured cells under conditions (time, concentration) at which the cells are reversibly arrested in an early phase.

Hood et al. (1988) made a study on teratogenic effects of the lipophilic cationic dye rhodamine 123 (R 123) on mitochondria.

Ranganathan and Hood (1989) studied the effects of *in vivo* and *in vitro* exposure to rhodamine (Rh) dyes (Rh 6G, Rh 123, Rh B) on mitochondrial function of mouse embryos.

Fowler and Greenspan (1985) described the use of Nile red as a hydrophobic fluorescent probe for the detection of neutral lipid deposits in frozen tissue sections of liver and aorta.

Bucana et al. (1986) reported that the hydroethidine was used as a vital dye uptake and accumulated in neoplastic cells.

Darzynkiewicz et al. (1986) studied the cytostatic and cytotoxic properties of pyronin Y and relation to mitochondrial localization of the dye and its interaction with RNA.

Kahn et al. (1987) introduced a method to detect mRNAs present at low concentration in rat liver during metabolic regulation and azo dye carcinogenesis.

Nesnow et al. (1988) examined aryl azo dyes for their ability to be reduced by rat liver microsomal azoreductase.

Okawa et al. (1989) investigated the effect of azo dyes on proliferation in mouse embryo culture cells.

Zabida et al. (1989) made studies on the mechanism of reduction of azo dye carcinogens by rat liver microsomal cytochrome P-450.

Ganote and Rosenthal (1968) compared the subcellular rat liver damage induced by methylazoxymethanol (MAM) with that produced by dimethylnitrosamine (DMN), hydrazine sulfate (HS), and carbon tetrachloride CCl_4.

Tullis et al. (1987) studied the characterization and properties of DNA adducts formed from N-methyl-4-aminoazobenzene in rats during a carcinogenic treatment regimen.

Although very few light and electron microscope studies dealt with some azo dyes, other than cyanines (**Price et al., 1952; Miller and Miller, 1966; Briere, 1970; Daoust and Calamai, 1971; Farber and Farmar, 1973; Karasaki, 1975; Lin et al., 1975; Flaks and Teh, 1976; Bumett and Squire, 1986; Scoazec et al., 1988; 1990**), in contrast, studies of the histopathological effects of monocyanine dyes on cell proliferation, particularly of liver and kidney in mice, are very scare: Moreover, no attention has been paid to the cytological or cytochemical changes, induced by cyanine dyes.

In none of these studies, however, has the sequence and progression of the histologic, cytologic and cytochemical changes, been described from the earliest alteration to the development of necrosis.

When rats are fed carcinogenic azo dyes they develop cirrhosis with hepatocellular nodules of different types, proliferated ductular cells, and cholangiofibrosis, later they develop multiple and often mixed hepatomas, cholangia carcinomas, and undifferentiated carcinomas (**Price et al., 1952**).

Biochemical studies indicated that, during feeding of DAB, hepatic glucose-6-phosphatase activity gradually decreased and ultimately disappeared in induced hepatomas (**Weber and Cantero, 1955**).

The term "neoplastic nodule" was recommended by **Bannasch (1976)** to replace the so-called "hyperplastic nodule" of the rat liver (**Squire and Levitt, 1975**).

Using the light and electron microscopes, **Bannasch (1976)** studied the cytology and cytogenesis of "neoplastic (hyperplastic) nodules" in rat liver induced by nitrosomorpholine or thioacetamide.

In routine hematoxylin-and eosin-stained sections of neoplastic nodules, at least four different types of altered hepatocytes can be distinguished with the light microscope: