EFFECTS OF METHOTREXATE ON TISSUES OF THE RAT

THESIS

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A C K N O W L E D G M E N T

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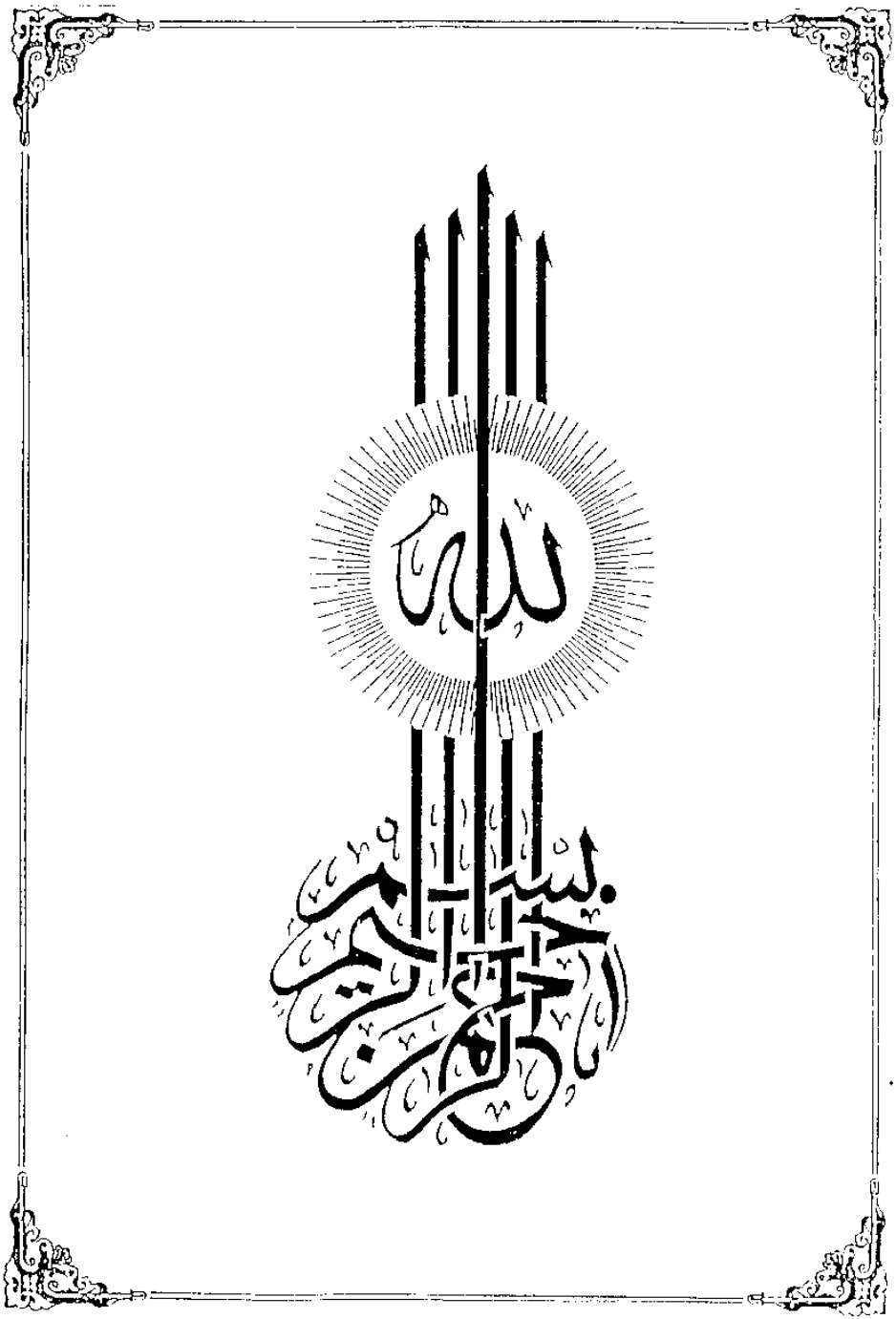

C O N T E N T S

AIM OF THE WORK

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The group of folic acid antagonists is one of the most important groups of cytotoxic drugs used nowadays. Methotrexate is the main member of this group. Many authors studied the clinical and pathological effects of the drug on various organs of the body. The aim of this work is to investigate the histopathological, histochemical and cytological changes which methotrexate induce in different tissues of the adult rat, taking the liver and small intestine as the material of study and using different doses ranging from the therapeutic up to the toxic doses.

INTRODUCTION

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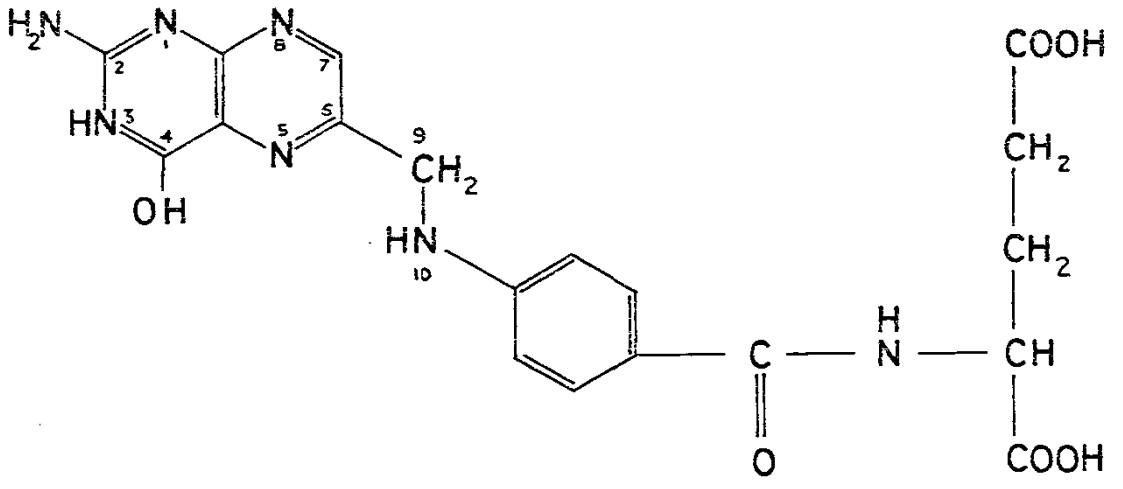
The nucleic acid metabolism is one of the most complicated cycles of cellular metabolism. All cells must synthesize nuclei acid in order to divide and perform their functions. Gubner (1951) reported that folic acid is a coenzyme in a complex enzymatic system concerned with synthesis of DNA and RNA inside the cell. In order to act as a coenzyme, folic acid must be changed to the biologically active form tetrahydrofolic acid by an enzyme called folic acid reductase (Harper et al., 1977). Folic acid antagonists are potent inhibitors of the reductase enzyme. So when the tetrahydrofolic acid production is blocked, all nucleic acid production is inhibited . As the synthesis of nucleoprotein is essential for chromosomal reduplication, the cells entering mitosis are unable to advance byond the metaphase stage and cannot enter the anaphase stage. In this way, mitosis and growth of both normal and pathological tissues are inhibited after the use of folic acid antagonists (Jacobson, 1954).

Laurance (1973) noted that normal cells that divide more rapidly than cancer cells like the gut mucosa and bone

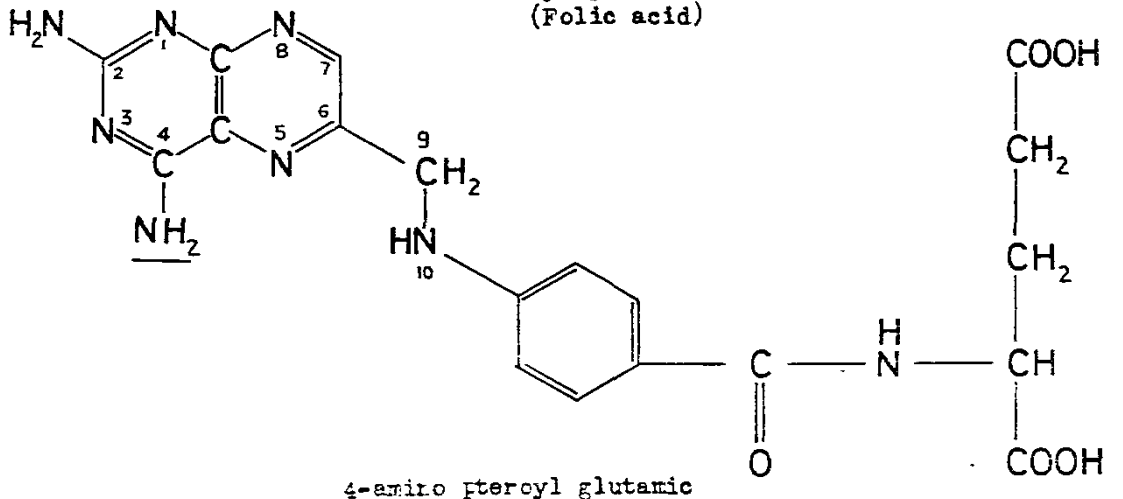
marrow, are greatly affected by folic acid antagonists. Therefore toxicity to gut and bone marrow cells are limiting factors to its use.

Jacobson (1954) stated that the most effective folic acid antagonists are those in which the OH group in 4-position of pteridine nucleus is replaced by amino group. This compound is called aminopterin (4 amino pteroyl glutamic acid). Another folic acid antagonist is called amethopterin and its structure is 4-amino-10 methyl pteroyl glutamic acid, the activity of which is about one fifth to one tenth of that of aminopterin. Amethopterin is now known as methotrexate and is the only folic acid antagonist used nowadays.

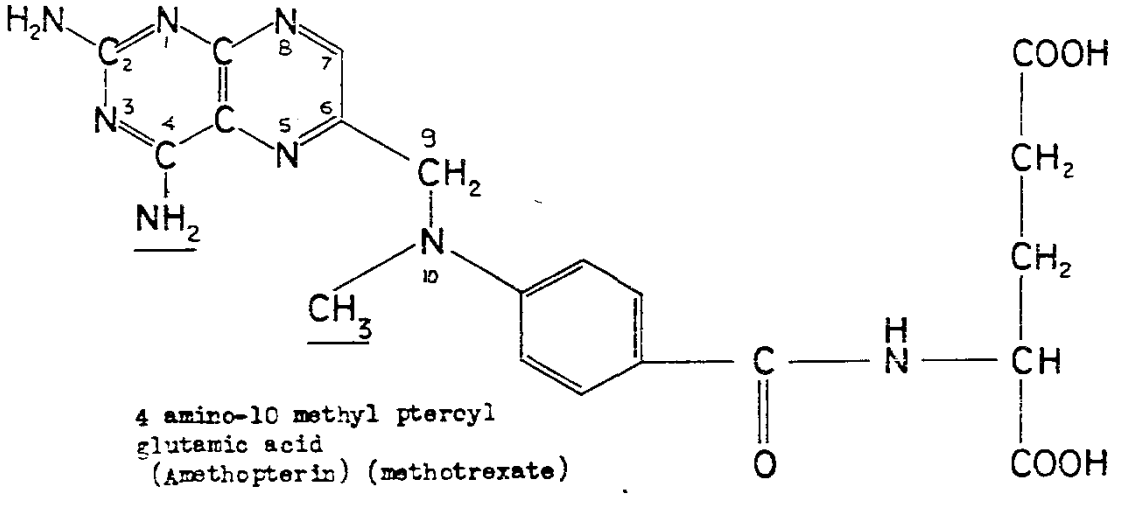
Methotrexate is widely used in treatment of acute leukaemia, choriocarcinoma, breast carcinoma, malignant testicular tumours and all forms of late malignant diseases. Recently it is indicated in the treatment of severe forms of psoriasis (Goodman and Gilman, 1975).



Pteroyl glutamic acid
(Folic acid)



4-amino pteroyl glutamic
acid (aminopterin)



4 amino-10 methyl pteroyl
glutamic acid
(Amethopterin) (methotrexate)

REVIEW OF LITERATURE

ACTION OF THE DRUG ON THE LIVER

Many authors described the clinical and histopathological changes produced by methotrexate in the liver.

Colsky et al. (1955) noticed hepatic fibrosis in children with acute leukemia after therapy with folic acid antagonists. They described the lesion produced in the form of marked portal fibrosis and small proliferating bile ducts. Brown pigment was prominent in the parenchymal cells near the central vein. The central zones of the lobules showed marked fatty changes. Extensive fibrosis in the portal areas and to a lesser degree in the centre of the lobules was observed.

O'rourke and Eckert (1964) described a case of hepatic injury in an adult after methotrexate treatment for psoriasis. There were peripheral hepatic fibrosis and mild fatty change.

Hersh et al. (1966) investigated hepatotoxic effects of methotrexate in 22 patients during intensive and intermittent therapy. During intensive treatment, they developed abnormal liver function tests. There was cellular inflammatory reaction of moderate degree characterized by infiltration of both polymorphonuclear and mononuclear cells

in the portal and periportal areas. There was no necrosis of liver cells. Mild interstitial fibrosis, bile stasis and mild fatty change were also observed. Intermittent therapy had slight effects on the liver function tests and liver biopsies. After cessation of intermittent therapy, recovery occurred in one month.

In the same year, Talerman and Thompson (1966) described a case of marked hepatic fibrosis probably resulted from prolonged treatment of acute leukemia with methotrexate. The normal architecture of the liver was distorted by numerous strands of connective tissue which was mainly present in the portal areas, and by their continuity, they produced numerous isolated liver nodules. There were slight proliferation of the bile ductules in the portal tracts, and slight infiltration by lymphocytes. The liver lobules showed marked fatty change which was mainly central. Von-Küpffer cells appeared normal. Some nodules showed the picture of regeneration.

Also Coe and Bull (1968) noted similar cases of cirrhosis associated with methotrexate treatment for psoriasis.

Ervin et al. (1969) investigated similar cases of severe psoriasis treated with methotrexate and developed

severe cirrhosis and centronodular hepatic cell necrosis.

Sharp et al (1969) studied the liver function tests and liver biopsies using the light and electron microscopes during methotrexate treatment. The ultrastructural changes included double-nucleated hepatic cells with minor nucleolar changes and decreased perichromatin. The cytoplasmic alternations included cellular variation in fat and glycogen with mitochondrial swelling.

Muller et al. (1969) studied similar cases showing the picture of postnecrotic cirrhosis after the treatment of psoriasis with methotrexate.

Barnardo et al. (1971) stated that in 59 psoriatic patients treated with methotrexate, 17 developed hepatic fibrosis and cirrhosis.

Dahl et al. (1971) studied the liver function and histological changes in liver biopsies of 37 patients who had been treated for psoriasis with methotrexate. Cirrhosis was found in 19 % and hepatic fibrosis in 27 % of the cases. Minor abnormalities in another 46 % consisted of fatty change and extensive vacuolation of their liver nuclei. The hepatic histology was normal in 8 % of the cases.

Filip et al. (1971) investigated liver disease associated with long term methotrexate therapy. Liver biopsies showed serious lesions in the form of widespread cellular infiltration and hepatic fibrosis.

Price (1971) noted that toxicity of methotrexate to the liver was not proportional to the dose but rather to the time for which a given plasma level was mentioned; the longer the time was, the greater the toxicity to the liver.

Zachariae and Schioât (1971) noted that methotrexate caused fatty infiltration and multi-focal necrosis, but no cirrhosis was observed.

Almeyda et al. (1972) reported that treatment of psoriasis with methotrexate carried a low but definite risk of producing histological abnormalities in the liver.

Millard-Sadler and Ryan (1974) studied methotrexate induced liver disease in psoriasis. Many patients showed no abnormalities after six months. Some showed minor abnormalities in the form of mild fatty change and small foci of necrosis in the centrilobular areas. These foci were composed of one or more degenerated hepatocytes surrounded by mononuclear cells. Also there were vacuolation of hepatocytes and oedema of the portal tract.Only two cases developed fibrosis out of 17 cases examined.There were proliferation