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The effect of methotrexate on DNA synthesis and its reversal by folinic acid

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SUMMARY

In suckling mouse liver methotrexate is a powerful inhibitor of DNA synthesis, as assessed by the incorporation of tritiated deoxyuridine. Folinic acid is an effective antagonist.

INTRODUCTION

Transient inhibition of DNA synthesis has been used as an experimental technique in a number of situations. Massive doses of methotrexate, a folic acid antagonist, have been used to inhibit humoral (Berenbaum & Brown, 1965) and cellular immune responses (Berry, 1969) and to delay the appearance of vertebral ossification centres (Berry, 1971). More recently its effects on the expression of a polygenically determined malformation have been described (Berry & Germain, 1972). Although thymidine deficiency has been demonstrated in animals exposed to methotrexate for 8 h (see Berry and Germain) there has been no direct demonstration of the effects of the drug on DNA synthesis in the tissues of rapidly growing animals, nor of the efficiency of the specific antagonist, folinic acid.

DNA is synthesized from purine and pyrimidine nucleotides. Methotrexate acts by inhibiting the enzyme dihydrofolate reductase, preventing the formation of tetrahydrofolate, the co-enzyme (as 5,10-methylene tetrahydrofolate) in the conversion of deoxyuridate (d-UMP) to thymidylate (d-TMP). Folinic acid counteracts this effect by supplying the product of the blocked reaction.

We have measured the rate of incorporation of deoxyuridine into rapidly growing mouse liver, shortly after birth. The inhibitory effect of methotrexate and its prevention by folinic acid are reported, together with the results of simple histochemical studies designed to assess the effects of therapy on certain cellular activities.

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Table 1. *Effects of methotrexate on incorporation of* [^3H]*deoxyuridine*

(100 % represents mean incorporation of five 8-day-old animals and two 15-day-old animals. Each figure represents a single animal.)

Activity in test livers	
8 h methotrexate	16 h methotrexate
8-day animals (%)	
10	50·8
3·6	20·1
2·4	14·4
14·3	6·2
13·9	5·6
15·3	23·2
16·2	7·3
16·2	
17·6	
18·5	
20·0	
15-day animals	
14·3	48·3
21·1	50·8
14·5	
25·1	
21·1	
20·6	
Mean 15·55 %	25·1 %
S.E. 1·44	3·32

MATERIALS AND METHODS

CBA mice, kept under standard conditions, were used throughout the experiment. Suckling mice aged 8 and 15 days were injected intraperitoneally with 0·7 μCi/gm body weight deoxyuridine-6-^{3}H (> 10000 mCi/mmol) obtained from the Radiochemical Centre, Amersham, 1 h before they were killed. The liver was removed immediately, quick frozen and stored at −20 °C until DNA extraction was carried out by the method of Shibko *et al.* (1967). Prior to this injection various procedures had been carried out. In controls, a short period of separation from the mother was the only manipulation. Other animals were injected intraperitoneally with 8 mg/kg body weight methotrexate at varying intervals before the tracer was used, a further group had received both methotrexate and folinic acid, the latter in a dose of 64 mg/kg body weight.

Scintillation counting was carried out using an Intertechnique 5 L 50. Results were expressed as counts per minute per 100 mg of wet weight liver, after subtraction of the background (35–40 counts). The activity of test extracts was expressed as a percentage of the mean activity of controls.

Table 2. *Effects of folinic acid on the inhibitory action of methotrexate on incorporation of [^3H]deoxyuridine*

(100% represents mean incorporation of five 8-day-old animals. Each figure represents a single animal.)

Activity in test livers in 8-day animals (%)		
4 h Mtx.	4 h Mtx. FA at 2 h	4 h Mtx. FA simultaneously
31·6	52·8	74·1
27·2	62·2	85·8
35·3		95·1

Mtx. = Methotrexate. FA = Folinic acid.

Histochemical studies carried out on cryostat sections of undecalcified teeth cut at 10 μm, from animals killed 1, 2 and 4 days after exposure to methotrexate for 8 h, with subsequent injection of folinic acid. After staining for succinic dehydrogenase (Pearse, 1960) and acid phosphatase (Barka & Anderson, 1962) the sections were compared with normal aged-matched controls.

RESULTS

The results of incorporation experiments are seen in Tables 1 and 2. It is evident from Table 1 that methotrexate is a powerful inhibitor of DNA synthesis at the dose used and that the effects of a single injection persist for 16 h after administration, although some spontaneous recovery is seen at this time.

From Table 2 it is evident that folinic acid effectively antagonizes methotrexate, and that the effect is quite rapid, since an 'improvement' in the rate of incorporation of deoxyuridine is seen 2 h after giving the acid. If given simultaneously the inhibitory effect of the cytotoxic agent is barely manifest.

Histochemically, acid phosphatase activity was not altered in any animal studied. Normal distribution of succinic dehydrogenase, a dense compact band at the base of the cells of the enamel organ of the developing tooth (Fig. 1), was found in all control and methotrexate and folinic acid treated animals.

DISCUSSION

The inhibitory effects of methotrexate on DNA synthesis may be exploited in a number of ways in developmental studies. The particular advantage of this compound is the capacity to reverse or prevent its action with large amounts of folinic acid. Animals treated with methotrexate and folinic acid show no obvious signs of ill health and grow normally (see Berry & Germain, 1972). Histochemically, no early evidence of cell death as assessed by changes in acid phosphatase activity was seen in any of the animals studied. Demonstrable succinic dehydro-

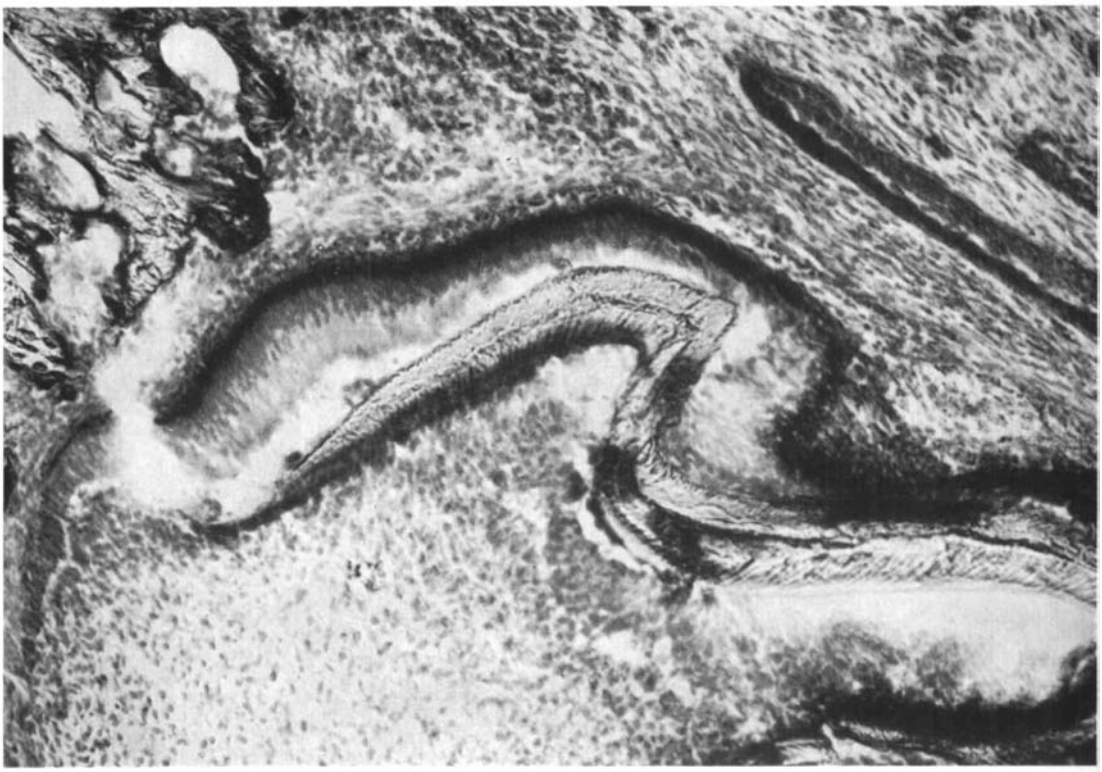

Fig. 1. Undecalcified section of tooth from methotrexate and folinic acid treated mouse. Dense band of succinic dehydrogenase activity seen in enamel organ. × 50.

genase activity in the cells of the developing tooth did not change in methotrexate treated animals; in both instances the developing tooth was studied since well defined bands of enzyme activity normally occur and are easily assessed histologically. The lack of effect of methotrexate on 'energy producing' enzyme activity (as assessed by this crude method) is a further indication of the value of this technique in producing a circumscribed metabolic injury. The specificity of folinic acid deficiency in this respect has also been demonstrated chemically by Chepenik & Moseley Waite (1972). These authors have shown no change in the facility with which mitochondria from the embryos of normal and folinic acid deprived rats carry out oxidative phosphorylation with succinate as substrate.

Using methotrexate and folinic acid well-defined periods of inhibition of DNA synthesis may be induced. The precision of the technique will allow better definition of the nature of the so-called 'critical' periods in teratogenesis.

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