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Effects of ethylenediaminetetraacetate and diethylenetriaminepentaacetate of DNA. Synthesis in kidney and intestinal mucosa of folate treated rats

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THE polyaminopolycarboxylic acid chelating agents ethylenediaminetetraacetic acid (EDTA) and diethylenetriaminepentaacetic acid (DTPA) are valuable drugs for the treatment of certain types of metal and radioelement poisoning in man.[1–5] However, studies in experimental animals have shown that at high doses these substances may cause damage to the kidneys and intestinal mucosa[6–11] and this has led to some concern about their prolonged use in man.

We have studied the effects of EDTA and DTPA salts on the initiation of DNA synthesis in the kidneys of folate-treated rats[12] and on the rate of DNA synthesis in the intestinal mucosa of the same animals. The folate-stimulated increase of DNA synthesis in the kidney is markedly depressed by $CaNa_2EDTA$ and by $CaNa_3DTPA$ and $MnNa_3DTPA$ but not by the corresponding Zn salts of EDTA and DTPA. In the intestinal mucosa, under the conditions studied, only $CaNa_3DTPA$ decreased the rate of DNA synthesis.

Male rats of the highly inbred August strain, aged 8–10 weeks and weighing about 200 g, were given a single intraperitoneal injection of 250 mg folic acid/kg body wt in 0·3 M sodium bicarbonate. Twenty-five hr later each animal received by intraperitoneal injection 250 μCi/kg of ^{3}H-thymidine (Radiochemical Centre Ltd., Amersham, Bucks.—sp. act. 25 Ci/mmole), all animals were killed 26 hr after folate injection. The left kidney and a sample of ileal mucosa were removed and the specific activity of the isolated DNA was determined as described previously.[13] The chelating agents, and in one experiment zinc acetate, at a dose level of 0·75 mmole/kg body wt were administered intraperitoneally at the same time as, or at various time before or after, the folate injection.

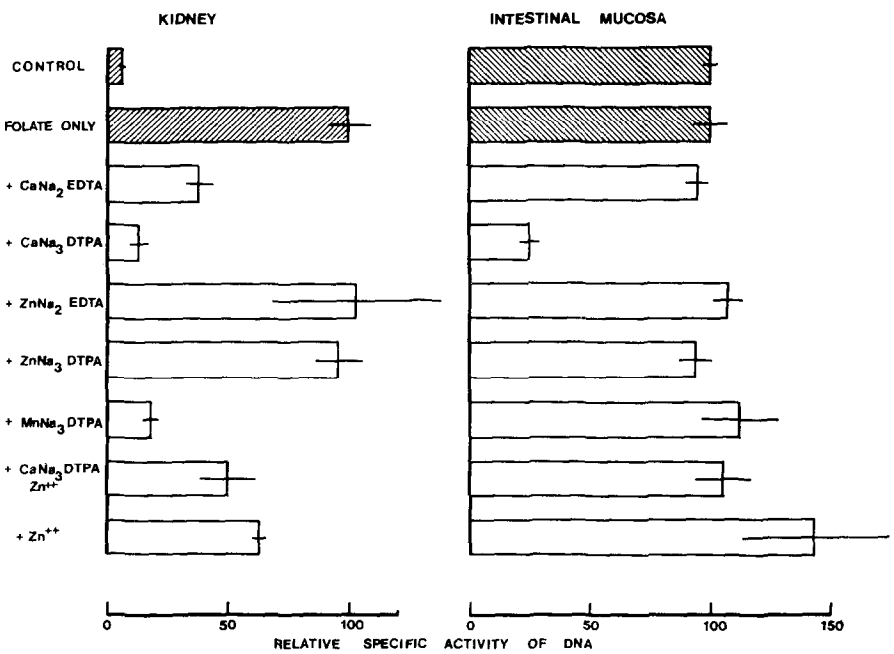

FIG. 1. The effects of EDTA or DTPA salts on DNA synthesis in rat kidney and in intestinal mucosa of folate-treated rats. Chelating agents, or Zn acetate, (0·75 mmole/kg) were administered by intraperitoneal injection simultaneously with folic acid (250 mg/kg). Rates of DNA synthesis were measured by ^{3}H thymidine incorporation at 26 hr after folate injection.

The effects of the chelating agents on the rates of DNA synthesis in kidney and intestinal mucosa are illustrated in Fig. 1 and Table 1. For ease of comparison the specific activities are expressed as the "relative specific activity" obtained by equating the measured specific activity in the kidney and mucosa of folate treated animals to 100. In the rats given only folate the actual rates of DNA synthesis, measured 26 hr after folate, were similar in the kidney (40,200 ± 2100 dis./min/mg DNA) and intestinal mucosa (39,200 ± 1450 dis./min/mg DNA).

TABLE 1. EFFECT OF DTPA SALTS (0·75 mmole/kg ON FOLATE-STIMULATED DNA SYNTHESIS IN RAT KIDNEY AND INTESTINAL MUCOSA‡

Time of treatment relative to time of folate administration	Relative specific activity of DNA: Kidney		Intestinal mucosa	
	$CaNa_3DTPA$	$ZnNa_3DTPA$	$CaNa_3DTPA$	$ZnNa_3DTPA$
−48 hr	113 ± 14	106 ± 13	95 ± 6	111 ± 16
−24 hr	114 ± 19	73 ± 13	136 ± 11†	103 ± 8
0	13 ± 1*	96 ± 7	25 ± 4*	94 ± 7
+24 hr	86 ± 7	137 ± 14	88 ± 6	106 ± 7
Folate only	100 ± 5	100 ± 5	100 ± 3	100 ± 3

* Significantly less than control. $P < 0{\cdot}005$.
† Significantly greater than control. $P = < 0{\cdot}035$.
‡ 250 mg folic acid/kg body wt i.p. at $t = 0$. 250 μCi/kg ^{3}H thymidine 1 hr before death at $t = 26$ hr.

Simultaneous administration of $CaNa_2EDTA$, $CaNa_3DTPA$ or $MnNa_3DTPA$ with folate markedly depresses the rate of DNA synthesis observed in the kidney 26 hr later (Fig. 1), the largest effects being produced by the DTPA salts. In the intestinal mucosa only $CaNa_3DPTA$ produced a depression of the normal rate of DNA synthesis. In both tissues the rates of DNA synthesis were not altered following administration of $ZnNa_2EDTA$ or $ZnNa_3DTPA$.

When the Ca or Zn salts of DTPA were administered 48 or 24 hr before, or 24 hr after folate the only significant change observed was a small increase in the rate of DNA synthesis in the intestinal mucosa of the animals given $CaNa_3DTPA$ 24 hr prior to folate (Table 1).

The data presented in Fig. 1 and Table 1 illustrate the effects of EDTA and DTPA on two different conditions of DNA synthesis. In the kidney the administration of folate initiates a marked increase in the rate of DNA which is near maximal, 15–20 times greater than control levels, 26 hr later.[14] Thus the effects observed in the kidney following EDTA and DTPA administration probably represent interference with the initiation of new DNA synthesis. In contrast the intestinal mucosa has a naturally high rate of DNA synthesis, which is unaffected by folate, and the effects produced by chelating agents represent interference with established DNA synthesis. However, in both tissues the effects of DTPA and EDTA may be manifested by the same mechanism. The effects of $CaNa_3DTPA$ on the intestinal mucosa have been studied by Bohne and her colleagues.[15,16] Maximal depression of DNA synthesis was found about 12 hr after administration of the chelating agent and Bohne[16] has suggested that this represents an effect on reactions preceding DNA synthesis rather than a direct effect on DNA assembly.

The observations that $ZnNa_3DTPA$ does not affect DNA synthesis in either the kidney or the intestinal mucosa is interesting in view of the greatly reduced toxicity of $ZnNa_3DTPA$ as compared to $CaNa_3DTPA$ which has been reported by Catsch.[7] Similarly $MnNa_3DTPA$ has been reported to have a lower general toxicity and to cause less renal tubular damage than the corresponding Ca salt.[6] However, our results show that $MnNa_3DTPA$ depresses DNA synthesis in the folate stimulated kidney to a level similar to that produced by $CaNa_3DTPA$. Since $CaNa_3DTPA$ and $CaNa_2EDTA$ are known to decrease tissue zinc levels, at least transiently,[17,18] the absence of effect of $ZnNa_3DTPA$ or the corresponding EDTA salt on DNA synthesis in the kidney or gut may provide further evidence for the requirement for Zn^{2+} in the initiation or continuation of DNA synthesis.[19–21] This is further supported by the observation (Fig. 1) that the administration of Zn acetate 15 min after $CaNa_3DTPA$ resulted in a much smaller reduction in the rate of DNA synthesis in the folate stimulated kidney and restored DNA synthesis in the intestinal mucosa to control levels.

The data shown in Table 1, together with those of Bohne and her colleagues,[15,16] suggest that the effects of EDTA or DTPA on DNA synthesis are transient phenomena which take up to 12 hr to

become manifest but have disappeared by 24 hr. This pattern of effect may reflect transient changes in intracellular levels of Zn^{2+} or other trace metal ions.

The absence of any effect of $ZnNa_3DTPA$ on DNA synthesis in kidney or intestinal mucosa adds further support to the view that this, less toxic, salt would be more suitable for prolonged administration to man than the Ca salt.

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