Effect of vitamin A deficiency on rat hepatic and colon epoxide hydrase[1]

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Summary. Rat liver microsomes and homogenized mucosal linings prepared from vitamin A-supplemented and deficient male rats were used in metabolic studies of 7-^{3}H-styrene oxide. The colon tissue in deficient animals exhibits a significantly higher value of V_{max} than the same tissue from vitamin-supplemented animals. The implications of this finding in addition to our earlier observation[10] is discussed in relation to colon carcinoma.

Studies during the past few years have shown that many compounds implicated as carcinogens and other toxicants are not harmful per se, but are biotransformed to active metabolites (arene oxides) in vivo through the action of various microsomal drug-oxidizing enzyme system of the liver, skin, lung, colon and other tissues[4-9]. Rat colon tissues are able to metabolize aflatoxin B_1 (AFB_1) and this ability has been associated with the existence of a cytochrome P420-like drug-oxidizing system in the rat colon mucosa which metabolized AFB_1 to 4 other products[10]. This finding gives reason to suspect that the metabolism of substrates in the colon and liver[11] could be influenced by vitamin A to form intermediary metabolites which may have harmful toxigenic properties. Tissue epoxide hydrase(s) are involved in the biotransformation of such 'activated', harmful intermediates to less harmful dihydrodiols, a detoxification function which has been recently assessed[12]. Since vitamin A deficiency permits the increase in the induction of carcinoma in rat colon[13], we have in this study investigated some kinetic parameters of epoxide hydrase in relation to vitamin A malnutrition.

Materials and methods. For producing vitamin A deficiency in experimental animals, male weanling Sprague-Dawley-derived rats (50–55 g) were fed for 45 days on a corn-based diet without the vitamin. Control animals received a diet supplemented with vitamin A (5 mg vitamin A palmitate per kg diet) for the same period both groups receiving water ad libitum. The rats were decapitated and their colons were dissected out and washed free of fecal substances using 0.9% saline. The mucosal linings of the colon were scrapped and pooled for each group and stored at 0 °C. The pooled samples were minced in 0.2 M phosphate buffer, pH 7.4, and homogenized in the buffer in a motor-driven Potter-Elvehjem Teflon-glass homogenizer (in an iced bath) at 600 rev/min. Pooled livers from each group of the animals were finely minced and homogenized at 6 complete strokes in 2 vol. of 0.2 M phosphate buffer, pH 7.4, using the same homogenizer at 600 rev/min. All manipulations were carried out at 0–3 °C. The liver homogenate was centrifuged at 9000 × g for 20 min in a Sorvall refrigerated centrifuge, and microsomes were obtained from the supernatant using the technique described in our previous paper[14]. Protein and vitamin A were determined by the technique of Lowry et al[15] and Neeld and Pearson[16], respectively.

The kinetics of epoxide hydrase determination was based on the conversion of 7-^{3}H-styrene oxide, an arene oxide (New England Nuclear), to 7-^{3}H-styrene glycol according to the method of Oesch et al.[17] which we slightly modified[18]. The K_m-and V_{max}-values were calculated using a Fortran computer program devised by Cleland[19]. Means and SE values were calculated using a Wang 600-14 programmable calculator employing the Wang statistical program for grouped data, and significance was calculated by the nonparametric technique of Wilcoxon and Wilcox[20].

Results and discussion. The causes of chemical carcinogenesis are still neither well-delineated nor agreed upon by workers in this area, and this difficulty has been assigned to a lack of proper definition of carcinogenesis on the molecular level[21]. For this reason, a study of this nature becomes relevant. The cancerogenic action of certain compounds can be reduced, completely inhibited or partially postponed by substances physiologically occurring in organisms of animals and human beings, as well as by several synthetically prepared compounds[22]. Vitamin A is copiously supplied in dietary oils and it may play a role in the etiology of carcinogens. The table shows the K_m- and V_{max}-values of colon and liver epoxide hydrase(s). The K_m-values are higher (39%) in the colon of deficient animals, but lower (< 8%) in the liver of vitamin A-supplemented animals. These data indicate that vitamin A dietary levels affect the rate constant (k_2) of the conversion of 7-^{3}H-styrene oxide to 7-^{3}H-styrene glycol. We wish to remark that these kinetic data are apparent values only, since the tissue preparations used are multicomponent, multisubstrate, membrane bound and ill-defined mechanistically. The observed difference in the metabolism of styrene oxide can be assigned theoretically to 2 possible factors:

a) the formation of a reaction product(s) or a complex between the arene oxide and vitamin A leading to conversion of styrene oxide to styrene glycol, and

b) the formation of the complex between witamin A and the arene oxide reduces the amount of the latter (available for formation of carcinogenic adducts with cellular proteins, DNA, RNA).

If these are possibilities, in both cases, the incidence of tumor development would be considerably decreased. The simple Michealis-Menten law[23] could be applied to examine the possibilities in detail. Since the reaction scheme applies to a one substrate system, uncomplicated by reverse reactions or inhibitors, the enzyme [E] substrate [S] interaction (between the epoxide hydrase and 7-^{3}H-styrene oxide respectively) is expressed simply as

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad (1)$$

$$ES \xrightarrow{k_2} E + P. \qquad (2)$$

Data in the table are examined applying the steady-state treatment of Briggs and Haldane[24], according to which the concentration of complex formed is constant in time, i.e. the net rate of formation of epoxide hydrase-7-^{3}H-styrene complex is 0. Under this steady state treatment $[ES] \ll [E]$, so that the rate of change of [ES], except perhaps during the early stages of the reaction is very much smaller than the rate of change of [S], i.e. [styrene oxide] which is the quantity of interest. The rate of formation of complexes is made up of 3 terms. Whether animals are supplemented or deficient in vitamin A, reaction (1) still proceeds at the rate k_1 [E] [S]; they are removed by the reverse reaction (1) of rate k_{-1} [ES] and by reaction (2) of rate k_2 [ES]. If [E] is the free enzyme concentration and the total concentration of enzyme available for the reaction is $[E]_0$,

$$[E]_0 = [E] + [ES] \qquad (3)$$

Effect of dietary vitamin A on kinetic parameters (K_m, V_{max}) of epoxide hydrase[a]

Animals	K_m[d] Colon	Liver	V_{max}[e] Colon	Liver	Tissue protein (mg/g) Colon	Liver	Tissue vitamin A Plasma[b]	Liver[c]
Deficient	$1.45^x \pm 0.15$ 25%	$1.80^x \pm 0.20$ 35%	$278^x \pm 20$ 39%	$290^x \pm 21$ 8%	$9.80^x \pm 1.20$	$4.90^x \pm 1.10$	$0.1^x \pm 0.01$	$0.05^x \pm 0.01$
Supplemented	$1.95^y \pm 0.10$	$2.80^y \pm 0.5$	$200^y \pm 19$	$314^x \pm 21$	$9.00^x \pm 1.20$	$5.00^x \pm 1.70$	$43.80^y \pm 3.15$	$15.00^y \pm 1.42$

[a] Values represent mean ± SEM of 5 experiments with 6–7 animals per experimental subgroup. Statistical significance between groups is indicated by lettered superscripts (x and y); data bearing the same superscripts are not significant ($pF < 0.05$). [b] Expressed as IU per 100 ml of plasma. [c] Expressed as IU per g wet weight of liver. [d] K_m (mM). [e] V_{max} (nmoles/mg microsomal protein/h).

and since the steady-state treatment is assumed, one can write that

$$k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0, \quad (4)$$

whence

$$[ES] = \frac{k_1[E]_0[S]}{k_{-1} + k_2 + k_1[S]}. \quad (5)$$

The rate of the reaction is therefore

$$v = k_2[ES]. \quad (6)$$

The table shows that the K_m-values of the reaction are lower in both tissues of deficient animals. From equation (6), such an experimental observation leads to the minimal rate and perhaps also minimal levels of [ES]. Under this condition, the following theoretical implications are also considerable:

1. The abundant free substrate, 7-^{3}H-styrene oxide can form adducts with RNA, DNA or cellular protein unconditionally.
2. In supplemented groups, where K_m-values are higher, only very little of the 7-^{3}H-styrene oxide is therefore available for adduct formation.
3. If the report of Newberne and Rogers[13] is considered, vitamin supplementation creates optimal K_m-parameters which delay the circulation of a preformed carcinogen.

Apart from these considerations, the rate (v) of the reaction in equation (6) may be very important. The rate can be expressed as

$$v = \frac{V[S]}{K_m + [S]}, \quad (7)$$

where V is the limiting rate at high substrate concentrations. When K [S] is equal to unity or [S] is equal to K_m, the rate is equal to

$$v = \frac{V_{max}}{Z}, \quad (8)$$

Under this condition, our data on the table indicate that the rate is about 39% less abundant in colon tissue of supplemented animals, but only 8% more active in the liver of the same group of animals. This implies that, under vitamin supplementation, free substrate would be available for complementation with the macromolecules RNA, DNA and tissue protein, but the higher K_m-value at which the reaction proceeds may reduce such possibilities; the reverse of this interpretation applied to the deficient groups may explain carcinogenesis in vitamin A-deficient animals under drug metabolism. The rate of vitamin A deficiency mechanism of [ES] formation or its specific initial role on the native pure enzyme needs further study especially in relation to other sources of arene oxides (epoxides) such as 3,4-benzopyrene[22], benzidine[25], aflatoxin[26,27], and a host of food additives, food colours and sweeteners[28]. This study would be relevant in view of the fact that vitamin A, as well as its analogs, have proved successful in organ culture in the reversal of keratinized squamous metaplastic lesions of vitamin A deficiency in tracheobronchial epithelium of the hamster[29].

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