To examine the possibility that the PE and PZE voltage responses of live skin or fresh skin preparations were caused mainly or partly by ionic transport or bioelectric phenomena normally associated with the living state, we repeated the PE and PZE measurements on dead, dry (several months old) skin preparations. The signals from the dry samples were PE and PZE in nature, but the peak voltages were 20 to 60 percent smaller than those observed in live or fresh skin. We therefore conclude that the PE and PZE effects are mainly due to a physical material property, not dependent on the living state, resembling that of synthetic organic PE polymers.

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Interference with Dimethylhydrazine Induction of Colon Tumors in Mice by ε-Aminocaproic Acid

Abstract. *The antifibrinolytic agent ε-aminocaproic acid given in the drinking water to Swiss ICR/Ha mice significantly counteracted the appearance of colorectal tumors induced by 21 weekly injections of 1,2-dimethylhydrazine. The drug affected both the number and the location of the tumors and, in some animals, altogether prevented their appearance. The low concentrations of ε-aminocaproic acid in the plasma of four control mice given the agent labeled with carbon-14 for 3 days suggest that the effect may depend not on inhibition of plasminogen activator activity, but on interference with the binding of some substance to the strong lysine binding site of plasminogen.*

The carcinogen 1,2-dimethylhydrazine (DMH) induces adenocarcinomas of the colon and rectum of rats (*1*) and mice (*2*) given weekly subcutaneous injections of the compound, with maximum uniformity of tumor induction being attained in Swiss ICR/Ha mice (*3*). These murine tumors are regarded as useful histopathologic models of the human disease. We recently demonstrated that human colon tumors contain significantly higher levels of plasminogen activator (PA) than corresponding normal mucosa (*4*), in agreement with evidence of the involvement of the fibrinolytic system in malignant transformation [for reviews, see (*5*)]. Plasminogen activators are serine proteases that enzymatically convert the plasma zymogen, plasminogen, to the active fibrinolytic enzyme, plasmin. We have also observed that colon tumors of DMH-treated mice contain at least ten times more extractable PA than normal colon mucosa, and that short-term explants of these tumors release large amounts of this enzyme into the culture medium (*6*). We are thus afforded a model system for the study of fibrinolytic enzymes in colorectal cancer. That it may be in the early phase of tumor induction that PA has a significant role is suggested by the observations that (i) the enzyme is induced with a high degree of consistency in cell cultures within hours after transformation by oncogenic viruses (*7*) or treatment with tumor promoters (*8*), and (ii) in passaged, transformed cultures continued elevation of PA production is not required for continued tumorigenicity (*9*). Since ε-aminocaproic acid (EACA) is known to inhibit the activation of plasminogen both in vivo and in vitro, we investigated its effect on the induction of murine colorectal tumors by DMH. The effects of both EACA and its analog tranexamic acid on the growth of various transplanted mouse tumors and on their metastasizing tendency were investigated earlier (*10*). In most of these experiments the agents significantly decreased the growth rate of the primary tumors, but the effects on metastasis formation were more variable. Tranexamic acid also has, in some instances, a favorable effect on the course of advanced disease in cancer patients (*11*).

For these experiments we used 51 ICR/Ha mice of both sexes, aged 12 to 15 weeks (average weight, 27 g). The animals were maintained in metal cages under room conditions, fed Tek-Lad laboratory pellets, and provided with sawdust bedding. The initial group was divided into four experimental subgroups. Group 1 (17 mice) received weekly subcutaneous injections of a 0.1 percent aqueous solution of DMH, *p*H 6.5, at a dose base of 15 mg/kg for 21 weeks; group 2 (16 mice) received DMH with a schedule identical to that of group 1 and received EACA (Sigma) at a concentration of 1 mg/ml in their drinking water. Since the average oral intake of water was 6 ml per day, each mouse received approximately 0.2 g of EACA per kilo-

Table 1. Effect of ε-aminocaproic acid on the number and location of DMH-induced colorectal tumors in mice. The mice in group 1 received weekly subcutaneous injections of DMH (15 mg/kg) in a 0.1 percent aqueous solution, *p*H 6.5 for 21 weeks. The mice in group 2 received, in addition to DMH, a daily dose of EACA (1 mg per milliliter of drinking water). In mice receiving DMH alone the tumors often occurred in clusters which made accurate counting of the number of tumors difficult. When more than 20 tumors were present, the number 20 was used for statistical analysis. Group 1 included seven males and ten females; group 2 included ten males and six females. Numbers of mice per group are shown in parentheses.

Group	Treatment	Time between first injection and death (weeks)	Number of tumors per mouse*	Distance of most distal tumor from anus (mm)*	Number of mice without tumors
1	DMH	22 to 27 (7)	5 ± 5.2	4.7 ± 0.8	0
		28 to 34 (10)	15.5 ± 7.8	5.4 ± 5.6	0
		All mice (17)	11.18 ± 8.5	5.12 ± 4.4	0
2	DMH plus EACA	22 to 27 (7)	0		7
		28 to 34 (9)	2.3 ± 3.0	25.3 ± 17.0	2
		All mice (16)	1.4 ± 2.6†	25.3 ± 17.0‡	9

*Data show means ± standard deviation. †$P < .001$ for the difference between the means of the number of tumors for all mice in groups 1 and 2 as determined by Student's *t*-test. ‡$P < .025$ for difference between the means of all mice for groups 1 and 2 as determined by Student's *t*-test.

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gram per day. This dose was derived from the recommended dosage for human use as an antifibrinolytic agent (*12*). The EACA treatment was continued for the duration of the experiment, with the water being changed weekly. Groups 3 and 4 (nine mice per group) were regarded as control groups: group 3 received only EACA in an identical regimen to that of group 2, and received no DMH injections; group 4 received no treatment (*13*). At various times after the initial injections the mice were killed by ether anesthesia and immediately autopsied. The pathology was systematically assessed macroscopically based on a number of objective criteria, which included (i) total number of colorectal tumors present, (ii) location of the most distal tumor (measured as distance in millimeters from the anus), (iii) presence of anal tumors, (iv) presence of uterine tumors, and (v) the size of the largest colorectal tumor present. Uterine tumors were rarely present in groups 1 and 2, with no significant difference between the groups. No tumors of any kind were found in groups 3 or 4. Three anal tumors were found: two in group 1 and one in group 2. The size of the individual colorectal tumors also showed no difference between groups 1 and 2.

Group 2 showed a significant reduction in total number of tumors compared to group 1 (1.4 ± 2.6 as opposed to 11.18 ± 8.5) (mean ± standard deviation) ($P < .001$) as well as a distinct preponderance of more proximally distributed tumors (25.3 ± 17 mm as opposed to 5.12 ± 4.4 mm from the anus) ($P < .025$) (Table 1). This difference was apparent in mice killed early as well as late in the experiment. In group 2, nine mice showed no macroscopic evidence of tumor, whereas in group 1, no tumor-free animals were observed. All tumors examined histologically were moderately to well-differentiated adenocarcinomas. In the limited population studied histologically, there appeared to be no significant differences in the neoplastic process between the mice in groups 1 and 2 that developed tumors; rather, the tumors in group 2 appeared to represent an early, preinvasive stage, whereas the tumors in group 1 had progressed to a more invasive form.

We also determined the concentration of EACA in the plasma of four control mice given ^{14}C-labeled EACA (New England Nuclear) in their drinking water (1 mg/ml) for three consecutive days; the plasma concentration of EACA was 0.057 ± 0.019 m*M* (± standard deviation). Unless the drug is actively concentrated in the tumor, its level in the tissues should be close to that in the plasma, since the drug readily penetrates cells (*12*). The inhibition of plasminogen activation by EACA is a complex phenomenon in that the drug has multiple targets; it can directly inhibit urokinase with a dissociation constant (K_d) of $10^{-2}M$ (*14*), and, by virtue of its structural similarity, it can interact with plasminogen through the latter's four to five lysine binding sites (*15*). Although only the first of these is a strong site (K_d = 9 μ*M*), it is the binding to the weaker sites (K_d = 5 m*M*) that is held responsible for changes in the rate at which plasminogen can be activated. The response in this region of EACA binding is biphasic: after an initial increase, the activation rate decreases and reaches zero at 0.1*M*. The latter effect may be due in large part to direct inhibition of plasminogen activator. The K_d for EACA for the strongest binding site in human plasminogen is 9 μ*M* (*14*) and the plasminogen concentration in human plasma is 0.14 mg/ml (1.65 μ*M*). Assuming that these values are valid for the mouse, we calculated that 97 percent of the total EACA is free, and that the amount bound is sufficient to cause an 86 percent saturation of the strong binding site. The weaker sites, whose saturation is responsible for the conformational change in plasminogen and thereby for changes in response to activators (*15, 16*), would be practically unaffected at this EACA level. Urokinase, and presumably other plasminogen activators, would also be unaffected. Saturation of the first site with EACA, however, aside from a rather modest inhibition of plasminogen activation by urokinase (~ 10 percent) has the important effects of decreasing the affinity of plasminogen for fibrin (antifibrinolytic effect) (*17*), α_2-antiplasmin (*18*), and histidine-rich glycoprotein (*19*).

Any of these mechanisms could be responsible for the drug effect described herein, or there may be some other mechanism that also involves interaction with the strong binding site of plasminogen. In any event, it seems likely that plasminogen has a role in some phase of tumor development by dimethylhydrazine. Indomethacin, an anti-inflammatory drug, also has an inhibitory effect on the induction of rat colon tumors by DMH (*20*). The effect of indomethacin was interpreted in terms of the inhibitory effects of the drug on prostaglandin synthesis by the tumors (*21*). A possible relation between prostaglandin synthesis and the fibrinolytic system was recently suggested (*22*).

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