prepared from octamer, which was mildly trypsinized (*11*). Lane 4 contains the protein from a few form II crystals. The bands characteristic of trypsinized H3 and H4 are apparent. Since SDS-gel electrophoresis yielded three bands, there might be some additional proteolysis in the crystal (not shown).

The amino acid analysis of the form II preparation is consistent with an equimolar H3-H4 complex with the amino termini of both proteins removed (Table 1). The amino acid analysis of the form I preparation indicates similar proteolysis.

Figure 1C shows a 10° screened precession photograph of a form II crystal about 0.4 mm across. This film shows reflections at 4.4-Å resolution, the limit set by the precession angle. Small-angle, unscreened photographs show reflections past 3.5-Å resolution that should be measurable. The crystals are surprisingly resistant to radiation damage. Form I crystals photographed at the Cornell High Energy Synchrotron Source (CHESS) with an extremely intense, focused monochromatic beam gave films comparable to that shown in Fig. 1C.

A number of studies demonstrate that chromatin briefly digested with trypsin has characteristics similar to that of undigested chromatin. Trypsinized chromatin yields 145–base pair repeats of DNA when treated with micrococcal nuclease. Trypsinized nucleosomes are stable particles that preserve the sensitivity of the nucleosomal DNA at ten base intervals to deoxyribonuclease I digestion (*6*, *7*) and that retain a buried sulfhydryl group (*13*). Trypsinized histones can be reconstituted with DNA into particles resembling nucleosomes (*14*). Therefore we believe that the structure of the tetramer with the amino termini removed is physiologically relevant.

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Epidermis of Human Skin: Pyroelectric and Piezoelectric Sensor Layer

Abstract. *The epidermis of live human skin has a permanent electric dipole moment perpendicular to its surface. Voltage responses to a rapid change of temperature are pyroelectric, while voltage responses to pressure pulses are piezoelectric in nature. The time course of the responses depends on* dX/dt (X, *temperature or pressure). The epidermal surface can react to all physical environmental influences to which nonbiological pyroelectric materials are known to respond. Epidermal voltage signals can be perceived through the intraepidermal and the superficial dermal nervous network. The pyroelectric and piezoelectric properties are also measurable on dead, dry skin samples.*

We examined the hair-free surface of the backs of the fingers of ten test persons. A measuring electrode and a reference electrode were applied to the skin surface. The electrodes (area, 10 mm^2) were placed approximately 1 cm apart; they consisted of 5 μl of colloidal graphite (Aquadag) held in place with adhesive rings (*1*). The measurements were carried out in a Faraday cage. Additional measurements were made on skin preparations examined within 1 to 2 hours after surgery. The specimens (area, ~ 2 cm^2) were prepared from "intact" skin (epidermis plus corium; thickness, 130 to 270 μm) and thin epidermal layers or corium layers (thickness, 60 to 90 μm). The specimens were attached with their inner (or outer) surfaces to the grounded electrode of a sample holder and were investigated in a shielded sample chamber. The front electrode (area, 10 mm^2) was identical to the measuring electrode used for the in vivo measurements.

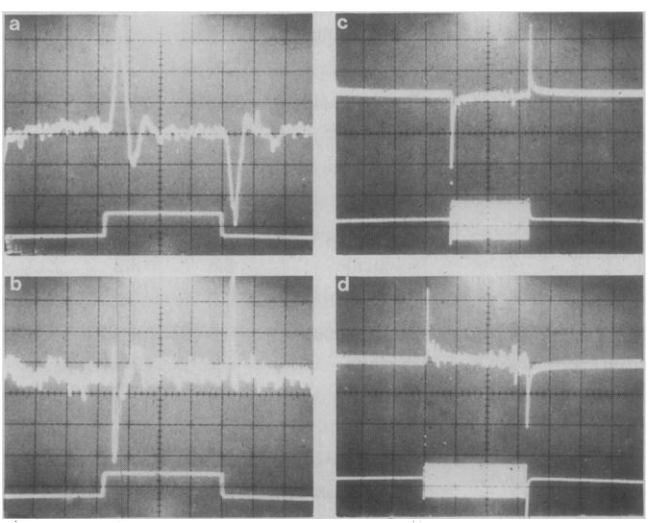

Fig. 1. Polar behavior of epidermis of human skin (fresh preparations). Opposite signs of PZE (a and b) and PE (c and d) voltage responses of outer (a and c) and inner (b and d) surfaces of the skin sample. (a and b) PZE responses to a square uniaxial pressure pulse; upper trace, skin response (100 μV per division); lower trace, signal of frequency generator (27.0 Hz). (c and d) PE responses caused by dielectric heating (radio-frequency pulse of 12.6 MHz and ~ 200-msec duration); upper trace, skin response; lower trace, radio-frequency signal.

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Quantitative measurements of pyroelectric (PE) and piezoelectric (PZE) responses were carried out by the following techniques. (i) Radiant heating method (*1–6*): absorption of a light pulse by a material causes a small temperature change (warming up) to occur, and this causes a voltage response of PE character if a polar material is present. The PE nature of the voltage responses was established by using the analysis of Simhony and Shaulov (*1, 7, 8*). Photoelectric effects were excluded by use of an edge filter, RG 695, which suppressed wavelengths shorter than 695 nm; nonuniform heating effects were diminished by use of very thin samples. (ii) Dielectric heating method (*9*): specimens were heated for 100 to 500 msec by applying radio-frequency voltages of 10 to 20 MHz (*1*). (iii) Piezoelectric methods: square mechanical pressure pulses (10 to 30 Hz) were applied on the surface of live skin, using a generator-driven loudspeaker membrane (*1*). The PZE coefficient d_{33} of fresh skin samples was determined by a quasi-static method (*10*).

The outer skin surface (live skin or fresh skin preparations) responded to rapid uniaxial stress as well as to rapid temperature changes with measurable voltage signals. The sign of the responses was positive on compression (and cooling) and negative on dilatation (and warming) (Fig. 1). The signs of the voltage responses of the inner skin surface (skin preparations) to mechanical or thermal influences were opposite to those of the outer surface; the skin is thus polar (Fig. 1). Investigations of fresh skin preparations showed that the polar properties represent the epidermis and that the polar axis of the epidermis is oriented perpendicular to the skin surface. The corium preparations showed no polar properties perpendicular to the outer skin surface.

Square uniaxial pressure pulses (10 to 30 Hz) acting on the outer surface of skin preparations produced voltage responses whose time course depended on dP/dt, where P is mechanical pressure. Application of such pulses to specimens with known PZE properties (such as quartz) resulted in responses similar to those observed in skin. Voltage responses of a predominantly PZE nature were also recorded from live skin. The PZE coefficient (d_{33}) along the polar axis of the skin was measured on skin preparations and values between 0.02 and 0.19 pC/N were found; the average of these values is 1/15 of the d_{11} for α-quartz (2.3 pC/N). Piezoelectric measurements on skin samples were previously carried out by Shamos and Lavine (*11*) who observed strong but uncalibrated PZE signals in the plane of specimens from human, cat, and pig skin. Fukada and Hara (*12*) observed PZE behavior in some other biological tissues; for instance, in pig aorta the values ranged from 0.02 to 0.2 pC/N.

Figure 2a shows a typical PE response of a skin specimen (outer skin surface) to a single square radiation signal. The main part of the response trace is in agreement with the points which were calculated for the course of a theoretical PE response according to Simhony and Shaulov (*7*). It is easier to establish the PE character of a response on isolated skin samples than on live skin, where disturbance effects can alter the PE signal (for instance, body and skin movements, which cause wobbling of the signal with time in the millisecond region, and the pulse and possibility of muscle twitch).

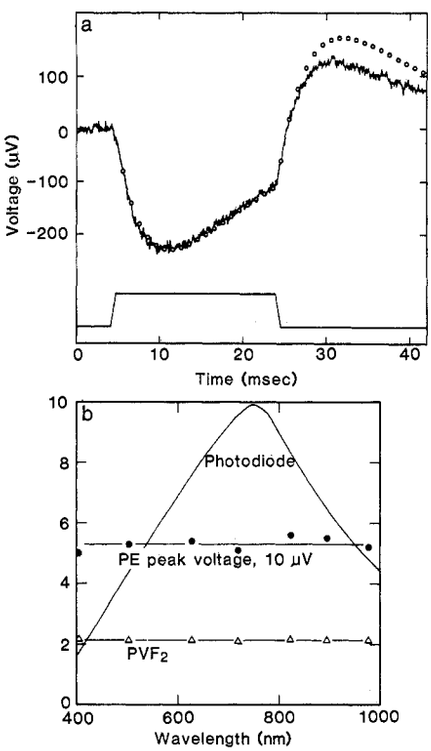

Fig. 2. Evidence of PE nature of skin responses. (a) PE voltage response of outer surface of human skin in vivo to a square light pulse of 19.5-msec duration. (Upper trace) Transient recorder trace (continuous line); (○) points calculated on the basis of the analysis in (*1, 7, 8*) and the experimental data: thermal radiation flux F_0 = 1.82 W/cm^2, electrical time constant τ_e = 4.3 msec; thermal time constant τ_T = 11 msec, initial slope of PE signal k = −0.098 V/sec; load resistance R_L = 10 megohms. (Lower trace) Photodiode signal. (b) Interference filter measurements (fresh preparation). The light intensity, F_0, of the monochromatic light pulses was adjusted to 100 mW/cm^2 with a pyroelectric radiometer. Under this condition the PE peak voltage V_p (*1, 7, 8*) remains nearly constant between 400 and 1000 nm. The effect is therefore a thermal (*PE*) one rather than a photoeffect. The behavior of the PE polymer polyvinylidene fluoride (*PVF_2*) is analogous. The different behavior of a photodiode is shown.

It was possible to show that the voltage responses of the skin to light pulses (radiant heating method) are not photoeffects but are due to a change in temperature, and thus are of a PE nature. Measurements with interference filters (Fig. 2b) showed that the effects were independent of the wavelength of the light. Heating of the skin samples by radio-frequency pulses (dielectric heating method) resulted in responses of a clear PE nature (Fig. 1). When the skin samples were reversed between the measuring electrodes, the responses were opposite in sign, indicating a PE effect.

The PE coefficient p was measured as described in (*1, 7, 8*). Fresh skin samples yielded p values of 2.1 to 27.0 pC/cm^2-K; for the live skin used in our trials, p values of 1.8 to 26.5 pC/cm^2-K were calculated. These values are of the same order of magnitude as those measured by Fürst and Liboff (*13*) on tusk, but much greater than the p values for dead, dry bone (*14*).

We assume that the polar behavior of the epidermis is caused by polar keratin filaments that are oriented perpendicular to the dermal-epidermal junction in the basal cell layer and possibly in some of the suprabasal cell layers of the stratum germinativum. The PE behavior of fully differentiated keratin structures (such as hairs and bristles) was shown to be independent of the living state (*10*). The PE properties of keratin structures seem to be physical properties of the material and are not due to ionic transport or other bioelectric phenomena.

The epidermis can react to the physical environment as a PE and PZE sensor layer. The polar texture of the epidermis makes it suitable for sensor functions related to the physical and chemical environmental influences to which nonbiological PE materials are known to react. The changes detected through the skin will result in uniform voltage responses, the voltage/time course of which depends on dX/dt (X, temperature or pressure). These effects could be perceived by the organism only if the entire epidermal surface were linked to the central nervous system. This is brought about through the intraepidermal nerve network (nerve fibers inside the epidermis) and the superficial dermal nerve network (nerve fibers mostly oriented horizontally in cutaneous surfaces).

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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