EVIDENCE FOR PYROELECTRIC AND PIEZOELECTRIC SENSORY MECHANISMS IN THE INSECT INTEGUMENT

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ABSTRACT Quantitative pyroelectric (PE) and piezoelectric (PZE) measurements were carried out on the insect integument of live *Blaberus giganteus* (cockroach) and on dry integument preparations of the same species. Voltage responses to optical pulses of 10–500 ms, absorbed in the live integument, were PE: interference filter measurements showed the responses to be proportional to the absorbed thermal radiation flux and independent of the wavelength. The voltage/time-course of the responses was in agreement with theoretically calculated PE signals. Voltage responses to mechanical pulses were PZE. The responses of the inner and outer integument surfaces always had opposite electric signs. The polar character of the integument was confirmed by means of a separate dielectric heating method. To explain these results, we hypothesize that the PE properties are for the most part localized in the two outermost layers (outer and inner epicuticle) of the integument, which consists mainly of polar lipids and proteins. Parallel alignment of these polar molecules perpendicular to the integument surface is very likely. PE and PZE responses, therefore, will not only occur in live insects but will also be measurable in dead, dry integument preparations as long as the polar tissue texture remains intact. Due to its polar texture, the insect integument will react to rapid changes in temperature, illumination, or uniaxial pressure in the same way as nonbiological PE materials, where the voltage responses depend on dX/dt (X, pressure or temperature). It seems clear, therefore, that the well-known physiological reactions of various arthropods to such physical outside influences may be related to the PE property of their integument.

INTRODUCTION

Previous investigations have shown pyroelectric[1] (PE) properties in various animal and plant tissues (1–8). The clear connection between these investigations and physiology became evident when it became possible to prove PE properties in live animals, that is, in the integument of live insects (9). The integument receptors of insects have long been used in sensory physiology as a favorite model for the function of the sense organs of the higher animals and of human beings.

The PE in vivo measurements on the insect integument of *Blaberus giganteus* (cockroach), complemented by measurements on dry integument preparations, are pursued further in this

[1]The pyroelectric (PE) effect has been investigated thoroughly on minerals that occur naturally, e.g., tourmaline (11). In recent years, PE materials (single crystals, ceramics, and polymers) have attained great technical significance, in particular as sensitive thermo detectors (11–13). The essential characteristic of PE materials is the presence of elementary electrical dipoles which are arranged parallel, which means that the materials also have a permanent dipole moment macroscopically (that is, a spontaneous polarization). All pyroelectrics are, without exception, piezoelectric.

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paper. The significance of these PE properties for the model of the live insect integuments is that they possibly may be generalized to higher animals and to the sense organs in the skin.

SAMPLE PREPARATION

For our measurements we used live ***Blaberus giganteus*** (156 specimens). Two different preparation methods, which yielded almost the same results (difference, ±5%), were applied (Fig. 1).

For additional measurements, integument preparations (thorax or abdomen segments) were used. Their inner or outer surface was attached to the grounded electrode of a sample holder. The radiation-receiving electrodes were identical to those used in the live insects. Most of the electrodes (A = 10 mm^2) were water-suspended colloidal graphite held in place with adhesive rings. The layer of graphite (thickness, ~30 μm) dried within a few minutes. The thickness d of the integument was 44–57 μm (abdomen segments) or 68–94 μm (mesothorax segments). For further details, see reference 9.

EXPERIMENTAL TECHNIQUES

We applied two PE measuring methods.

Radiant Heating Method (10–12)

The experimental apparatus is shown in Fig. 2. The PE character of the voltage responses to single square radiation signals absorbed in the samples was determined using the analysis of Simhony and Shaulov (9, 14–16).

A light pulse absorbed in the sample causes a small rise in temperature T and changes the sample's inherent polarization. Free charges arising on the surface of the sample, due to the PE effect, are picked up by two electrodes. The amount of charge per unit time is measured as a voltage drop $V(t)$:

$$V(t) = k \cdot (1/\tau_\epsilon - 1/\tau_T)^{-1} \cdot [a_T \cdot \exp(-t/\tau_T) - a_\epsilon \cdot \exp(-t/\tau_\epsilon)], \qquad (1)$$

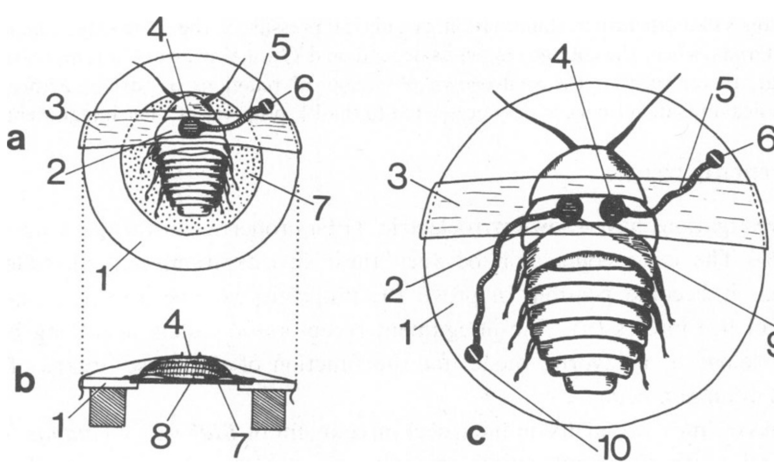

FIGURE 1 The two preparation methods used in our measurements on live ***Blaberus***. In both methods the radiation-receiving electrode is situated on the dorsal mesothorax (*2*). In method 1 (*a* and *b*) the reference electrode was applied to the ventral abdomen (*8*). In method 2 (*c*) the two electrodes were applied to the same dorsal mesothorax segment. *1*, Teflon holder; *3*, adhesive tape with window around electrodes; *4*, radiation-receiving electrode connected by an aluminium foil lead (*5*) to a contacting pole (*6*); *7*, gilded copper disk (ground electrode); *9*, reference electrode with contacting pole (*10*). The live animals were attached to the sample holder by a few strips (not shown) of adhesive tape, so that they could breathe but not move their abdomen and legs.

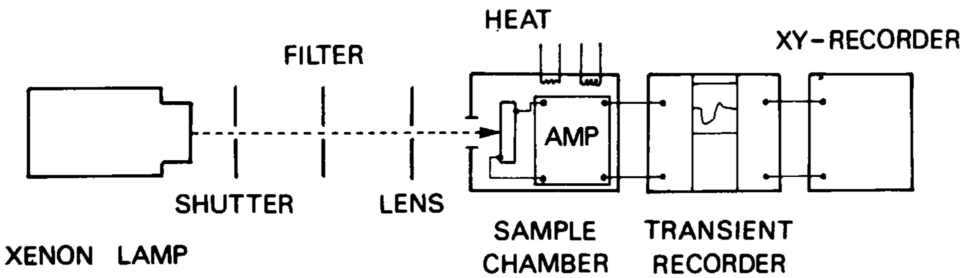

FIGURE 2 Diagram of the experimental apparatus of the radiant heating method.

where k is the initial slope ($k = p \cdot A^2 \cdot F_0/(C \cdot C_T) = dV/dt|_{t=0}$, volts per second); $a_T = a_e = 1$ for $0 \leq t \leq t_1$; $a_T = 1 - e^{t_1/\tau_T}$; and $a_e = 1 - e^{t_1/\tau_e}$ for $t_1 \leq t$.

SYMBOLS

t_1 is the duration of illumination, seconds;
τ_e is the electrical time constant ($= RC$), seconds;
τ_T is the thermal time constant ($= C_T/G_T$), seconds;
p is the pyroelectric coefficient, coulombs per square centimeter · kelvin
A is the area of the electrode, square centimeters;
F_0 is the thermal radiation flux (intensity of the light pulse), watts per square centimeter;
C is the capacitance of sample, farads;
C_T is the heat capacity (thermal mass) of sample, joules per kelvin;
G_T is the thermal conductance for heat loss of sample, watts per kelvin; and
R is the electrical resistance (composed of the resistance of sample and of external load), ohms.

The transient voltage curve $V(t)$ was measured for a number of interchangeable load resistors lying between 1 MΩ and 10 GΩ (see references 14–16 for details). For each $V(t)$ corresponding to a particular load resistor the following parameters were determined: initial slope, k; peak voltage, V_p; electrical time constant, τ_e; thermal time constant, τ_T.

A xenon lamp (450 W) was used as the light source. An edge filter RG 695 (Jenaer Glaswerke, Schott, Mainz, W. Germany) in the course of light beam suppressed wavelengths >695 nm to exclude photoelectric effects.

The light intensity F_0 was determined for each sample using a PE radiometer (Laser Precision model RK-5100, Laser Precision Corp., Utica, N. Y.) covering the wavelength range 250–1,600 nm. The effective heat capacity of the specimen was calculated from the value of the specific heat capacity of *Blaberus* integument samples, measured with the Perkin-Elmer DSC-2 differential calorimeter (Perkin-Elmer Corp., Norwalk, Conn.). Further details of the experimental equipment and the theoretical background of the analysis are given in reference 9.

Dielectric Heating Method

The method using dielectric heating published by Sussner et al. (17) was used in a modified construction for additional investigations on fresh integument samples. Single abdomen segments (d = 44–57 μm) with electrodes applied to the outer and inner surfaces were heated for a short period (100–500 ms) by applying radio-frequency voltages (10–20 MHz). This method volume heats the sample, largely eliminating heat gradients and the tertiary PE effect.

Piezoelectric (PZE) Method

The PZE coefficient d_{33} of fresh integument preparations of *Blaberus* was determined by a quasistatic method (2). Single mechanical impulses were applied, using a generator-driven loudspeaker membrane with an attached Teflon staff that touched the insect integument at the electrode surface.

EXPERIMENTAL RESULTS

Polar Behavior

The outer integument surface of the live *Blaberus* reacts sensitively to every temperature change with measurable voltage responses; the electric sign is, without exception, negative upon heating and positive upon cooling.

Integument preparations react in the same way. The electric sign in the case of the inner integument surface is opposite to that of the outer surface. This polar behavior was found in all integument samples of *Blaberus*. Fig. 3 compares results from the radiant heating method and the dielectric heating method.

Between the outer and inner surfaces of the wings, no polar behavior was found. Two cuticle layers are present, with their polar axes orientated in opposite directions; thus, the responses of both wing layers may be compensating. (This result corresponds to the anatomical structure of the insect wing that arises as an integument pocket and consists of two cuticle layers that have grown together.)

Piezoelectric Behavior

The integument of live *Blaberus* also reacts to uniaxial mechanical stress with measurable voltage responses. In the case of compression (corresponding to cooling), they have a positive electric sign, in the case of dilatation (corresponding to heating), a negative sign. The voltage/time-course of the responses corresponds to the correlation dP/dt (P, mechanical pressure). In the integument preparations the sign of the inner integument surface response is opposite to that of the outer surface responses (Fig. 4).

The behavior of the integument of live *Blaberus* and of isolated integument preparations

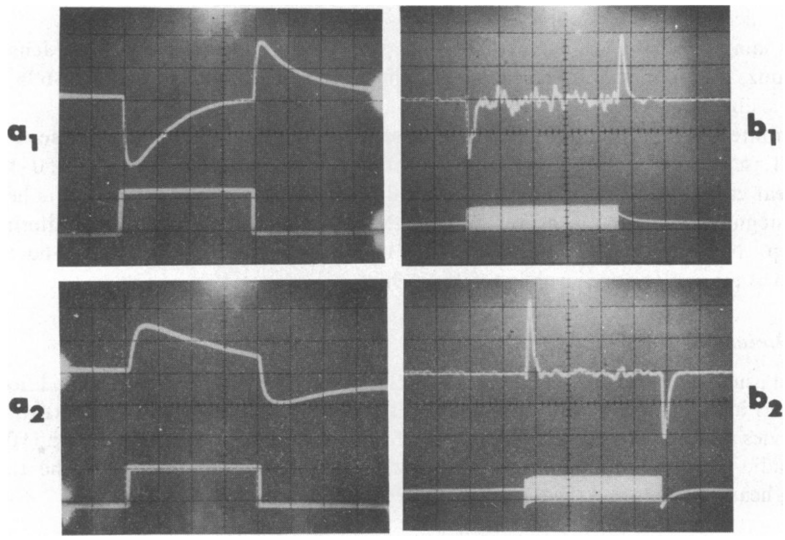

FIGURE 3 Opposite signs of the voltage responses of outer (a_1 and b_1) and inner (a_2 and b_2) surfaces of *Blaberus* integument (isolated abdomen segments). The responses (upper beam) are caused by radiant heating (a_1, a_2; square light pulses of 400 ms) and by dielectric heating (b_1, b_2; radio-frequency pulses of 20 MHz and 400 ms).

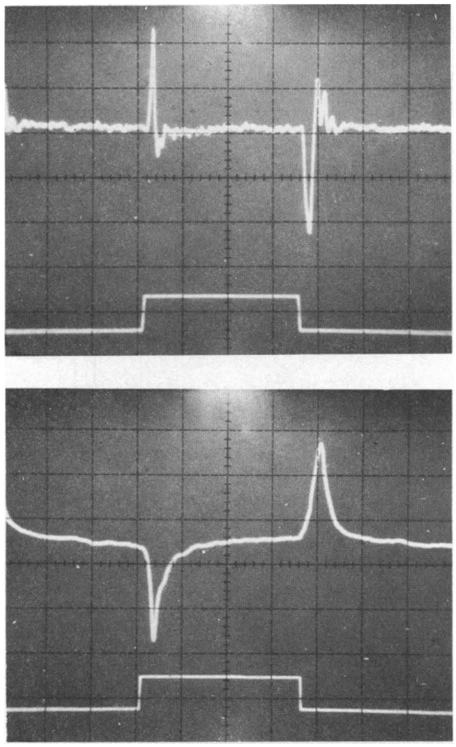

FIGURE 4 Piezoelectric voltage responses of *Blaberus* integument (integument preparation) to a square mechanical pressure impulse acting on the outer surface (upper photo) and on the inner surface (lower photo). Upper trace, integument response (0.2 mV/div). Lower trace, signal of the frequency generator.

corresponds to the behavior of nonbiological PZE materials (e.g., quartz) under the same conditions, and can therefore be considered to be a PZE effect. The PZE coefficient in the direction of the polar outside-inside axis of the integument ("d_{33}") was measured only on dry integument preparations. For dorsal mesothorax segments the coefficient varied between 1.8 and 2.7×10^{-13} C/N. These values had an average of 0.1 of the d_{11} of α-quartz (2.3×10^{-12} C/N).

Proof of the PE Nature of Responses

INTERFERENCE FILTER MEASUREMENTS We used interference filters for wavelengths between 400 and 975 nm (Jenaer Glaswerke Schott, Mainz, W. Germany). The light intensity reaching the sample was uniformly adjusted to 100 mW/cm^2 using a PE radiometer. Fig. 5 shows that the values for the PE coefficient p and the peak voltage V_p of live *Blaberus* are constant for wavelengths between 400 and 1,000 nm. This means that the voltage responses are independent of the wavelength, and thus are due to a change in temperature and are PE in nature. The PE polymer polyvinylidene fluoride (PVF_2) behaves in an analogous way (Fig. 5).

CORRESPONDENCE OF MEASURED RESPONSES WITH THEORETICALLY CALCULATED PE SIGNALS Fig. 6 compares the measurement (transient recorder trace) of $V(t)$ of a live *Blaberus* with the calculation of $V(t)$ according to Eq. 1. Note the excellent agreement

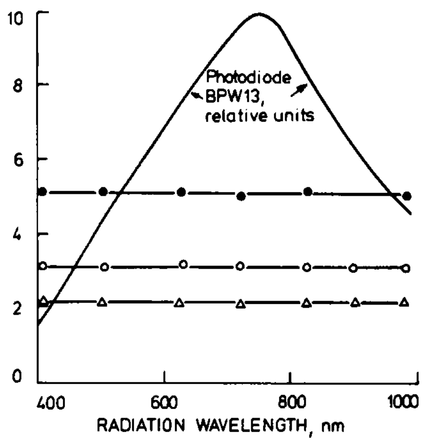

FIGURE 5

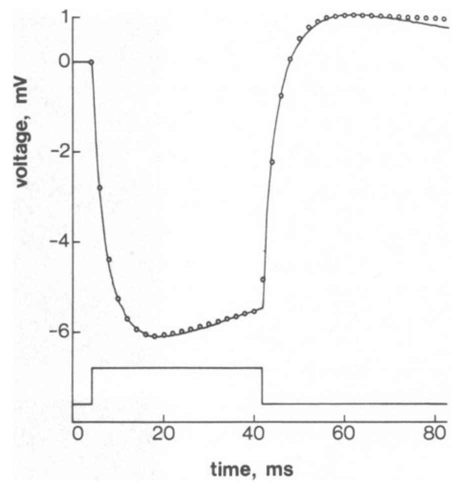

FIGURE 6

FIGURE 5 Interference filter measurements on the integument of live *Blaberus* (specimen 32). The light intensity F_o of the monochromatic light pulses were adjusted to 100 mW/cm^2 with a pyroelectric radiometer. Under this condition, the peak voltage V_p and the pyroelectric coefficient remained nearly constant at wavelengths between 400 and 1,000 nm. The effect, therefore, is thermal (pyroelectric) and not a photoeffect. The behavior of PVF_2 is analogous. The different behavior of a photodiode is shown. (•) *Blaberus*, PE peak voltage, 100 μV. (o) *Blaberus*, PE coefficient, 100 pC/cm^2K. (Δ) PVF_2, PE coefficient, nC/cm^2K.

FIGURE 6 Voltage responses of live *Blaberus* integument to a single square light pulse of 37.8 ms (specimen 105). The continuous line is the transient recorder trace. The points were calculated with Eq. 1. Values of this measurement: $F_o = 1.98$ W/cm^2; $R_L = 106$ MΩ; $\tau_e = 193$ ms; $\tau_T = 3.5$ ms; $k = -1.87$ V/s.

between the oscilloscope trace and the calculated points. This agreement was found in our measurements of *Blaberus* integument (live as well as dead) when the radiation time did not exceed ~500 ms. Again, this tends to confirm the PE nature of our results.

PE Coefficient

The PE coefficient p was determined using the radiation heating method and according to the analysis of Simhony and Shaulov (13) from

$$p = k \cdot C \cdot C_T/A^2 \cdot F_o. \tag{2}$$

C was measured with an impedance Wheatstone bridge; the values of C were ~1 nF/cm^2. The light intensity reaching the radiation-receiving electrode ($A = 10$ mm^2) was in the range 1.7–2.0 W/cm^2. C_T was 2.06×10^{-3} J/K with live *Blaberus* and 1.65×10^{-3} J/K with dead integument samples. The value of p of the dorsal thorax segment of live *Blaberus* varied between 260 and 750 pC/cm^2K; it was 30–110 pC/cm^2K in dead preparations.

Additional Results

LIVE AND DEAD INTEGUMENT PE and PZE voltage responses of the integument of live animals were considerably higher than those of the same animals after their death (Fig. 7). Between 1 and 3 d after death, without changing the position of the specimen, the responses gradually dropped to a lower value, which then remained almost constant over a period of months: the voltage/time-course is analogous in the case of live animals and air-dried (dead) integument preparations.

INTEGUMENT FROM DIFFERENT PARTS OF THE BODY In the same animal the thorax segments always showed higher PE responses than the segments from the abdomen. To obtain comparable results we have analyzed the PE values of the dorsal mesothorax. Without recognizable relationship to the stage of development of the animals, the PE coefficient varied between 260 and 750 pC/cm^2K from one individual to another.

POSITION OF THE POLAR LAYERS WITHIN THE INTEGUMENT The polar properties of the live and of the dead *Blaberus* integument are predominantly localized in the outermost layers, that is, in the cuticulin layer and the inner epicuticle. After mild abrasion of the cuticle surface and repetition of the PE measurement within a few hours, the values of the remaining integument (identical with the procuticle) dropped to 10–20% of the values of the intact integument of the same animal (Figs. 8 and 9).

DISCUSSION

The Whole Arthropod Integument Shows PE and PZE Properties

Our investigations show that the whole integument surface (including the upper and lower layers of the wings) reacts with PE or PZE voltage responses to various physical influences. It

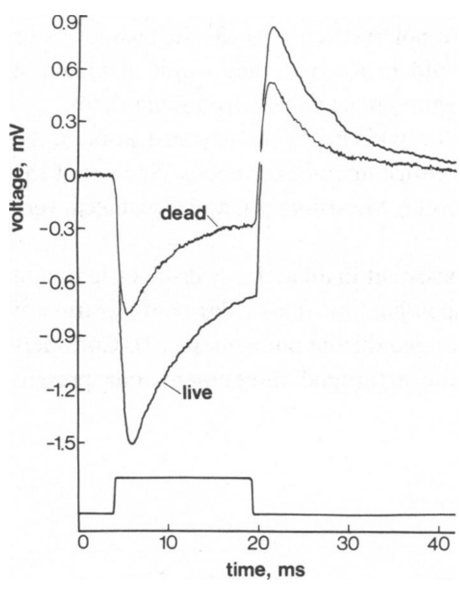

FIGURE 7

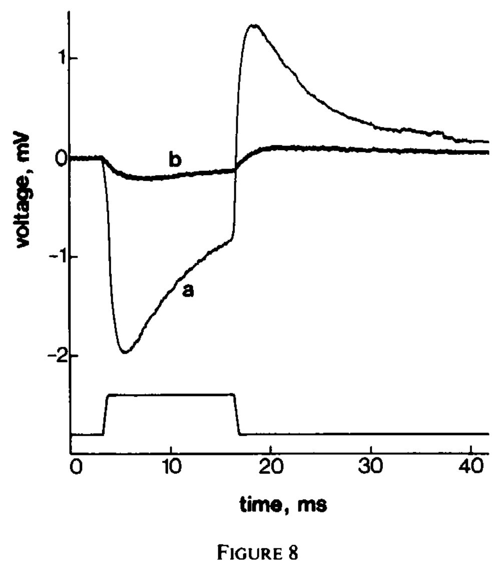

FIGURE 8

FIGURE 7 Voltage responses of the live and the dead *Blaberus* integument to a single square light pulse of 15.6 ms (specimen 81). (*a*) Live animal: $F_o = 1.94$ W/cm^2; $R_L = 1$MΩ; $\tau_e = 0.6$ ms; $\tau_T = 16$ ms; $k = -2.85$ V/s. (*b*) The same animal as in *a*, dead: $F_o = 1.86$ W/cm^2; $R_L = 1$MΩ; $\tau_e = 0.5$ ms; $\tau_T = 13$ ms; $k = -1.75$ V/s.

FIGURE 8 Voltage responses of the integument of live *Blaberus* to a single square light pulse of 12.6 ms (specimen 9). (*a*) Intact integument: $F_o = 1.84$ W/cm^2; $\tau_e = 0.7$ ms; $\tau_T = 11.5$ ms; $k = -3.35$ V/s. (*b*) Integument after mild abrasion of the epicuticle: $F_o = 1.85$ W/cm^2; $\tau_e = 1.7$ ms; $\tau_T = 24$ ms; $k = -0.14$ V/s.

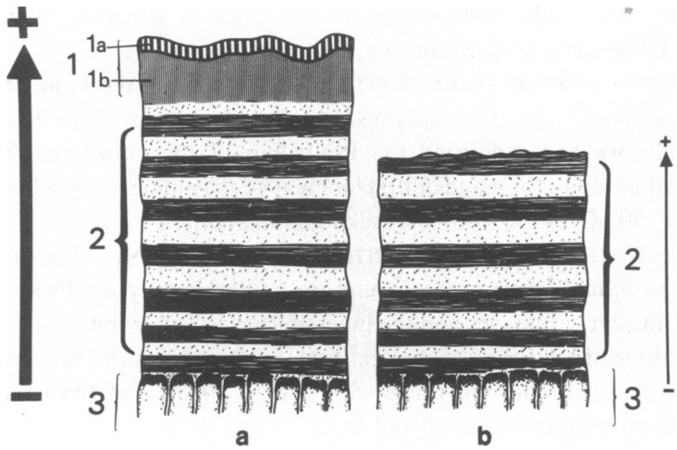

FIGURE 9 The polar axis of *Blaberus* integument shown schematically (see 18–21). (*a*) The orientation of the polar axis is the same in all cuticle layers; the outer surface of the integument becomes positive upon cooling (arrow, left) and negative upon heating. (*b*) After the outermost layers are destroyed mechanically or chemically, the pyroelectric coefficient of the remaining cuticle yields only 10–20% of the intact integument (arrow, right). (*1*) Epicuticle with cuticulin layer (*1a*) and inner epicuticle (*1b*); (*2*) procuticle; (*3*) epidermal cells.

therefore seems that PE and PZE characteristics are not restricted to the surface areas or structures of specific integument receptors. This would mean that they would not have a "special physical status" when compared with the integument surface surrounding them.

At the beginning of our investigations (2) on the arthropod cuticle (fresh preparations), the species were selected so as to be representative of the entire line of arthropods. Species of the Coleoptera, Orthoptera, Blattaria, Chilopoda, Chelicerata, Merostomata, and Crustacea were examined.

The orientation of the polar integument axis was consistent in all arthropods investigated in that the outer integument surface became negative upon heating. This polar configuration of the arthropod integument was shown to remain unchanged during ontogenesis (2). Considering the uniformity of PE and PZE properties in the arthropod integument, our present

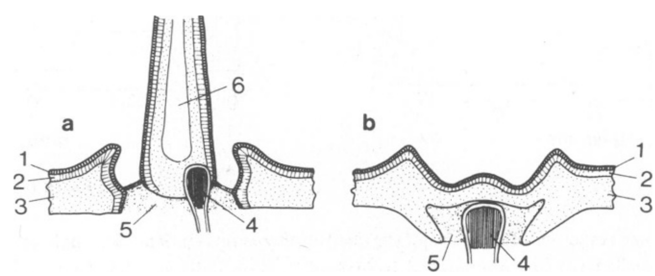

FIGURE 10 Integument receptors of arthropods and their possible pyroelectric and piezoelectric function (see 22). The outermost cuticulin layer with its pronounced pyroelectric and piezoelectric properties completely covers an insect, including the surfaces of its finest hairs. (*a*) Hair type receptor; (*b*) campaniform type receptor; (*1*) cuticulin layer; (*2*) inner epicuticle; (*3*) procuticle; (*4*) tubular body; (*5*) spongy layer; (*6*) hair.

measurements on live *Blaberus* may possibly serve as a model for the integument of all arthropods.

The PE and PZE effects occur not only in live but also in dead, air-dried integuments. In earlier investigations (2) PE and PZE properties were found to exist in the integument of beetles that had been kept in zoological museums for more than 10 years.

PE and PZE responses thus are not dependent on the living state. The signals must be a consequence of the tissues' polar texture, arising from a parallel alignment of polar molecules in perpendicular direction to the integument surface. As long as the polar texture remains intact, the PE properties are demonstrable.

Analogous Electrical Reactions of the Arthropod Integument and of "Technical" Pyroelectrics

The voltage/time-course of the integument responses is similar to that of the PE and PZE responses of nonbiological pyroelectrics (e.g., of PVF_2).

The following conclusions can be drawn from our findings. Every outside influence that causes voltage responses with a nonbiological PE material (e.g., with PVF_2) can also cause analogous electrical reactions with the integument structures of live arthropods. The voltage responses will be all the greater the quicker the influence takes place, since they correspond to the relationship dX/dt (X, type of outside effect). The following can be effects of this kind: (*a*) changes in temperature (heating, cooling); (*b*) changes in light intensity (assuming absorption of the light), (*c*) variation of the external electric field, (*d*) variation of hydrostatic pressure (air pressure, hydraulic pressure), and (*e*) changes in mechanical stress (compression, dilatation). These relationships have been referred to previously (2–4, 6).

The Insect Integument, a PE and PZE Sensor?

The well-known physiological reactions of various insects and other arthropods (e.g., spiders) to the above-mentioned physical environmental influences seem to be related to the polar texture and PE property of their integument and their integument receptors.

The arthropod integument appears to function in a manner similar to nonbiological materials such as PE thermo- or photodetectors or PZE mechanoelectrical sensors (12, 13). One of the most convincing facts supporting this supposition is the common dependence of the effects on dX/dt. We therefore assume as a starting point for future investigations that the established PE and PZE effects are due to a physical "material property" of the integument and that they are not caused by ionic transport or other bioelectrical phenomena normally associated with the living state.

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