The pulsations of the heart were monitored simultaneously in the thorax and in the abdomen by silver electrodes implanted on each side of the heart. The electrodes were wired to a pair of Biocom impedance converters, the signals of which were recorded on separate channels of a polygraph. The movements of the heart, observed through the transparent integument, coincided with the polygraph records. The impedance measurements recorded the timing of the heart pulsations as well as their relative amplitude.

The frequency, amplitude, and duration of continuous pulsations of the heart increased when the thorax was heated to 40° to 43°C, even though the abdominal temperature initially did not change and remained relatively low (Fig. 1). In moths with heated thoraxes, the pulses usually moved from the tip of the abdomen into the thorax. These pulses were always at the same frequency in the thorax and in the abdomen, even though the temperatures of thorax and abdomen were made to differ by more than 20°C. Individual pulses in the abdomen were usually recorded a few milliseconds before those in the thorax.

It can be inferred from the heart pulsations (Fig. 1) that the flow of blood through the heated thorax was relatively strong. When the heat input to the thorax was not excessive, the temperature of the thorax often stabilized. This stabilized thoracic temperature was always lower than that observed during thoracic heating of dead moths. Since the thorax of the moth shown in Fig. 1 was deliberately overheated, the rate of heat transfer from thorax to abdomen was probably maximum; the temperature of the second abdominal segment rose up to 8°C in the dorsum and 11°C in the ventrum (Fig. 1). However, when the ventral nerve cord was transected (posterior to the second abdominal segment) and the thorax was heated as before, the increase in abdominal temperature was only 2° to 3°C, as in dead moths.

Transection of the nerve cord immediately reduced the frequency of the heart pulsations. In operated moths, in contrast to normal ones, heating of the thorax did not evoke either increased rates or amplitudes of pulsation of the heart in the thorax or in the abdomen. Unlike the situation observed in unoperated moths with high thoracic temperatures, the pulses of the heart in operated moths sometimes moved from thorax to abdomen.

Four out of seventeen unoperated moths failed to show increases of heart rate, pulse amplitude, abdominal temperature, or stabilization of thoracic temperature in response to thoracic heating. It is inferred, in these cases, that the ventral nerve cord, or nerves innervating the heart or its alary muscles were damaged by one of the many implanted thermocouples or electrodes.

A number of anatomical features of sphinx moths contribute to temperature regulation. The thick layer of thoracic scales (*9*) and the hollow space (*7*) between thorax and abdomen (Fig. 1) aid in the retention of heat in the thorax, and should therefore be useful whenever the moths are flying in low (<15°C) air temperatures, when blood flow from the abdomen to the thorax appears to be nearly cut off (*8*).

The blood from the thorax flows under and around the ventral diaphragm. This membrane, extending along the midventral line of the abdomen, undulates laterally and posteriorally (*3, 6*). It aids in stirring and propelling the blood which collects in the heart after passing through the hemocoel, where heat is lost by convection and evaporation (*9, 10*). The blood appears to have cooled maximally by the time it has entered the heart (Fig. 1). It is then impelled into the thorax where the pulsatile vessel loops through the flight musculature (*7, 11*). This loop should act as a cooling coil.

It can be concluded that the pumping of the heart is affected by impulses from the thorax via the ventral nerve cord. This affords a mechanism for control of the movement of heated blood from the well-insulated thorax into the poorly insulated abdomen, and allows the abdomen to serve as a radiator where heat from the thorax is unloaded via the blood.

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1 April 1970; revised 17 June 1970

Junctional Membrane Permeability: Restoration by Repolarizing Current

Abstract. *In cells of the* Chironomus *salivary gland, junctional membrane conductance, depressed by various chemical treatments, is restored to its normal high level by currents passed inward through nonjunctional cell membrane.*

The high membrane permeability at the junction of a variety of cells is depressed by cell injury (*1–3*), by intracellular injection of Ca^{2+} (*3*), by anisotonic media (*3*) or media free of Ca and Mg (*4*), by media in which propionate substitutes for Cl^- (*2*) or Li^+ substitutes for Na^+ (*4*), by low temperature (*5*), by chemical metabolic inhibitors (*6*), and by electric current going outward through the nonjunctional membrane (*7*). Under most, if not all, of these conditions depolarization takes place at various rates and to various degrees. Thus the question arose whether repolarizing (inward-going) current, applied at times of low junctional permeability (uncoupled state), will restore high junctional permeability. I have examined this question for three of the aforementioned conditions of uncoupling and found that restoration indeed takes place.

Salivary glands of mid-fourth instar larvae of *Chironomus thummi* were isolated in a physiological solution [normal medium (*8*)] and then exposed continuously to one of the following uncoupling media: dinitrophenol (DNP) medium, medium free of Ca and Mg, and medium free of Ca and Mg but with Li (*8*). Two adjacent cells I and II (Fig. 1) were impaled, each with one voltage-recording (E_I, E_{II}; filled with KCl) and one current-recording microelectrode [i_I, i_{II}; filled with KCl,

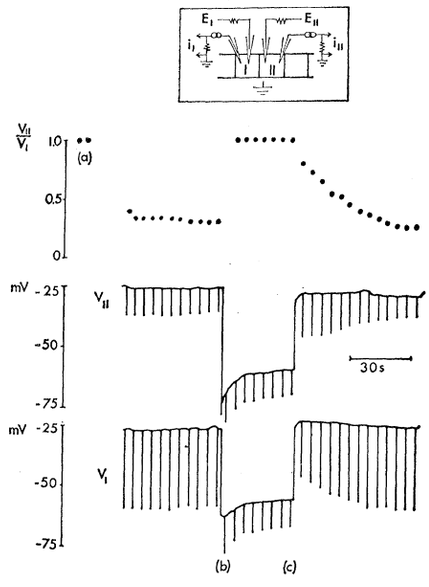

Fig. 1. Junctional recoupling by constant inward current. Cells uncoupled in Ca- and Mg-free Li medium. Coupling is tested by passing inward pulses of 4×10^{-8} ampere, 100 msec long, at a rate of 0.2 per second from a current source in cell I, and recording the resulting changes in membrane voltage (V_I, V_{II}) in this cell and a contiguous one (II). Between (*b*) and (*c*), constant inward-going currents of 4×10^{-8} ampere are applied to both cells I and II. (All electrodes filled with KCl. Tracing from chart recorder; currents not shown.) The upper curve gives the coupling ratios V_{II}/V_I including those (*a*) while preparation is still in normal medium.

EDTA or EGTA (*9*)]. Coupling was probed by passing inward-going current test pulses (100 to 200 msec long) at low frequency (0.2 per second or less) into cell I, and measuring the resulting voltage drops (V_I, V_{II}) across the nonjunctional membranes of the two cells to the grounded medium. The ratio V_{II}/V_I (coupling ratio) served as an index of intercellular communication (*10*). To provoke recoupling, inward current was applied in one of the following modes: as a long current step into one or both cells (in the latter case test pulses were superimposed) or as brief repetitive pulses into one cell at rates of 2.3 to 6 per second (pulse duration 100 to 200 msec).

Figure 1 illustrates the results of an experiment in which junctional conductance in a preparation treated with Ca- and Mg-free Li medium is restored by a steady inward current (KCl electrodes). Upon simultaneous application of equal inward current to the two cells, the coupling ratio, which had fallen from its normal value of nearly 1.0 to 0.3 in the uncoupling medium, increases to 0.75 within 3 seconds and to nearly 1.0 within 8 seconds. Termination of the current is followed by gradual uncoupling to the previous low level within 1 minute. This result of fast restoration of junctional conductance is typical for all cases tested. These include 27 cases uncoupled in Ca- and Mg-free Li medium, two cases uncoupled in medium free of Ca and Mg, and two cases uncoupled in DNP medium. In all these cases uncoupling was associated with depolarization in at least one of the cells probed. At the time the coupling ratio had fallen to 0.3, depolarization ranged from 10 mv to complete abolition of membrane potentials (*11*).

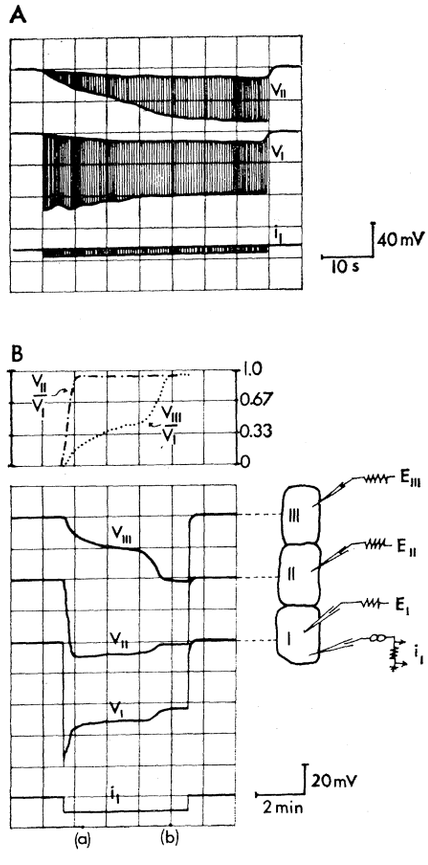

Fig. 2. (A) Partial junctional recoupling by repetitive inward-going current pulses. Cells uncoupled in Ca- and Mg-free Li medium, resting potential about 0 mv. Current pulses of 2×10^{-8} ampere (i_I), 200 msec long, frequency 2.3 per second, are passed into cell I, and voltage changes are recorded in cells I and II. (All electrodes filled with KCl. Tracing of a storage oscilloscope record.) (B) The same cell junction, I/II, and the next junction, II/III, recoupled at times (*a*) and (*b*), respectively, while an inward constant current of 2×10^{-8} ampere is passed into cell I. (Tracing of a storage oscilloscope record.) The curves in the upper graph give the corresponding coupling ratios V_{II}/V_I and V_{III}/V_I.

The restorative effect outlasts the current. This is particularly evident from experiments in which recoupling results cumulatively from the application of brief inward current pulses. Figure 2A shows this for a preparation, uncoupled in Ca- and Mg-free Li medium, in which coupling improves with each of a series of pulses (passed into one cell only; duration 200 msec, rate 2.3 per second, 2×10^{-8} ampere) until a maximum coupling ratio of 0.85 is reached. A steady current of the same intensity, passed into the same cell, reestablishes full communication (Fig. 2B). The experiment in Fig. 2B shows also that recoupling is not confined to immediate neighbors of the cell containing the current source. The junction I/II, nearest to the current source, recouples within 1 minute [Fig. 2B(a)]; the next one, II/III, recouples within 4 minutes of uninterrupted flow of current inward.

The recoupling is similar in all respects, including duration, when EDTA (two cases) or EGTA (16 cases) instead of Cl (27 cases) in the micropipettes carried the repolarizing current. Thus, here the phenomenon of recoupling appears to be the consequence of nonspecific current flow through the nonjunctional membrane. The recoupling is unlikely to be simply due to a change in cytoplasmic osmolarity resulting from the injected current: current passed across a junction, bypassing the extracellular medium, with a pair of KCl-, EDTA-, or EGTA-filled micropipettes (sink and source) placed on either side of a coupled junction, promotes rather than prevents uncoupling in a medium free of Ca and Mg. (Such current passage does not uncouple in a normal medium.)

The uncouplings produced here by three different means are reversed by a common procedure. This suggests, as did earlier results from this laboratory (*1, 3, 4, 6*), that the uncouplings are mediated by a common factor. Loewenstein's hypothesis (*12*) postulates cytoplasmic Ca^{2+} as such a mediating factor. The implication of the present results for the hypothesis will be discussed elsewhere.

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13. I thank Prof. W. R. Loewenstein and Dr. S. J. Socolar for interest and valuable discussions. Supported by PHS and NSF research grants. B.R. is on leave from the University of Munich.

21 April 1970

Double-Helical Polynucleotides: Immunochemical Recognition of Differing Conformations

Abstract. *Rabbit antibodies to double-helical RNA react by complement fixation with synthetic or natural double-strand RNA but not with native DNA. In turn, human (from systemic lupus erythematosus patients) antibodies to native DNA do not react with double-strand RNA. Both types of antibodies show cross-reactions (from 1 percent to 50 percent) with RNA-DNA hybrids, but antibodies to the hybrids do not react at all with double-strand RNA or with native DNA. Antibodies to polydeoxyguanylate·polydeoxycytidylate also failed to react with native DNA.*

Serums from rabbits immunized with complexes of methylated bovine serum albumin (MBSA) and the double-strand polyribonucleotide copolymer poly A·poly U (*1*) reacted, by precipitation and complement fixation, with either synthetic or naturally occurring double-strand RNA (*2*); they cross-reacted to a variable extent with RNA-DNA hybrid molecules, but did not react with either single-strand or double-strand DNA. This type of serum, used at a suitable dilution in quantitative complement fixation experiments, was an effective reagent for studying the small amount of double-strand RNA in the total RNA extracted from arbovirus-infected mammalian cells (*3*). Similar serums have been prepared by Lacour *et al.* (*4*) and by Plescia *et al.* (*5*), although the former group found a wider reactivity in some serum samples.

I now report some immunochemical relationships among a wider variety of double-helical polynucleotides. For this purpose, in an attempt to obtain antibodies that would react with native DNA, I injected rabbits with MBSA complexes of the synthetic hybrid poly A·poly dT (*6*), of the polydeoxyribonucleotide copolymers poly dG·poly dC, poly dAT·poly dAT, and of calf thymus DNA. Histone-DNA complexes were also used as immunogens. In a given course of immunization, animals were given two intradermal injections of complex (in complete Freund's adjuvant) a week apart and one intravenous injection a week after the second intradermal dose; the rabbits were bled a week after the intravenous injection. Each dose contained 30 to 50 μg of synthetic polymer or 50 to 200 μg of native DNA.

Of the polynucleotides used, the poly A·poly dT and poly dG·poly dC elicited specific antibody after a single course of immunization. The antiserums that they induced were compared with antiserum to poly A·poly U and antiserum to poly I·poly C (*7*), and with serums from patients with SLE, which react with native DNA (*8*). The complexes containing native DNA or poly dAT·poly dAT did not elicit specific antibody to the nucleic acid, in five and two animals, respectively, that received immunizing injections for as long as 6 months.

The immunochemical reactivities of the antiserums were selective for double-strand RNA, RNA-DNA hybrids, and double-strand DNA. At suitable dilutions, serums reacted by complement fixation with only one class of polynucleotide (Fig. 1). Although poly dG·poly dC reacted effectively with the

Table 1. Cross-reactions of antibodies to double-helical polynucleotides. Each serum was tested at varying dilutions with each antigen. The maximal complement fixation was plotted as a function of serum dilution and the dilution required for 50 percent maximal complement fixation was read by interpolation. Blank spaces indicate that there was no complement fixation at any dilution tested, the lowest dilution being 1/50.

Serum	Immunizing copolymer	Serum dilution required for 50 percent maximal complement fixation with:							
		Poly A·poly U	Poly I·poly C	Reovirus RNA	Poly A·poly dT	Poly dG·poly dC	Poly dA·poly dT	Poly dAT·poly dAT	Calf DNA
					Rabbit antiserums				
170	A·U	16,000	2,800	2,500	720				
171	A·U	12,000	2,000	850	< 50				
172	A·U	13,000	575	500					
201	A·U	7,000	1,550	2,400	< 50				
S-179	I·C	910	940	450	450				
S-180	I·C	480	580	370	300				
217	A·dT				10,000				
228	dG·dC					250			
229	dG·dC					2,000			
					Human SLE patients' serums				
SLE L					50	170	225	180	260
SLE R					350	800	1,200	1,000	1,450
SLE P					75	220	160	160	300
SLE E					45	60	85	85	85