Creg: unmatched, $P = .002$; matched, $P = .001$). These results are, however, more subject to error than those cited in Table 1 (*15*). A total of 41 allergic people possessed an antigen of the HL-A7 "Creg" in the present study; of these, 13 (32 percent) were highly Ra5-sensitive.

Serotypes HL-A10, 9, and 13 showed some degree of negative association with Ra5 sensitivity (although none were significant at the $P = .05$ level). For 2nd locus antigens, this is not unexpected since specificities showing negative associations are allelic with those showing positive associations. Negative associations for 1st locus antigens can, in part, be explained in terms of the linkage disequilibria between 1st and 2nd locus antigens (*21*).

We have shown a highly significant association between an allergic individual's ability to develop marked skin sensitivity to allergen Ra5 (following exposure to ragweed pollen) and his having the major histocompatibility antigen HL-A7 or one of the group of immunologically related, presumably chemically closely similar, antigens comprising the HL-A7 "Creg." Skin sensitivity to a particular allergen has previously been shown to correlate well with an allergically predisposed individual's ability to synthesize specific IgE antibody after inhalation of immunogenically limiting low doses of the allergen (*1, 3, 17*). More recently, in passive leukocyte and skin sensitization experiments (*13*), we have shown that allergic sensitivity to Ra5 is IgE-mediated. Thus, we have demonstrated an association between a specific immune response and HL-A serotype in man. Even though most P values are highly significant, in view of the fairly small population sample, it will be necessary to confirm our results in a second series of patients.

The genetic locus controlling responsiveness to Ra5 appears to be analogous to the mouse Ir-1 locus, which maps closely to, and normally segregates with, the K locus of the major histocompatibility (H-2) region of the mouse (*4, 18*). Furthermore, since HL-A7 "Creg" antigens form part of the 2nd locus of HL-A, there appears to be a good correlation between the K locus of H-2 and the 2nd locus of HL-A. This conclusion is compatible with several findings that man's susceptibility to certain immunological diseases is associated with HL-A antigens of the 2nd locus (*20*). It seems probable that this genetic control is operative predominantly, if not exclusively, at the thymus-derived (T) cell level (*4*), rather than in the bone marrow–derived (B) cells where the IgE antibody is actually synthesized.

We have presented strong evidence for a histocompatibility-associated Ir gene or genes in man. Under extremely limiting immunogenic stimulation with allergen Ra5, there is a highly significant association between immune response and a specific group of closely related HL-A antigens, a situation analogous to that described for inbred animal strains. Further studies of specific allergic hypersensitivities in man should forward our understanding of the genetic control of immune responsiveness.

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9. Short ragweed pollen contains approximately 5000 μg of antigen E per gram of pollen (radial immunodiffusion analysis). The maximum yield of Ra5 so far obtained is 15 μg per gram of pollen.
10. The sample of antigen E was supplied by Dr. T. P. King, Rockefeller University. Gel electrophoresis revealed that it consisted essentially of fraction IV-C with minor proportions of other isoallergens of the antigen E complex. It was homogeneous by ultracentrifugal analysis and gave a single precipitin line on immunodiffusion analysis against hyperimmune rabbit antiserum to antigen E and antiserum to whole ragweed extract.
11. The sample of Ra5 gave a single protein-staining band on acrylamide gel disc electrophoresis at *p*H 4.5 and 6.5 when 90-μg samples were used. It was homogeneous by equilibrium ultracentrifugal analysis ($\log_{10} c$ plotted linearly against r^2) and gel filtration. It gave a single precipitin line against hyperimmune rabbit antiserum to Ra5, but no line against antiserum to antigen E.
12. A skin reaction of a 0.8- to 1.0-cm diameter wheal without pseudopodia and with erythema of 2.0- to 4.0-cm diameter (variable from individual to individual) was graded as "2 plus." In some cases it was necessary to interpolate the allergen concentration yielding a 2-plus reaction from larger and smaller reactions at two allergen concentrations differing by tenfold.
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15. Two types of experimental problems exist: (i) possible trace, physicochemically undetectable, allergenic impurities in Ra5, totaling, say, 0.1 percent, might lead to the erroneous impression that certain Ra5 insensitive individuals are weakly sensitive to Ra5; (ii) positive skin sensitivity end points are subject to an error of $\pm$ one tenfold dilution.
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Retzius Cells: Neuroeffectors Controlling Mucus Release by the Leech

Abstract. *Mucus release from the skin of leeches is under the control of the pair of large Retzius cells in each segmental ganglion. The rate of mucus release increases with the impulse activity of Retzius cells and with the concentration of their putative neurotransmitter, 5-hydroxytryptamine, to which the skin is exposed.*

The largest cells among the approximately 175 pairs of bilaterally symmetrical neurons in the iterated segmental ganglion of the leech ventral nerve cord are the two "kolossal" cells of Retzius (*1*). Because their large size renders them accessible to study, Retzius cells have been the subject of histological, histochemical, electron microscopic–microchemical, neuropharmacological,

and electrophysiological investigations (*2–5*). But despite these studies, the function of these cells has remained unknown. However, it has been suggested, on the basis of the following findings (*4*), that Retzius cells are inhibitory motor neurons: (i) their function must be intra- rather than intersegmental since their processes do not enter the longitudinal connective; (ii) branches of their axons project to the body wall through all the major ipsilateral ganglionic roots; (iii) action potentials travel along their axons centrifugally; and (iv) they contain a high concentration of the putative neurotransmitter serotonin, or 5-hydroxytryptamine (5-HT), perfusion with which reduces the amplitude of excitatory junction potentials observed in leech muscle fibers.

I have carried out a variety of experiments designed to reveal an effect of Retzius cell activity on the musculature of the leech to test the validity of this suggestion. The results were uniformly negative. For instance, simultaneous intracellular recordings from Retzius cells and individual muscle fibers failed to show any correlation between action potentials and the inhibitory or excitatory postjunctional potentials in longitudinal, circular, oblique, or dorsoventral flattener muscles. Moreover, bursts of impulses induced by injection of current into Retzius cells caused no observable contractions. When longitudinal and circular muscles were made to contract by stimulating the longitudinal connective, Retzius cell activity did not affect the rate of tension increase, the maintenance of tonus, or the rate of relaxation by either muscle layer.

In view of the intrinsic likelihood that Retzius cells have peripheral effects and the failure to demonstrate any effect on the musculature, I examined the possibility that Retzius cells govern the release of mucus from the globose glands, which lie between the skin and the muscle layers (*6*). Most of these glands range in size from 30 to 80 μm, and many nerve terminals are in close apposition to them. Like many aquatic animals, leeches secrete large amounts of mucus, although the precise role of slime in the life of the leech has not been elucidated. Two preliminary experiments demonstrated that the release of mucus by the leech is under neural control, as has been shown to be the case for bullfrogs (*7*). First, one or two body segments of the intact leech were disconnected from the nerve cord by cutting the ganglionic roots. Visual

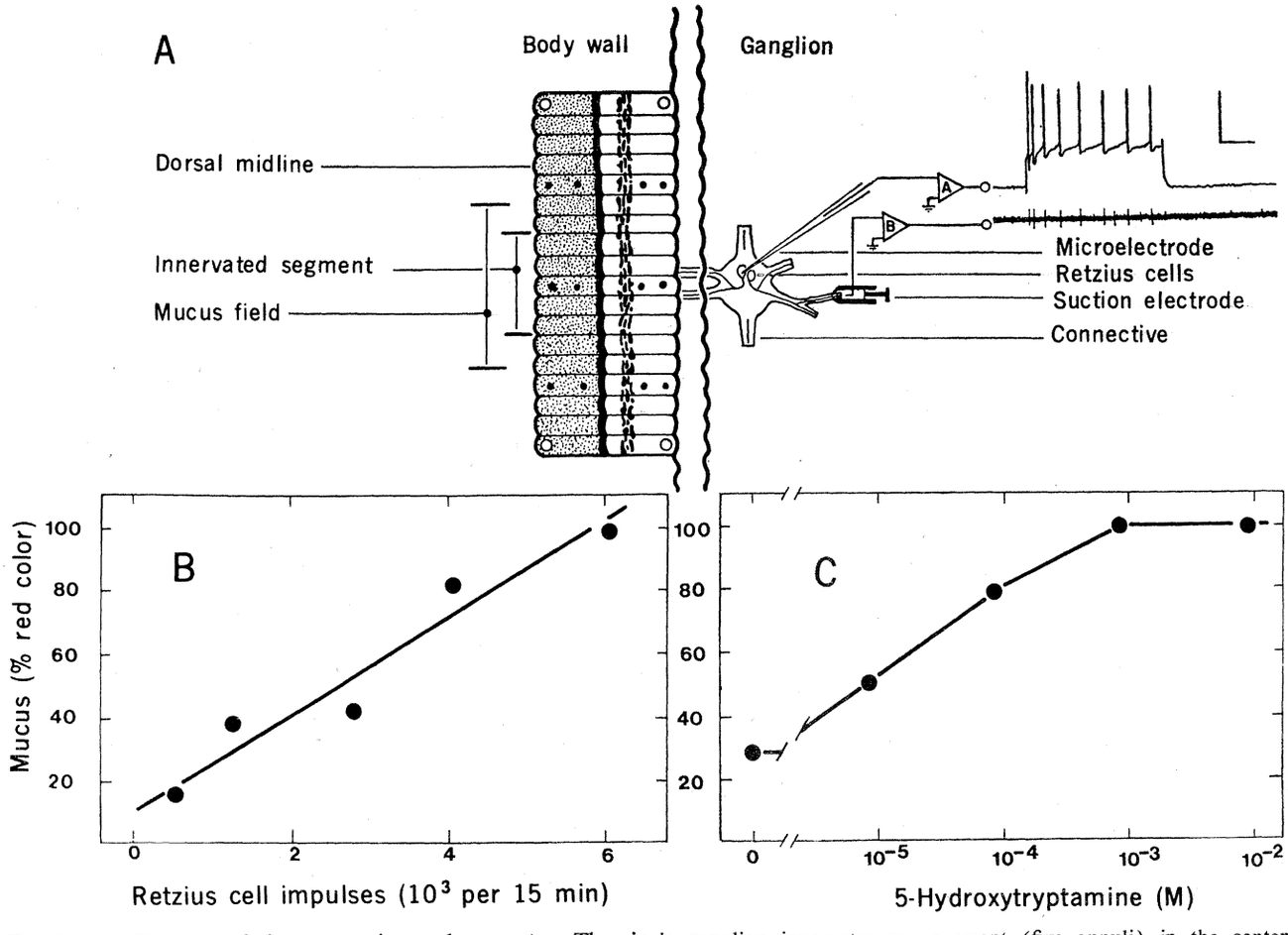

Fig. 1. (A) Diagram of the preparation and apparatus. The single ganglion innervates one segment (five annuli) in the center of the three-segment preparation. Retzius cell impulses are produced by depolarizing currents through the microelectrode, and the efferent activity of the ganglion is monitored by the suction electrode. The vertical calibration is 25 mv on the intracellular trace, *A*, and 100 μv on the extracellular trace, *B*. The horizontal calibration represents 1 second. (These are retracings of actual records.) During stimulation, eight or nine annuli produce mucus by the overlap of three or four annuli with adjacent segments. (B) The percentage of mucus produced as a function of Retzius cell impulses generated in 15-minute intervals. The depolarizing pulses producing the spikes were delivered at a frequency of 0.1 hertz and a duration of 4.5 seconds. The current strength of the depolarizing pulse determined the number of impulses produced. (C) The percentage of mucus produced as a function of the concentration of 5-HT. Data are taken from a single experiment on five denervated half sections of body wall, each 15 annuli long, bathed in high-Mg^{2+} saline.

inspection showed that the denervated, and only the denervated, segmental skin remained free of slime. Second, the connective of another group of leeches was severed near its midpoint, and either the anterior or the posterior cut end was stimulated with a suction electrode. The stimulated half-animals—whether anterior or posterior—invariably released more mucus than the unstimulated control halves. The results presented below show that it is the Retzius cells which mediate this neural control of mucus release.

Quantitative experiments on the relation of Retzius cell activity to mucus release were conducted on a preparation from the medicinal leech, *Hirudo medicinalis*, shown diagrammatically in Fig. 1A. This preparation consisted of a single ganglion connected unilaterally by its roots to a corresponding half body wall extending over about three segments. The ganglion, pinned down in a dish and viewed by transmitted light, was separated from the body by a petroleum jelly dam, so that the ganglion and wall could be bathed in salines of different composition. The body wall compartment was bathed in leech physiological saline (*8*), whereas the ganglion was usually bathed in a saline with an Mg^{2+} concentration of 20 m*M*. Thus, while the high Mg^{2+} in the ganglion compartment abolishes chemical synaptic transmission in the central nervous system of the preparation (*8*)—presumably by inhibiting the release of neurotransmitters at the axon terminals—the saline in the body wall compartment allows transmitter release in the periphery. This procedure has the advantage that, being deprived of synaptic inputs, the Retzius cells lose most of their spontaneous impulse activity and remain quiescent until depolarized experimentally. Furthermore, any increase in the rate of mucus release as a consequence of such depolarizations can be attributed to a direct effector action of Retzius cells on the periphery rather than to synaptic activation of other central neurons. Since leeches often secrete copious amounts of mucus during their dissection, the preparation was removed from the animal in a high-Mg^{2+} saline. This reduces central nervous activity and also prevents to some extent the loss of releasable stores of mucus from the body wall prior to the experiment.

The Retzius cell innervating the body wall was impaled with a micropipette containing 4*M* potassium acetate. Depolarizing current pulses were passed into the cell by means of a balanced bridge circuit, and the spike activity was recorded through the same electrode on a penwriter (Fig. 1A) and summed automatically on an electronic counter. The efferent spike activity from the ganglion was monitored by a suction electrode on the dorsal branch of the posterior root, usually at the cut end of the contralateral root, but occasionally en passant on the intact ipsilateral root. Since the Retzius cell pair is so strongly coupled by an electrotonic junction that their spikes are nearly synchronous (*5*), either side provides a good measure of the activity traveling from the neuron to the innervated half body wall.

The amount of mucus released was assayed by taking advantage of its adsorption of carmine red. For this purpose, the skin was inundated with an excess of carmine suspension in saline at a concentration of about 75 mg/ml. Five minutes later, the unadsorbed carmine was removed by gentle washing with saline. The mucus and its adsorbed carmine were collected with a suction pipette, transferred to a test tube, and sedimented into a pellet in a clinical centrifuge. After removal of the supernatant, the pellet was resuspended in a fixed volume of dilute sulfuric acid, which dissolved the carmine. The concentration of carmine was assayed colorimetrically by the intensity of the red color in solution, and this was taken to be proportional to the amount of mucus. Since the rate of mucus release by different preparations is variable, the results are reported as percentages of the maximum mucus released by a particular preparation.

Figure 1B shows the results of an experiment in which the Retzius cell was depolarized by current pulses at levels sufficient to produce different numbers of impulses during successive 15-minute periods. As can be seen, the rate of mucus release is roughly proportional to the number of impulses, rising at least eightfold from the control period (where the 500 impulses were spontaneous) to the period of maximum depolarization (where 6000 impulses were produced). The maximum amount of mucus released by this preparation corresponded to a dry weight of about 10 mg. In this experiment, the number of impulses was increased in successive stimulation periods, to ensure that any exhaustion of mucus reserves during the experiment would result in an underestimate of the dependence of the rate of mucus release on spike activity. Since it takes a few minutes for the accelerated rate of mucus release to return to its low spontaneous level, the successive stimulation periods were separated by intervals of at least 10 minutes. In other experiments, not reported here in detail, the number of impulses was decreased in successive stimulation periods. Since here, too, the rate of mucus release followed the total impulse number, it can be concluded that the data presented in Fig. 1B cannot be attributed to any cumulative effect of successive collections of mucus in the assay procedure. Thus, these results show that the impulse activity of Retzius cells controls the rate of mucus release from the dermal glands.

No increase in the rate of mucus release with impulse activity was seen when the body wall was bathed in high-Mg^{2+} saline, which suggests that the Retzius cell exerts its effect by the release of 5-HT at its terminals in the body wall. To test this possibility, the effect of the direct application of 5-HT on the body wall on mucus release was examined. A preliminary experiment showed that a drop of concentrated 5-HT on the leech skin resulted in an immediate formation of a glob of mucus in the treated area. For a more quantitative study of this effect, equal-sized denervated sections of body wall (lateral halves, three segments long) were each bathed in high-Mg^{2+} saline to which different concentrations of 5-HT had been added. After 45 minutes, the mucus was collected and assayed. Figure 1C shows the amount of mucus produced as a function of 5-HT concentration. Exposure of the body wall to $10^{-3}M$ and $10^{-2}M$ 5-HT increased the rate of mucus release more than threefold over the control with no 5-HT, and intermediate concentrations produced submaximal effects.

The finding that the direct application of 5-HT to the body wall caused mucus release offers further support for the inference that the Retzius cell controls the process without synaptic transfer within the central nervous system, as the only axons in the ganglionic roots containing this neurochemical are those from the Retzius cells (*3*).

Since 5-HT releases mucus in the presence of Mg^{2+}, the 5-HT from the Retzius cells most likely activates the glands directly without synaptic transfer through a peripheral nerve plexus. The data reported here do not, however, allow any decision as to whether the process of mucus release is medi-

ated by a synaptic or neurosecretory route. Nor do the data demonstrate that the control of mucus release is the only effector role of these large neurons. But the control of mucus release by cells secreting 5-HT as a transmitter may be a more general phenomenon, for it has been reported that the direct application of 5-HT mediates the secretion of mucus in the mammalian stomach (*9*).

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Quantitation of RNA Tumor Viruses and Viruslike Particles in Human Milk by Hybridization to Polyadenylic Acid Sequences

Abstract. *RNA tumor viruses and viruslike particles from human milk are quantitated by hybridization of the polyadenylic acid regions in their 60S to 70S RNA to radioactive polyribouridylic acid of known specific activity. The length of the polyadenylic acid region in the 60S to 70S RNA of the human milk particle is identical to that of the known oncogenic RNA viruses.*

Particles that have several biochemical and biophysical characteristics unique to the RNA tumor viruses have been isolated from human milks (*1*). Utilization of the simultaneous detection technique (*2*) permitted the demonstration that these particles, with a density of 1.16 to 1.19 g/ml, contain an RNA-instructed DNA polymerase and a high-molecular-weight (HMW) RNA (60*S* to 70*S*), which serves as the template in an endogenous reverse transcriptase reaction (*3*). Numerous investigators have found that the 60*S* to 70*S* RNA of oncornaviruses contains polyadenylic acid [poly(A)] regions (*4–6*) that represent approximately 1.5 percent of the viral genome (*4, 5*). We report here that the 60*S* to 70*S* RNA from human milk particles also contains poly(A) stretches and that they are the same length, approximately 200 nucleotides long, as those found in the mouse mammary tumor virus (MMTV) RNA and in other RNA tumor viruses. Finally, we have used hybridization to poly(A) segments (*5*) as an assay for the detection and quantitation of these particles in human milks and for the quantitation of RNA tumor viruses in biological fluids.

The assay used in our study for the detection of poly(A) stretches in HMW-RNA is a modification of that of Gillespie *et al.* (*5*). Any viruses present were purified from biological fluids by velocity and equilibrium gradient centrifugations (legends to Figs. 1 and 2). RNA was extracted from particles with a density of 1.16 to 1.19 g/ml and then sized by means of glycerol velocity gradient centrifugation with the aid of appropriate external markers. Each fraction of the velocity gradient is tested for poly(A) regions by hybridization to [^{3}H]poly(U) (tritiated polyribouridylic acid). Ribonuclease and deoxyribonuclease treatment is used to ensure that the remaining acid-insoluble radioactivity in a given fraction is due to the hybridization of [^{3}H]poly(U) to the poly(A) region of an RNA molecule. Acid-insoluble radioactivity in the 60*S* to 70*S* region of the gradient constitutes a positive.

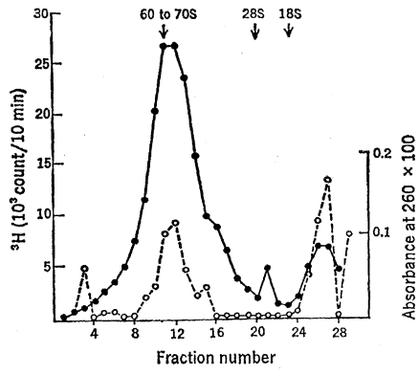

Fig. 1. Detection of polyriboadenylic acid regions in high-molecular-weight RNA from AMV. The AMV was purified by filtration and differential centrifugation (*14*). RNA was extracted from 2 mg of viral protein by treatment with sodium dodecyl sulfate (SDS), 1 percent; Pronase (self-digested), 750 μg/ml; and mercaptoethanol, 1 percent; for 30 minutes at 37°C in TNE buffer [0.1*M* NaCl, 0.01*M* tris(hydroxymethyl)aminomethane, *p*H 8.3, 0.015*M* EDTA]. The RNA solution was then mixed (1 : 1 : 1) with chloroform containing 1 percent isoamyl alcohol and phenol-cresol (7 : 1), *p*H 7.5, containing 8-hydroxyquinoline (0.37 g/100 ml) and shaken at 4°C for 10 minutes, and then centrifuged at 8000*g* for 10 minutes. This and all subsequent centrifugations were at 4°C. The aqueous phase was reextracted with the chloroform-phenol mixture, as described above, and then precipitated overnight at −20°C after addition of 0.4*M* NaCl and two volumes of cold ethanol. The RNA was then sedimented by centrifugation at 16,000*g* for 20 minutes; it was then dissolved in 0.5 ml of 0.003*M* EDTA and layered over a linear glycerol gradient (10 to 30 percent) in 0.01*M* tris (*p*H 8.3), 0.15*M* NaCl, 0.002*M* EDTA, and centrifuged at 40,000 rev/min for 3 hours (Spinco SW-41 rotor). The gradient was dripped and absorbancy (260 nm) was read for each 400 μl fraction. The poly(A) content of each fraction was then determined by hybridization to [^{3}H]poly(U). A 4-μl sample from each fraction was brought to 600 μl, the final solution containing 0.45*M* NaCl and 0.045*M* sodium citrate (3 × SSC), 0.003*M* tris (*p*H 7.0), 50 percent formamide, and 5000 count/min of [^{3}H]poly(U) (17 c/mmole or 9.3×10^3 count/min per picomole) and incubated at 36°C for 48 hours. Two milliliters of solution containing 0.01*M* tris, 0.01*M* $MgCl_2$, 0.5*M* NaCl, 10 μg of pancreatic ribonuclease A (Sigma) (boiled 10 minutes in 6 × SSC), and 40 μg of deoxyribonuclease I (Sigma) were added and the incubation was continued for 2 hours at 30°C; the mixture was then placed at 4°C for 15 minutes and precipitated with 0.5 ml of 100 percent trichloroacetic acid containing 0.2 percent uridine (P-L Biochemicals). After incubation for 30 minutes at 4°C, acid-precipitable radioactivity was determined by collection on membrane filters, followed by washing with 10 percent trichloroacetic acid containing 0.02 percent uridine. Closed circles, ^{3}H, 10^3 count/10 min; open circles, absorbancy at 260 nm × 100.