gous at several *H* loci, but homozygous *H-2*^{b} (Table 1). Therefore, such F_1 hybrids were considered nonresistant to the parental C57BL/10 marrow graft. On the other hand, mice that were heterozygous at the *H-2* locus, but homozygous at other *H* loci, showed deficient growth of grafted C57BL/10 marrow and were classified as resistant (Table 1). No difference among them in strength of resistance was detected, since in all instances 10^6 transplanted C57BL/10 cells failed to grow, but the possible existence of differences could be tested by grafting serial dilutions of marrow cells.

It has been shown that *H-2* is a complex gene locus including at least four regions denoted in order of linkage as D, C, V and K (*6*, *9*). Because of the demonstrated association of hybrid resistance with heterozygosity at the *H-2* locus, it was of interest to establish whether heterozygosity of the entire *H-2* locus was necessary for the manifestation of resistance to grafted C57BL/10 marrow. In the course of studying the genetic structure of *H-2* (*6*), several variants were identified and were presumably derived from cross-overs within *H-2*. The exceptional alleles were found in the offspring of *H-2*^{a}/*H-2*^{b} heterozygotes in which *H-2*^{a} was from strain A and *H-2*^{b} from C57BL/10. The *H-2* "recombinant" alleles were transferred, by repeated backcrosses, to a genetic background approximately congenic with C57BL/10. A preliminary account of these recombinants has been reported (*6*) and a more detailed description is forthcoming.

Two of the recombinant lines congenic with C57BL/10, were used in an experiment described here. The serotype of mice homozygous for the *H-2*^{a} allele is D+M+C+H+K+ (*6*) while the serotypes of the two recombinant lines are either D+M+C+H+K− (type 1) or D−M−C+H+K+ (type 2) and resemble the *H-2*^{i} and *H-2*^{h} alleles described by Gorer and Mikulska (*9*). The components of specificities M and H are thought to be closely associated with the determinants of D and V (*6*, *9*). F_1 hybrids from mice which possess the type 1 or type 2 recombinant alleles and from C57BL/10 are heterozygous for either the D or K regions of the *H-2* locus, respectively. When tested by the I^{131}UdR method, the hybrids heterozygous for the K region of *H-2* were resistant and indistinguishable from resistant *H-2*^{a}/*H-2*^{b} F_1 mice, while hybrids heterozygous for the D region of *H-2* were not resistant to C57BL/10 marrow grafts (Table 2).

Thus, C57BL/10 mice possess in the K region of *H-2* a genetic determinant whose expression is required for the growth of transplanted marrow cells. F_1 hybrid mice heterozygous for this determinant are incompatible for transplanted C57BL/10 hemopoietic cells; for example, they do not support optimal growth of minimal numbers of infused C57BL/10 marrow cells. Allogeneic strains homozygous for *H-2* alleles other than *H-2*^{b} do not display such resistance (*1*, *2*, *10*), a finding that is contrary to expectation if the factor associated with *H-2*^{b} of C57BL/10 were recessive. The long-term persistence of non-minimal C57BL marrow grafts may, however, be controlled by factors different than the *H-2* locus, not necessarily genetic in nature, especially when sufficient cells are initially transplanted to override the hybrid resistance (*11*).

It was reported that transplanted C57BL lymphomas (*5*) and sarcomas (*12*) exhibited deficient growth in F_1 hybrids. Homozygous lymphoma and carcinoma cells originating in mice of types other than *H-2*^{b}/*H-2*^{b} were also found to grow deficiently in *H-2* heterozygous F_1 hybrids (*12*, *13*). The various findings suggest that the hybrid resistance to transplantable hemopoietic cells associated with the *H-2* locus probably applies also to transplantable tumor cells. It may be inferred, therefore, that the phenomenon of hybrid resistance to parental grafts is not peculiar to hemopoietic cells or only to cells carrying *H-2*^{b}. Perhaps the relative instability of the tumor cell genome provides a variety of such noncodominant *H-2* histocompatibility factors which may be useful for elucidating the inheritance of histocompatibility in more stable cell lines.

GUSTAVO CUDKOWICZ
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee*
J. H. STIMPFLING
Jackson Laboratory, Bar Harbor, Maine

References and Notes

1. E. A. Boyse, *Immunology* **2**, 170 (1959); E. A. McCulloch and J. E. Till, *J. Cellular Comp. Physiol.* **61**, 301 (1963).
2. G. Cudkowicz and J. H. Stimpfling, *Genetics* **48**, 886 (1963); ———, *Immunology* **7**, 291 (1964).
3. G. Cudkowicz and J. H. Stimpfling, *Federation Proc.* **23**, 202 (1964).
4. Pedigreed breeders of the isogenic-resistant lines were made available by G. D. Snell.
5. G. D. Snell, *J. Natl. Cancer Inst.* **21**, 843 (1958); ——— and L. C. Stevens, *Immunology* **4**, 366 (1961).
6. J. H. Stimpfling and G. D. Snell, in *International Symp. Tissue Transplantation* (Universidad de Chile, Santiago, 1962) p. 37; J. H. Stimpfling, in preparation.
7. G. Cudkowicz, A. C. Upton, L. H. Smith, G. D. Gosslee, W. L. Hughes, *Ann. N.Y. Acad. Sci.* **114**, 571 (1964).
8. Nuclear Science and Engineering Co.
9. S. L. Allen, *Genetics* **40**, 627 (1955); D. B. Amos, P. A. Gorer, Z. B. Mikulska, *Proc. Roy. Soc. London Ser. B* **144**, 369 (1955); P. A. Gorer and Z. B. Mikulska, *ibid.* **151**, 57 (1959); O. Pizzarro, G. Hoecker, P. Rubinstein, A. Ramos, *Proc. Natl. Acad. Sci. U.S.* **47**, 1900 (1961).
10. G. Cudkowicz and J. H. Stimpfling, unpublished observations.
11. R. A. Popp, *J. Natl. Cancer Inst.* **26**, 629 (1961); R. A. Popp, G. E. Cosgrove, D. M. Popp, *Ann. N.Y. Acad. Sci.* **114**, 538 (1964); R. A. Popp, *J. Natl. Cancer Inst.*, in press.
12. K. E. Hellström, *Science* **143**, 477 (1964).
13. ———, *Nature* **199**, 614 (1963).
14. Supported in part by research grant CA-01329 from the National Cancer Institute, USPHS. We thank G. D. Snell and A. C. Upton for support, and Mrs. L. C. Satterfield and Mrs. A. Richardson for technical assistance.
* Operated by Union Carbide Corporation for the AEC.

3 April 1964

Intercellular Channels in the Salt-Secreting Glands of Marine Turtles

Abstract. *Long, pleomorphic microvilli project from the walls of adjacent secretory cells in the lacrymal glands of sea turtles, and a substance identified histochemically as a mucopolysaccharide fills the intercellular channels. These features are not characteristic of the principal secretory cells in the salt glands of marine birds.*

The extra-renal glands for salt secretion in marine birds and reptiles have different embryonic origins; those of marine birds are modified nasal glands (*1*), while those of reptiles are modified lacrymal glands (*2*). In both classes of vertebrates the glands secrete a sodium chloride solution that is hypertonic to blood (*3*, *4*) and on histological examination they show essentially the same pattern of organization (*3*, *5*). However, electron microscopic and cytochemical techniques disclose unique intercellular channels in the reptilian salt glands, that are not present in the salt glands of marine birds (*5–7*). These differences may reflect the separate embryonic origins of the glands, and provide two possible designs for cells in which electrolytes are concentrated.

Tissue from the salt glands of two loggerhead (*Caretta caretta*) and four green (*Chelonia mydas*) turtles was fixed in phosphate-buffered 2-percent osmium tetroxide (*8*) and dehydrated with acetone; the fixed tissue was embedded in Epon (*9*), sectioned, and

stained (*10*) for electron microscopy. Larger blocks of fresh or appropriately fixed tissue from the same salt glands were used for cytochemical tests.

The histological features of the salt glands of the two species of marine turtles were found to be essentially similar in all details. The secretory lobules consist of myriads of closely packed, branched tubules radiating outward from a central duct or canal. At the blind, peripheral ends of the tublues are small terminal cells with scant cytoplasm. The principal secretory cells line the remainder of the tubules; these cells are largest near the central canal. In the loggerhead turtle, the tubules average 1 mm in length and bifurcate two or three times within this span. Near the periphery of the lobule the lumen of each tubule is extremely small; centripetally it increases slightly in size and reaches its largest diameter where the tubule joins with the central canal. The fine bore of the lumen indicates that the pyramidal secretory cells have a very limited secretory surface.

When observed with the electron microscope, the walls of the principal cells are fringed with a profusion of pleomophic tall sinous microvilli which are occasionally branched. In some regions the microvilli are truly digitiform, in other areas they appear as flat folds. The microvilli of adjacent secretory cells intermesh loosely with one another and are linked by occasional desmosomes. Irregular, clear spaces appear between them and the tortuous intercellular channels thus formed average 1.5 μ in width (Fig. 1*A*). At the base of each cell the microvilli are sparse, and may be flattened against the basement membrane. No fibrous elements or other cytoplasmic organelles extend outward into the microvilli but small, membrane-bound vesicles are evident in the cytoplasm, subjacent to the pleomorphic processes.

Scant, irregular, short microvilli extend outward from the apical surface of the cell into the lumen of the tubule. No open or direct connection was observed between the intercellular channels and the lumen of the secretory tubule. Without exception, the lateral and luminal surfaces of the cell are separated by a continuous system of prominent terminal bars and intermittent desmosomes that resemble the junctional complexes found in other epithelia (*11*).

The cytochemical studies revealed in the intercellular channels a substance that resists digestion with diastase or saliva and stains pink with the periodic acid Schiff reaction (Fig. 1*B*). After sulfation (*12*), the substance stains metachromatically with toluidine blue at *p*H 5.0; without sulfation it colors metachromatically with the method of Hess and Hollander (*13*) (Fig. 1*C*). These reactions indicate that a mucopolysaccharide is localized along the intercellular channels.

The fringed sides as well as the irregularities in shape greatly increase the surface area of the secretory cell of the salt gland (Fig. 1*A*). Microvilli are sparse on the basal surfaces of the secretory cells, which suggests that this region may be less active in absorption than the lateral margins. Since passage from the intercellular channels to the lumen of the tubule is blocked by the junctional complexes, the intercellular channels are not equivalent to the inter- or intracellular canaliculi observed in some other secretory epithelia.

Many mucopolysaccharides have the capacity for binding cations and some can act as ion exchange resins (*14*). Bennett has suggested that the "glycocalyx" of mucopolysaccharide that envelopes many cells may serve as an ion trap (*15*). Performing in this capacity, the mucopolysaccharide might facilitate greatly the concentrating of electrolytes by the cell. A glycocalyx has been demonstrated in association with

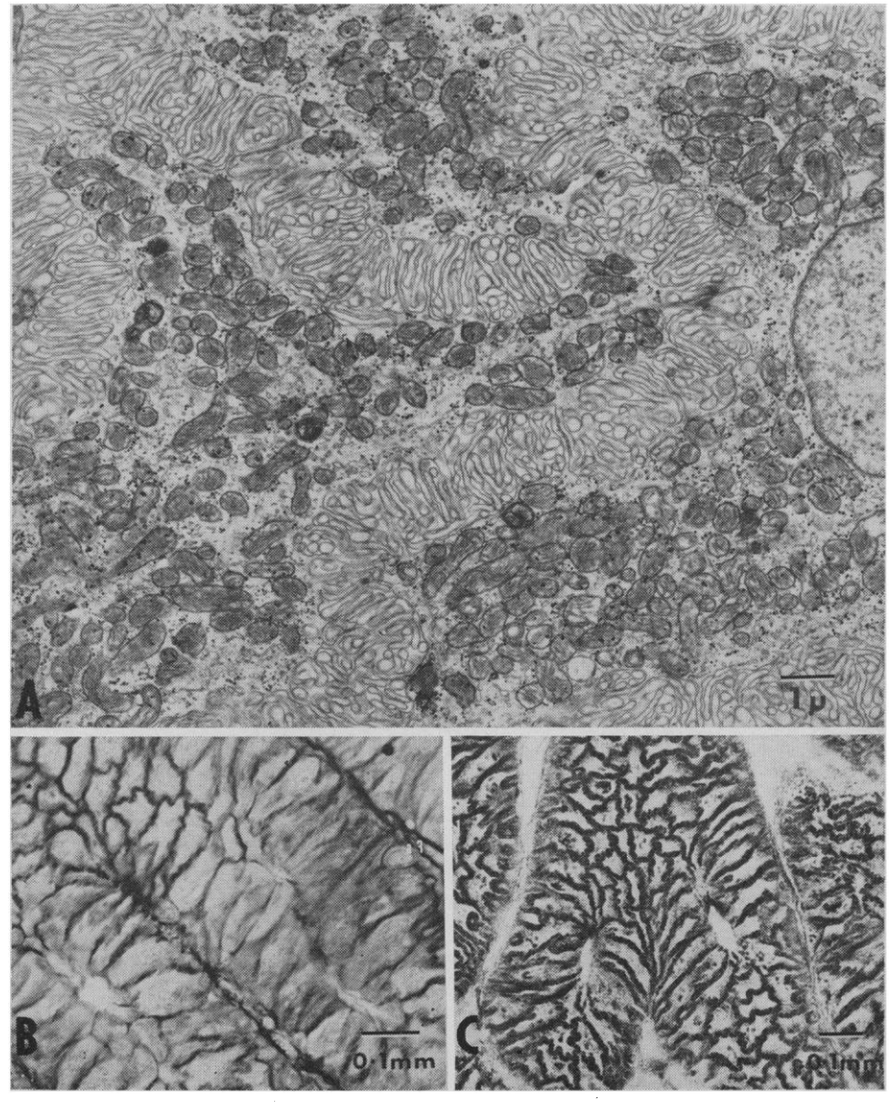

Fig. 1. *A*, An electron micrograph showing the abundant microvilli lining the intercellular channels. Portions of three secretory cells from the salt gland of a loggerhead turtle are included. Phosphate-buffered osmium fixation. *B*, The irregular intercellular channels are stained by the periodic acid Schiff reaction after digestion with diastase. Formalin fixation. *C*, The intercellular channels are stained metachromatically with toluidine blue by the method of Hess and Hollander (*13*). Zenker fixation.

a variety of cell types (*15*), but not as a component of an epithelium engaged solely in electrolyte secretion. The abundant mucopolysaccharide bordering the salt-secreting cells in the lachrymal gland of the turtle suggests strongly that it is linked with electrolyte secretion. Staining reactions indicative of mucopolysaccharide have also been reported in the secretory cells of the salt gland of the duck (*7*) and at the borders of the secretory cells in the rectal gland of the dogfish (*16*).

Intercellular channels bearing some resemblance to those described in this report have been observed in the ciliary epithelium of the eye (*17*) and between the clear cells of eccrine sweat glands (*18*). The tubule cells of the rectal gland of elasmobranchs (*18*, *19*) as well as the cells lining the central ducts or canals in the salt gland of the herring gull bear interdigitating processes along their margins (*20*). All of these cells are involved in the secretion of electrolytes. In these epithelia, however, the intercellular channels are less elaborate than those in the salt glands of marine turtles; and mucopolysaccharide has been reported only between the secretory cells in the rectal gland of the dogfish (*18*). Thus the abundance, the complexity, and the mucopolysaccharide content of the intercellular channels in the salt gland of the turtle seem to make them unique.

In the salt glands of marine birds the principal secretory cells have deep clefts along their basal and lateral surfaces (*6*). Adjacent cells bear clefts that complement each other, and this results in an extensive intermeshing of the cell surfaces. These interfolded cell processes containing both mitochondria and agranular endoplasmic reticulum serve to increase greatly the absorptive surface of the secretory cell (*6*); and they are analogous to the microvilli which fringe the salt-secreting cells in turtles. Thus, the architecture of the salt-secreting cells of marine birds and reptiles seem to represent two independent solutions to the problem of electrolyte excretion. The cells lining the convoluted tubules of the kidney (*21*) resemble the salt-secreting cells of birds, while the secretory cells of the rectal glands of elasmobranchs (*16*, *19*) resemble those of reptiles. The major differences in the fine structure of these cells are restricted primarily to the cell surface, while their contents are essentially similar.

The bulk of the evidence available from studies of fine structure shows that abundant mitochondria and an intracellular system of agranular membranes are the hallmark of cells that from physiological investigations are known to concentrate electrolytes. These organelles are both conspicuous and abundant in the secretory cells of the salt glands of marine birds (*6*), the rectal glands of elasmobranchs (*16*, *19*), the chloride cells of fishes (*22*), the salt cells of crustacean gills (*23*), the cells of the anal papillae of mosquito larvae (*24*), and the cells of the convoluted tubules of the kidney (*21*). The secretory cells of the salt glands of marine turtles are no exception to this rule and offer further evidence that these organelles participate directly in the secretion of electrolytes.

RICHARD A. ELLIS
JOHN H. ABEL, JR.
Department of Biology,
Brown University,
Providence, Rhode Island 02912

References and Notes

1. B. Marples, *Proc. Zool. Soc. London*, p. 829 (1932).
2. A. Peters, *Arch. Mikr. Anat.* **36**, 192 (1890).
3. K. Schmidt-Nielsen and R. Fänge, *Nature* **182**, 783 (1958).
4. K. Schmidt-Nielsen, *Circulation* **21**, 955 (1960).
5. R. Fänge, K. Schmidt-Nielsen, K. Osaki, *Biol. Bull.* **115**, 161 (1958).
6. W. Doyle, *Exptl. Cell Res.* **21**, 386 (1960); H. Komnick, *Protoplasma* **56** (1963).
7. R. Scothorne, *Quart. J. Exptl. Physiol.* **44**, 329 (1959); R. Ellis, C. Goertemiller, Jr., R. DeLellis, Y. Kablotsky, *Develop. Biol.* **8**, 286 (1963).
8. G. Millonig, *J. Appl. Phys.* **32**, 1637 (1961).
9. J. Luft, *J. Biophys. Biochem. Cytl.* **9**, 409 (1961).
10. E. Reynolds, *J. Cell Biol.* **17**, 208 (1963).
11. M. Farquhar and G. Palade, *ibid.*, p. 375.
12. R. Mowry, *J. Histochem. Cytochem.* **6**, 82 (1958).
13. M. Hess and F. Hollander, *J. Lab. Clin. Med.* **32**, 905 (1947).
14. E. Balazs, *Federation Proc.* **17**, 1086 (1958); S. J. Farber, *Circulation* **21**, 941 (1960).
15. S. Bennett, *J. Histochem. Cytochem.* **11**, 14 (1963).
16. R. Bulger, *Anat. Rec.* **147**, 95 (1963).
17. G. Pappas and G. Smelser, *Am. J. Ophthalmol.* **46**, 299 (1958).
18. R. Hibbs, *Am. J. Anat.* **103**, 201 (1958); B. Munger, *J. Biophys. Biochem. Cytol.* **11**, 385 (1961); R. Ellis, in *Advances in Biology of Skin*, W. Montagna, R. Ellis, A. Silver, Eds. (Pergamon Press, Oxford, 1962), vol. 3, p. 30.
19. W. Doyle, *Am. J. Anat.* **111**, 223 (1962).
20. H. Komnick, *Protoplasma* **56**, 274 (1963).
21. S. Clark, Jr., *J. Biophys. Biochem. Cytol.* **3**, 349 (1957); J. Rhodin, *Am. J. Med.* **24**, 661 (1958); A. Christensen, *J. Cell Biol.* **19**, 13A (1963).
22. D. Copeland and A. Dalton, *J. Biophys. Biochem. Cytol.* **5**, 393 (1959); C. Philpott and D. Copeland, *J. Cell Biol.* **18**, 389 (1963).
23. D. Copeland, *J. Cell Biol.* **19**, 16A (1963).
24. D. Copeland, in preparation.
25. This investigation was supported by PHS research grant GM-08380-03 and research career development award AM-K3-4938 from the National Institute of Arthritis and Metabolic Diseases.

5 May 1964

Geotropism: Its Orienting Force

Abstract. *Seeds of* Pisum sativum *L. and other species were germinated and grown for five or more days on a continuously rotating vertical-axis turntable that developed a maximum centrifugal force of 1.79*g. *Shoot (epicotyl) orientation in darkness was parallel to the resultant gravitational field. This is presented as confirmation of the hypothesis that the orienting force of geotropism of the higher plants is the inertial force of gravity.*

When a plant organ grows vertically (or at some particular angle to the vertical) this oriented growth phenomenon is referred to as geotropism. The purpose of this study is to establish on a firm footing that the normal geotropic response of the higher plants is a response to the inertial force of gravity and not to some other stimulus —known or unknown—parallel to it. It has been demonstrated repeatedly, and perhaps most elegantly by Fitting (*1*), that the geotropic response is to a vertical force. That this force is gravity has been accepted almost intuitively, but surprisingly, with almost no experimental verification.

The hypothesis that gravity is the orienting force for geotropism is perhaps best tested experimentally by growing a plant in the reoriented gravitational field attained by the use of a centrifuge. Should the geotropic orienting force be other (or partially other) than gravity, then the orientation of the plant would, of course, not be parallel to the resultant gravitational force (*2*). Such experiments have been reported apparently only seven times (*3–9*), with curious results. Two of the seven investigators (*4*, *6*) became convinced that they could reject the hypothesis, while the remaining five felt that it could be accepted. A careful analysis of the seven sets of data indicates that confirmation of the hypothesis was not justified by the experimental results in at least four of the cases (*3*, *4*, *6*, *9*), nor perhaps in some of the others. The reports in three cases (*3*, *4*, *9*) indicate that phototropic responses exerted an unrecognized influence on the orientation of the plants; in two cases (*6*, *8*) the centrifugal forces actually involved seem to have been reported incorrectly; in one case (*4*) the data were miscalculated and misinterpreted. There