1959); A. Tiselius, *Nova Acta Regiae Soc. Sci. Upsal.* **7** (4), 1 (1930).
11. W. J. Archibald, *J. Phys. Colloid Chem.* **51**, 1204 (1947).
12. S. M. Klainer and G. Kegeles, *J. Phys. Chem.* **59**, 952 (1955).
13. H. K. Schachman, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1957), vol. 4, p. 32.
14. In this equation, the coefficient of c will, of course, be influenced by any of the factors which affect protein aggregation, for example, ionic strength and temperature.
15. At *p*H 2.1 (0.21*M* NaCl, 0.07*M* phosphate buffer), the investigated range of meniscus concentrations corresponded to an optical density range of 2.2 to 11.4 at 280 mμ in cells 1.00 cm in length. At *p*H 10.5 (0.21*M* NaCl, 0.07*M* glycinate buffer), the investigated range of meniscus concentrations corresponded to an optical density range of 2.1 to 11.6 at 280 mμ in cells 1.00 cm in length. At each *p*H value, the respective ranges of meniscus concentrations were attained by the procedure described in the text accompanying Fig. 3; the respective series of rotor speeds used in each study were identical.

24 December 1964

Neurosecretory Processes Extending into Third Ventricle: Secretory or Sensory?

Abstract. *"Dendrites" projecting into the third ventricle from hypothalamic neurosecretory neurons of the Pacific tree frog,* Hyla regilla, *appear to be morphologically equipped to serve both secretory and sensory functions. These cell processes contain elementary neurosecretory granules, Golgi membranes, mitochondria, and endoplasmic reticulum, suggesting synthesis and possibly release of hormonal material. Each "dendrite" is provided with a single cilium which has an accessory centriole and a striated rootlet.*

"Dendrites" extending from hypothalamic neurosecretory cells toward the lumen of the third ventricle have been described repeatedly in various vertebrates by light microscopists (*1*, *2*). Stainable neurosecretory material in such processes often appears to project into the ventricle between ependymal cells, suggesting that these neurons may release their hormonal products into the cerebrospinal fluid as well as into the blood stream in the neurohypophysis (*2*). Dierickx has proposed instead that the "dendrites" act as receptors responding to osmotic changes in the cerebrospinal fluid (*3*).

In the course of an ontogenetic study of the neurosecretory system in the Pacific tree frog, *Hyla regilla*, the "dendrites" of adult preoptic neurons were observed with the electron microscope. The processes appear to be particularly prominent in this species (*2*) (Fig. 1), occurring as stout continuations of the perikaryon and usually ending in bulbous expansions at the luminal surface of the ventricle (Figs. 2 and 3). There is variation in the shape of the endings, however; some are not expanded, whereas others appear to spread over the surface of adjacent ependymal cells. The smooth surfaces of the processes contrast with the irregular outlines of ependymal cells which have numerous microvilli at their free surfaces and extensive interdigitations at the lateral and basal borders (Figs. 2 and 3).

All the organelles of the neurosecretory perikaryon, including endoplasmic reticulum, ribosomes, mitochondria, Golgi membranes, and elementary neurosecretory granules, are found throughout the process (Figs. 2 and 3). Typical terminal bars and desmosomes occur between the processes and adjacent ependymal cells. Extending into the lumen from the tip of the process is a single cilium provided with a striated rootlet (Figs. 3 and 4) and an accessory centriole (Fig. 4). The major branch of the rootlet has been followed to a depth of over 5 μ into the process. Occasionally, tenuous branches of the rootlet are also seen (Fig. 4). It has not been determined whether these cilia have the two central fibrils characteristic of kinocilia (*4*).

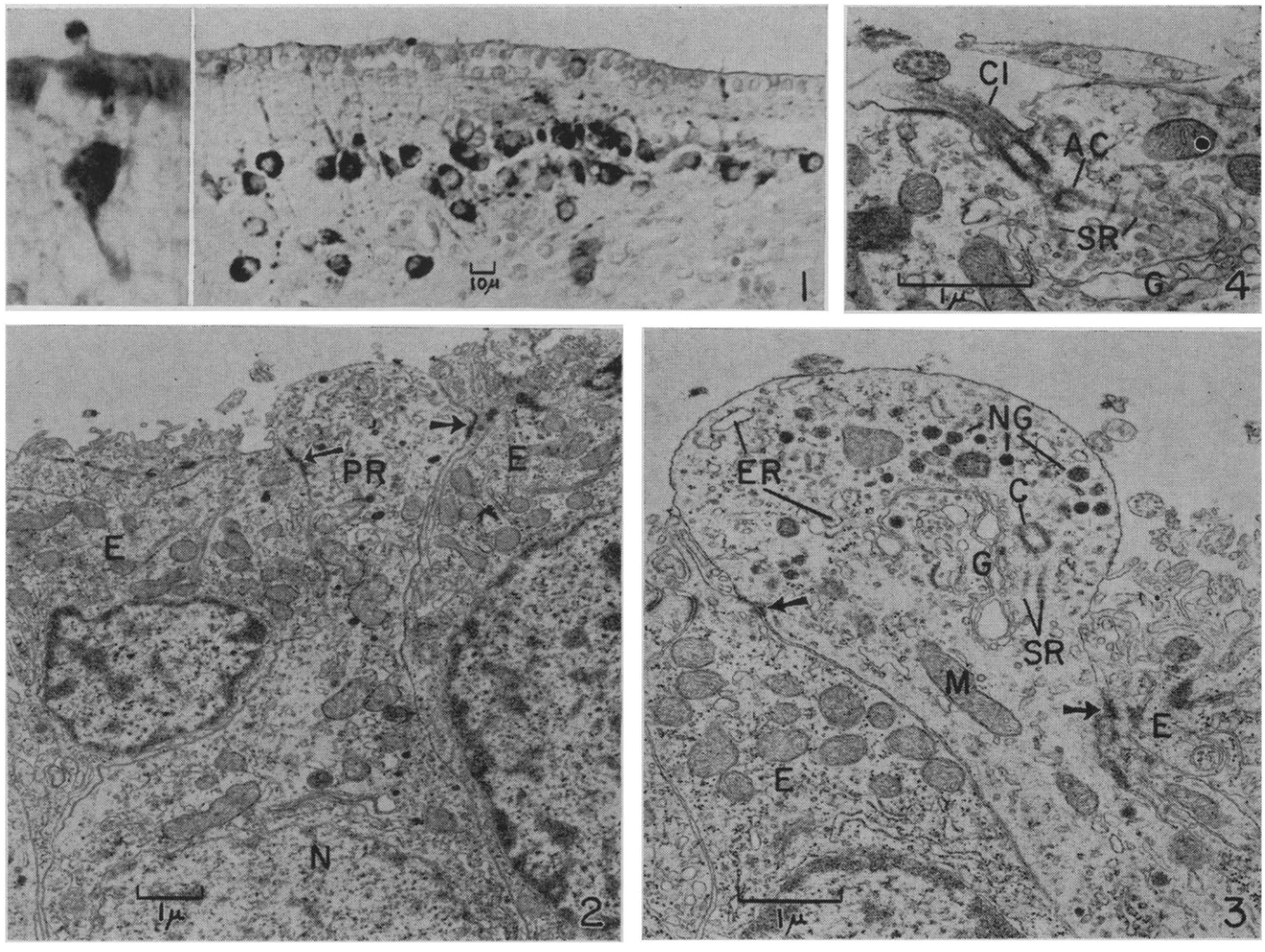
The finding of elementary granules in the processes may indicate secretory activity or merely that synthesis occurs here as well as in the cell body proper. Inasmuch as all of the structural components normally associated with synthesis are found in the processes, granules could be manufactured in the "dendrites" and then could move centripetally to the perikaryon and down the axon to be released in the neurohypophysis. On the other hand, although no intact granules were observed in the lumen or passing through the plasma membrane, release from the "dendrite" into the ventricle could occur by diffusion, as is apparently the case in the axon endings of these cells in the neurohypophysis. Furthermore, an apocrine type of secretion remains a distinct possibility. It is postulated that the granules and droplets described by light microscopists as representing secretion into the ventricle lumen (*2*, *5*) are really portions of the cytoplasm of the processes, containing secretory granules and thus staining with paraldehyde fuchsin. In many instances, the light microscope would not reveal a connection to the cell body because of the low concentration of secretory granules in the proximal part of the process. In other instances, cytoplasmic fragments, even under the electron microscope, appear to be disconnected from the surface; serial sections are required to establish whether these are in fact free in the lumen.

The whole question of secretion into the ventricle is difficult to assess, for there is a surprising lack of data on the content of neurohypophysial hormones in the cerebrospinal fluid (*6*), considering the numerous proposals of secretion into the ventricle, either from "dendrites" (*1*, *2*, *5*) or from axon endings in the neurohypophysis (*7*).

Dierickx's suggestion that the dendrites serve as osmoreceptors has some cogency, and, as he pointed out, this function could coexist with secretion into the ventricle (although this is unlikely if secretion is of the apocrine type). Unfortunately, the small amount of evidence is inferential. It is known that osmoreceptors exist somewhere within the vertebrate body, and perfusion experiments indicate location in the brain itself (*8*). Because hypothalamic neurosecretory cells respond to osmotic manipulation by changes in content of hormone and of stainable secretory material (*9*), the "dendrites" are considered the most likely candidates as such receptors.

Presence of a cilium at the tip of the process is certainly compatible with a sensory function, as many known receptors are associated with a ciliary structure (*4*). However, survey of the literature shows that no specific modification is invariable in sensory cilia: they may or may not have two central fibrils; they may or may not have accessory centrioles; they may or may not show structural modifications of the distal portion of the cilium (for example, *4*, *10*). In addition, cells not known to be sensory may have cilia lacking central fibrils and also may have accessory centrioles (*11*). Thus, observed morphologic features of neurosecretory "dendrites" leave their possible sensory function unresolved.

It is conceivable that the preoptic neurosecretory cells are derived from ependyma (*12*) and that therefore the "dendrites" merely remain attached to ependymal cells when the neurosecretory cell bodies proper sink into the underlying brain tissue. Further studies



Figs. 1–4. Fig. 1. Cross section of preoptic nucleus in hypothalamus of *Hyla regilla*. Several processes from neurosecretory cell bodies extend toward lumen of third ventricle. Material fixed in Bouin's fluid and stained with paraldehyde fuchsin (× 350). Inset on left: Higher magnification of single neuron showing "dendrite" projecting into lumen, and axon leaving other pole of cell (× 1050). Fig. 2. Electron micrograph of neurosecretory cell with its process (*PR*) extending into third ventricle (above). "Dendrite" cytoplasm does not differ from that of cell body. Desmosomes (arrows) are located between process and ependymal cells (*E*); *N*, nucleus of neurosecretory cell. Fixed in phosphate-buffered osmium tetroxide, embedded in Maraglas, stained with uranyl acetate and lead citrate (× 9500). Fig. 3. Neurosecretory process contains elementary neurosecretory granules (*NG*), mitochondria (*M*), Golgi membranes (*G*), and endoplasmic reticulum (*ER*). Smooth surface of process contrasts with microvilli-studded surface of ependymal cells (*E*). Desmosomes at arrows; *C*, centriole; *SR*, striated rootlets (× 14,700). Fig. 4. Portion of neurosecretory cell process at lumen of ventricle (above), showing cilium (*CI*) leaving cell from indentation of plasma membrane. Accessory centriole (*AC*) and two branches of striated rootlet (*SR*) are indicated. *G*, Golgi membranes (× 19,200).

of the ultrastructure of embryonic brains should yield information on this point, but even in the adult some of the neurosecretory neurons are literally within the ependymal layer. The differences between ependymal cells and neurosecretory processes in regard to abundance of ribosomes and density of mitochondria, as shown in Figs. 2 and 3, may result from secondary differentiation. Structure of the cilia provides arguments both for and against an ependymal origin. Accessory centrioles have sometimes been observed close to cilia in ependymal cells as well as in "dendrites." However, although ependymal cells in this region seem to be less heavily ciliated than in other nearby areas, they do appear to have several cilia each, whereas the "dendrites" have not been seen to have more than a single cilium.

An ependymal origin of neurosecretory neurons would exclude neither a secretory nor a sensory function. For example, it has been suggested that such well-known receptors as the retinal rods derive from ependyma rather than from neuroblasts (*13*). Special ependymal areas such as the subcommissural organ show considerable secretory activity (*14*).

My observations, although preliminary, indicate that morphologic studies alone will not reveal the function of neurosecretory "dendrites." Experimental evidence combined with electron microscopy will be required to determine whether sensory [osmo-, photo-, chemo- (*7*)] reception or secretion, or both, take place at the ventricular surface. The "dendrites" appear to be equipped for both functions.

CAROLYN G. SMOLLER
Department of Zoology and
Cancer Research Genetics Laboratory,
University of California,
Berkeley

References and Notes

1. W. Hild, *Z. Anat. Entwicklungsgeschichte* **115**, 459 (1951); W. D. Roth, *Anat. Record* **124**, 437 (1956); R. Diepen, in *Handbuch der mikroskopischen Anatomie des Menschen*, W. H. W. von Möllendoerff, Ed. (Springer, Berlin, 1962), vol. 4, pt. 7.
2. L. D. Wilson, J. A. Weinberg, H. A. Bern, *J. Comp. Neurol.* **107**, 253 (1957).
3. K. Dierickx, *Arch. Intern. Pharmacodyn.* **140**, 708 (1962); ———, F. Vandesande, D. Roggen, *Z. Zellforsch. Mikroskop. Anat.* **62**, 98 (1964).
4. M. A. Sleigh, *The Biology of Cilia and Flagella* (Pergamon Press, New York, 1962), pp. 11–76.
5. M. Okada, T. Ban, T. Kurotsu, *Med. J. Osaka Univ.* **6**, 359 (1955).
6. H. Heller, in *Neurosecretion*, H. Heller and R. B. Clark, Eds. (Academic Press, London, 1962), p. xv.
7. D. Bodian, *Bull. Johns Hopkins Hosp.* **89**, 354 (1951); R. S. Nishioka, H. A. Bern, L. R. Mewaldt, *Gen. Comp. Endocrinol.* **4**, 304 (1964).
8. E. B. Verney, *Proc. Roy. Soc. London Ser. B*, **135**, 25 (1947); I. Leusen and E. Lacroix, *Endocrinology* **68**, 719 (1961).
9. W. Hild and G. Zetler, *Arch. Ges. Physiol.* **257**, 169 (1953).
10. E. de Robertis, *J. Biophys. Biochem. Cytol.* **2**, 319 (1956); A. J. de Lorenzo, *ibid.* **3**, 839 (1957); A. Flock, *J. Cell Biol.* **22**, 413 (1964).
11. B. G. Barnes, *J. Ultrastruct. Res.* **5**, 453 (1961); H. H. Spoendlin, *Z. Zellforsch. Mikroskop. Anat.* **62**, 701 (1964).
12. Y. Sano and M. Kawamoto, *Z. Zellforsch. Mikroskop. Anat.* **51**, 56 (1959); R. Olsson, *Intern. Congr. Zool., 16th, Washington, D.C., 1963*, abstracts, vol. 3, p. 38.
13. P. Glees and S. LeVay, *J. Hirnforsch.* **6**, 355 (1964).
14. A. Oksche, *Z. Zellforsch. Mikroskop. Anat.* **54**, 549 (1961).

15. This work was supported by an NSF predoctoral fellowship and NSF grant GB 2484. I am indebted to Prof. H. A. Bern for advice and encouragement, to A. Blaker for the photomicrograph, and to J. Soubier for other photographic aid.

14 December 1964

Species and Geographic Differences in the Transferrin Polymorphism of Macaques

Abstract. *Eleven molecular forms and 34 phenotypes of transferrin have been detected in 372 serums from six macaque species. The tendency to polymorphism varies from species to species and from one local population to another. The most extensive polymorphism was observed in* Macaca mulatta, *which showed at least ten transferrins and 24 phenotypes. Transferrins present in stump-tail macaques* (M. speciosa) *from Thailand were also found in crab-eating macaques* (M. irus) *from Thailand, but not in crab-eating macaques from the Philippines. The results suggest that macaque species are semispecies rather than complete species.*

The question of whether the "species" of macaques cross-breed with each other remains open (*1*, *2*). Our analysis of similarities and differences in transferrins between populations of macaques suggests a resolution of this question. Transferrin is the serum protein which picks up iron from the iron-storage protein ferritin, transports the iron throughout the body, and delivers it to tissue sites where hemoglobin and the different iron-containing enzymes are synthesized. Since distinguishable genetic variants of transferrin exist within and between various mammalian populations (*3*), knowledge of these transferrins can be applied to questions of the genetic origins of natural populations. Rhesus monkeys and certain other groups of macaques show an extensive polymorphism of transferrin (*4*, *5*). We have already described nine molecular forms of transferrin and 19 transferrin phenotypes among 199 serums from four species of macaques. Subsequent work (*6*) on serums from the Wisconsin colony of rhesus monkeys furnished genetic evidence that a series of codominant alleles controlled the observed transferrin variations. Our analysis of 173 more macaque serums revealed two additional molecular forms of transferrin and 15 additional phenotypes. Altogether, 11 molecular forms and at least 34 phenotypes have been observed by starch-gel electrophoresis in 372 serums from six species. Not only are there sharp differences among macaque species in the distribution of transferrin phenotypes, but also marked geographic differences within a macaque species in the degree of the transferrin polymorphism and in the frequencies of the particular transferrin alleles.

The principal method employed was starch-gel electrophoresis (*7*) in the borate–tris buffer system (*8*), gels 14 cm wide, 16 cm long, and 1.4 cm deep being used. In this method up to 15 samples inserted into a starch gel on small squares of filter paper can be run side by side and compared. The transferrins of macaque serums, unlike those of human and chimpanzee serums, stand out after electrophoresis in an area of gel devoid of other serum proteins. Thus typing of macaque transferrins can readily be done simply by staining the starch gels with amido black.

The photograph of a starch gel in Fig. 1*A* shows the mobility differences among the 11 macaque transferrins, and the developed (*9*) autoradiograph in Fig. 1*B* demonstrates iron binding in the transferrins (*10*). Going from slowest to fastest electrophoretic mobility, the 11 macaque transferrins are

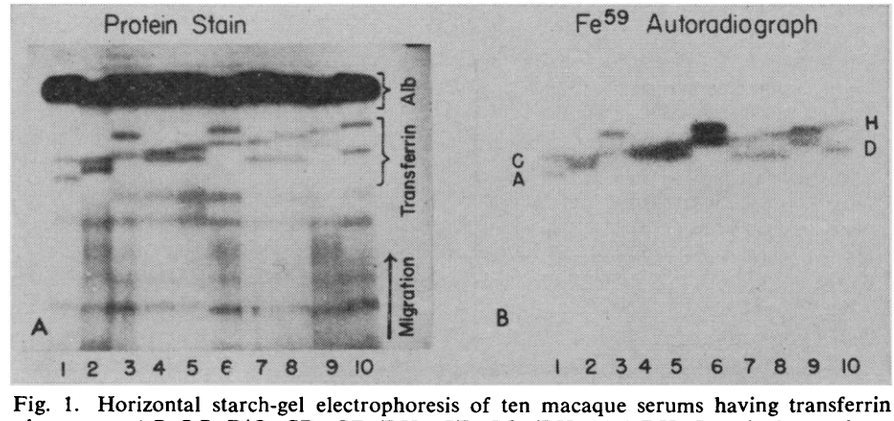


Fig. 1. Horizontal starch-gel electrophoresis of ten macaque serums having transferrin phenotypes AC, BC, D'G, CD, CE, F'H', CF, CG, F'H', and DH. Sample 2 was from a crab-eating macaque; samples 6 and 9 were from stump-tail macaques; and all other samples were from rhesus monkeys. Fe^{59} was added prior to electrophoresis according to Giblett *et al.* (*9*). The electrophoresis, however, was by the horizontal method at 6 cm for 4 hours. The gel half shown on the left (*A*) was stained with amido black. The other half was applied to x-ray film to make the autoradiograph shown on the right (*B*). (A transferrin such as F' in sample 9 might not be typed accurately from the kind of results shown here, since in this experiment sample 9 was not placed adjacent to a serum containing known E, F', or F transferrin. In the original typing suspected F's were run several times between known E and known F and several times alongside of known F' to confirm their classification. By similar procedures all the transferrins in samples 1 to 10 had previously been typed.)