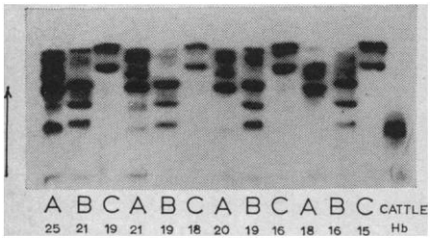

Fig. 1. Starch-gel electrophoresis of hemolymphs from individual third-instar larvae of three *Chironomus* species. (A) *C. tentans* (American). (B) *C. thummi*. (C) *C. atrella*. Numbers designate length in millimeters.

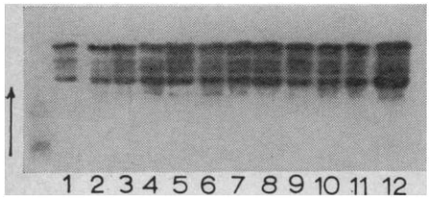

Fig. 2. Electrophoretic pattern from third-instar larvae of *C. tentans* (German), comparable in size and degree of pigmentation. 1–3, 21 mm; 4–6, 22 mm; 7–9, 23 mm; 10–12, 24 mm.

accumulation which may be comparable in basis to the fetal-adult transition in mammals. The entire pattern fades and disappears during the late prepupal stage (fourth instar) when the hemoglobins are apparently degraded.

The consistency of pattern within species, when individuals identical in size and pigmentation are compared (Fig. 2), apparently eliminates genetic heterogeneity as a primary factor in the multiplicity of hemoglobins. Although there are occasional variations in pattern which may be the result of allele substitution, it is clear that in general there is a standard pattern which probably reflects a standard genotype.

By the same token, heterozygosity is not a factor. The only conceivable way of obtaining a standard pattern with heterozygosity would be the establishment of a rigid system of balanced lethals. In view of the evidence (see *12*) that *Chironomus* populations contain extensive chromosomal polymorphism, but without the prevalence of any single heterozygous type, this is very unlikely.

The possibility that multiple hemoglobins could be generated by a single-locus system like that which controls haptoglobin structure in humans also appears slender. In the case of the haptoglobins, it appears that at least one allele of a single-locus system specifies a haptoglobin that is capable of several degrees of polymerization, each polymer having its own distinctive mobility in electrophoresis (*13*). In the case of *Chironomus*, however, no variation from the dimeric form or a molecular weight of 31,400 has been reported during a long period of study.

Thus it appears that the multiplicity of hemoglobins among these various species of *Chironomus* must be due to the independent specification of globin chains at several loci. Although such situations are now widely known among the more extensively studied mammals, and their genetic and evolutionary implications have been reviewed thoroughly (*14*), the concurrent production of eight or more molecular species in *Chironomus* certainly represents one of the most striking instances of control by what we assume to have been independently evolving "repeats" or duplications of an ancestral globin factor. The lack of conservatism in this case may derive from the limited involvement of the *Chironomus* hemoglobin in actual oxygen transport in any but the most adverse conditions (*15*).

There are aspects of the integration of gene products in *Chironomus* which are still difficult to understand. Studies in progress on the hybridization of European and American races of *Chironomus tentans* (which may be seen from Figs. 1 and 2 to have quite different electrophoretic patterns) have shown that starch-gel patterns among the F_2 are quite variable, as if factors heterozygous in the hybrids were segregating. On the other hand, the number of separable bands in the F_1 is not increased as markedly as one might expect from extensive heterozygosity. It may ultimately be found that these racial hemoglobin differences are largely quantitative rather than qualitative, as with certain of the human conditions (*16*).

PETER E. THOMPSON
DARREL S. ENGLISH
Department of Genetics,
Iowa State University, Ames

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7 January 1966

Electron Microscopy: Tight Junctions in Synapses of the Chick Ciliary Ganglion

Abstract. *Examination of the synaptic membranes in calyciform endings of the chick ciliary ganglion has shown tight junctions selectively located on axon hillocks of postsynaptic neurons. Observations of similar membrane fusion in other junctions that involve electrotonic transmission suggest the possibility of identifying electrotonic junctions by electron microscopy.*

Examination of synaptic junctions with the electron microscope has revealed fundamental new evidence linking structural characteristics to electrotonic, as contrasted to chemical, synaptic transmission. A number of junctions are now known to be electrotonic,

and high-resolution electron microscopy of these regions has demonstrated a unique fusion of the synaptolemma (synaptic interface): for example, the giant motor synapse in crayfish (*1*), mormyrid spinal electromotor neurons (*2*), and Mauthner cell synapses in goldfish (*3*). Fusion of unit membranes also occurs in vertebrate smooth muscle (*4*) and vertebrate cardiac muscle (*5*). It now seems possible to identify electrotonic junctions by electron microscopy.

The ciliary ganglion of the chick is particularly interesting because the oculomotor nerve endings seem to mediate both electrical and chemical transmission (*6*, *7*). However, examination of these synaptic junctions have not yet revealed structural specialization of the synaptolemma (*8*, *9*). The findings reported here are of interest in that they show that "tight junctions" (fusion of the synaptolemma) do occur. Furthermore, these membrane specializations occur only at the axon hillock of the postsynaptic ganglion cell.

Ciliary ganglia of newly hatched chicks and 1-month-old chickens were fixed with buffered 1.5 percent glutaraldehyde, treated with osmium tetroxide (1 percent), embedded in Araldite or Shell Epon 812, and stained with lead citrate. Synaptic junctions were characterized according to types, that is, by the endings, whether calyciform or basket; by the location on the postsynaptic surface, by dimensions of synaptic cleft, and by type of synaptic vesicles.

The large nerve cells in the ciliary ganglion are structurally unique in that they are myelinated and have synapses (*8*, *9*). There are at least two other kinds of myelinated neurons, the cells in the cochlear and vestibular ganglia; but unlike the ciliary ganglion they do not have synaptic endings. In the ciliary ganglia the myelin covers the entire perikaryon and the synapses. Only the axon hillock (region where the axon originates) is devoid of myelin. The afferent fibers which synapse upon the postsynaptic cell are likewise myelinated but lose their myelin as they divide and form the large calyciform terminal (*8*). Thus the myelin seems to part in order to permit entry of the afferent fiber and the exit of the ganglion cell axon. The calyx itself is structurally interesting, since it covers a large surface of the postsynaptic cell (as much as 65 percent). Our attention was directed to the synaptolemma of the calyx because this ending is specifically involved in both electrotonic and chemical transmission (*6*, *7*). Examination of 1000 ganglion cells with calyciform synapses revealed no significant fine structure differences with respect to the size of endings, of synaptic vesicles, or of dimensions of the synaptic cleft that might account for the two modes of transmission. However, in 22 ganglion cells the plane of the ultrathin sections passed through both the calyx and the axon hillock of the postsynaptic cell. High resolution of these regions revealed "tight junctions" between the terminal calyx and the postsynaptic axon hillock (Fig. 1). The ganglion cell (*GC*) is located in the upper right corner and the axon hillock region (*AH*) extends down the lower right-hand side. A portion of the presynaptic calyx (*CE*) is seen at the left. The region of cell membrane contiguity (synaptolemma)

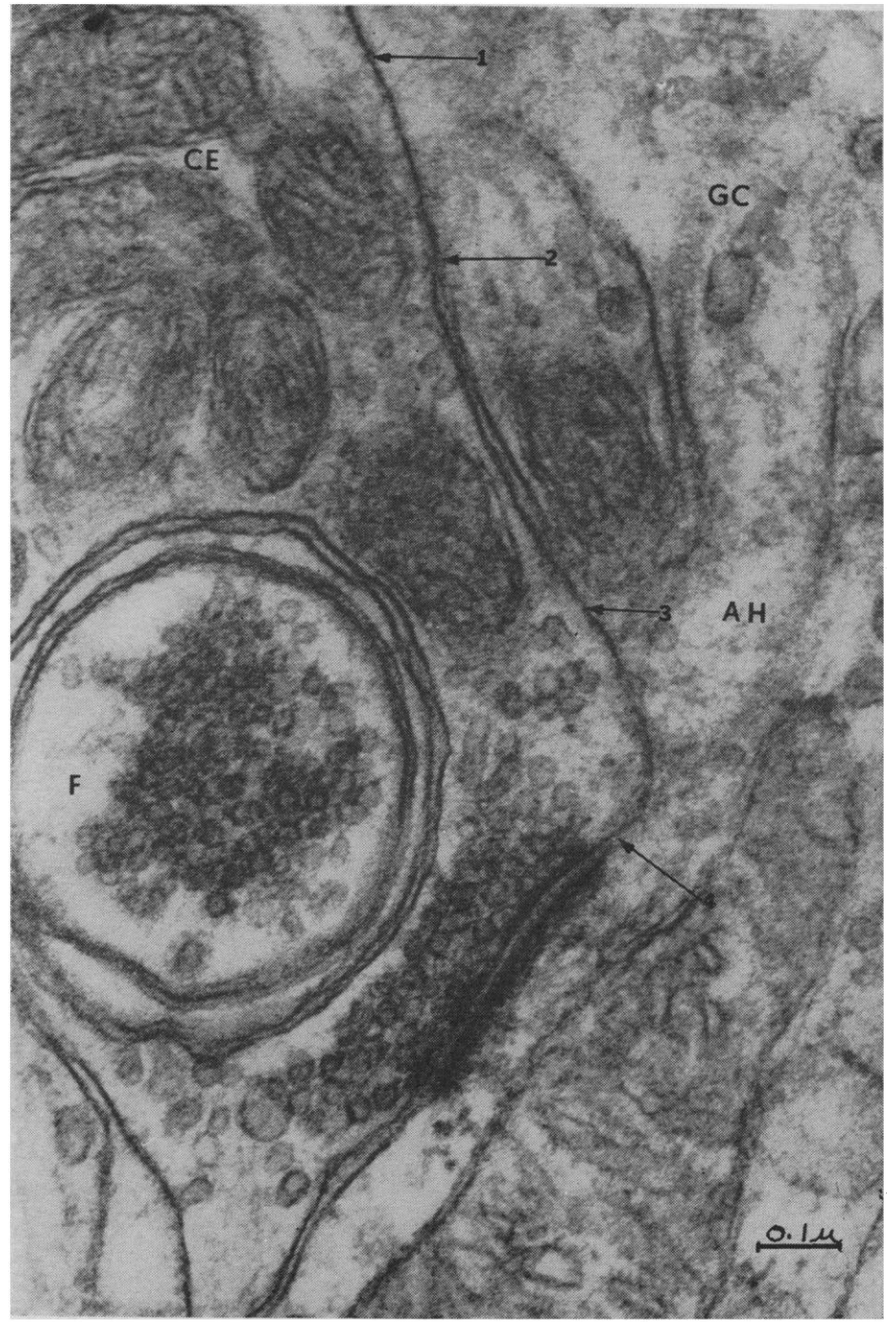

Fig. 1. High magnification of a region through the axon hillock: *GC*, ganglion cell; *AH*, axon hillock; *CE*, calyciform ending; *F*, nerve fiber containing synaptic vesicles. Arrows 1, 2, 3, and 4 indicate pre- and postsynaptic membrane specializations (× 135,000).

is identified by the arrows. Below arrow 4 one can see the synaptic complex usually associated with chemical synapses; that is, the presynaptic cluster of synaptic agranular vesicles measuring 250 to 400 Å, synaptic cleft 200 to 300 Å wide, and a postsynaptic density or web. Following the synaptic membranes, one can see in the regions between arrows 3 and 4 and arrows 1 and 2 that the synaptic cleft has been obliterated and the synaptolemma has an appearance similar to tight junctions seen elsewhere. Between arrows 2 and 3 the intercellular space is again of conventional magnitude. Note the paucity of synaptic vesicles in the regions of the tight junctions. A further synaptic relationship is represented by the nerve fiber (F) which appears to end (judged by the presence of synaptic vesicles) upon the calyx. Perhaps this may be a mechanism for modulating the function of the calyx itself.

Of the 22 ganglia observed, I distinguished membrane tight junctions in 15, and in each calyx an F-type fiber was also observed, in agreement with the electrophysiological incidence of electrical transmission (*7*). It does not, however, preclude the possibility that the "spots" of pre- and postsynaptic membrane fusion were missed when not observed. In any event, the observations suggest that membrane fusions do occur in calyciform synapses and in the present study have been found only in the axon hillock region of the postsynaptic cell. Thus the synaptic membrane exhibits morphological characteristics of both electrical and chemical synapses.

A. J. DARIN DE LORENZO
Johns Hopkins University, School of Medicine, Johns Hopkins Hospital, Baltimore, Maryland 21205

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Increased Incidence of Lymphoma after Injections of Mice with Cells Differing at Weak Histocompatibility Loci

Abstract. *Newborns of two congenic strains of mice, in which the incidence of leukemia is low, differing only at the weak* H-1 *locus, were injected at birth with adult spleen cells of the parent and of the congenic strain. A marked increase in the incidence of lymphoma ensued in those mice of both strains injected with cells from the other strain. The experiment lends some support to the idea that transplantation immunologic mechanisms may play a role in the genesis of lymphomas.*

Injection of newborn C3H mice with adult spleen cells from a congenic C3H.K partner strain is associated with an increased absolute incidence of lymphoma and with a marked shift in the incidence of lymphoma toward a younger age (*1*). These strains differ from each other in histocompatibility only at the weak *H-1* locus. The C3H mice are $H\text{-}1^a$, and the C3H.K are $H\text{-}1^b$. As controls in this prior experiment C3H newborns were injected with adult C3H spleen cells. Thus test animals were those in which adult $H\text{-}1^b$ cells were injected into neonatal $H\text{-}1^a$, and controls were those in which adult $H\text{-}1^a$ cells were injected into neonatal $H\text{-}1^a$. This earlier work left almost entirely open the question whether induction of lymphoma was occasioned by oncogenic viruses, transplantation immune mechanisms, or other factors, although some inferential evidence was presented favoring the second possibility. We have now injected newborns of both strains with spleen cells of the strain differing only at the *H-1* locus and with cells of their parent strain as controls, with a resulting great increase in the incidence of lymphoma in each group receiving the differing cells.

The plan of the experiment is indicated in Table 1. For purposes of exposition, the two congenic strains are referred to by their *H-1* histocompatibility genes as $H\text{-}1^a$ or $H\text{-}1^b$, respectively. The two strains were obtained initially from Snell at the Jackson Memorial Laboratory, and have been further inbred here for about 5 years. The mice are considered to be essentially genetically identical at all histocompatibility loci except the *H-1* locus. About 2.5×10^6 spleen cells from 2- to 3-month-old apparently normal male mice were injected into recipients less than 24 hours old by the intracardiac route. A small population of noninjected mice was also retained. The females were discarded at time of weaning because of the high incidence and early deaths from mammary carcinoma in female C3H mice. Five or six mice were housed in a cage. They were fed commercial mouse chow, and all were allowed to live out their natural life spans. The total weights of body and spleens were determined at death, and a gross necropsy was performed on all animals. Microscopic examination of liver, spleen, and other organs, as seemed appropriate, was accomplished for all animals except when advanced autolysis interfered.

The incidence of lymphoma was markedly increased by injection of the newborns with spleen cells differing only at the *H-1* locus (Table 1). This increase occurred in both directions (that is, when $H\text{-}1^b$ cells were injected into $H\text{-}1^a$ or when $H\text{-}1^a$ cells were injected into $H\text{-}1^b$) as compared to the controls who received cells from the parent strain. Also, injection of $H\text{-}1^b$ cells into $H\text{-}1^b$ newborns yielded no increase in lymphoma incidence as compared with noninjected $H\text{-}1^b$ controls. There was, however, an intermediate augmentation of lymphoma incidence in the $H\text{-}1^a$ set treated similarly. In separate experiments, it was found that injection of viable liver or kidney cells in either differing strain did not increase the lymphoma incidence over that in noninjected mice.

The lymphomas in all mice were characterized by marked splenomegaly, liver infiltration by lymphoid cells, and frequent lymphadenopathy. The thymus was rarely involved. The histologic pattern for animals that received the differing spleen cells has been described (*1*), and the same pattern was seen in all the present combinations. The lymphomas resembled that designated as lymphocytic neoplasm (*2*).

In C3H mice in general, and in both of the present strains as well, reticuloendothelial neoplasm is quite uncommon. Indeed, C3H strains are commonly used in studying virus-induced lymphomas or leukemias in mice precisely