aration of porcine TCT conjugated to albumin reacts specifically with the extracted polypeptide in vitro and with the hormone as it is situated in the thyroid epithelial cell in tissue section. The specificity of the latter reaction is shown by (i) localization of fluorescent staining in the cytoplasm of the thyroid cells, (ii) inhibition of this fluorescent staining by prior reaction of the thyroid tissue with nonconjugated antiserum to TCT, and (iii) failure of fluorescent antiglobulin to TCT to produce specific staining after it has been incubated with a preparation of homogeneous TCT. The presence of fluorescence in all discernible thyroid epithelial cells suggests that, in all cells, TCT is synthesized or stored or both. The observed variation in the intensity of fluorescence among the cells may indicate variation in the quantity of TCT synthesized or stored, but more sensitive techniques will be necessary before this finding can be evaluated. The granular appearance of the fluorescence in many cells suggests that TCT is localized in some intracellular organelle. Improved microscopic resolution of fluorescent tissue sections should clarify this observation.

Thus, TCT is present in all porcine thyroid epithelial cells. It is not present in the follicular colloid but must be synthesized or stored in the same cells that elaborate thyroglobulin.

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Multiplicity of Hemoglobins in the Genus Chironomus (Tendipes)

Abstract. *The number of hemoglobins found in individual mature larvae of several* Chironomus *species is six to nine. The consistency of this number and of the electrophoretic mobilities of these separable forms seems to indicate the synchronous production of polypeptide chains by several genetic loci.*

Multiple forms of hemoglobin within species may result from allelic variations, or from the independent action of several gene loci. The total number of variant hemoglobins from allele substitutions is limited only by the rate and retention of mutations, but in single individuals having a two-locus system of genetic control the number of forms can be no greater than four (with heterozygosity at both loci). This degree of multiplicity has been reported for the major adult hemoglobin of humans (*1*). Further multiplicity may result from the expression of alternative loci in the formation of polypeptide chains. In many mammals, for example, a fetal hemoglobin is replaced near birth by adult hemoglobin through the substitution of one globin chain (β) for another (γ) (*2*). It has also been shown that in a number of mammals, more than one hemoglobin is produced during adult life (*3*). In most such cases, the number of hitherto separable forms is two or three, with at least one "minor" component. Among the lower vetebrates, the lamprey *Petromyzon planeri* has two forms each of both adult and fetal hemoglobins (*4*).

With regard to invertebrates, a recent report has shown that the hemoglobins from a single insect species may take several molecular forms. From homogenates of numbers of larvae of *Chironomus thummi*, Braunitzer and Braun (*5*) obtained four major hemoglobins, of which three have been characterized extensively. In agreement with earlier studies (*6*), all were found to be dimers of around 31,400 molecular weight, having two hemes and two dissimilar polypeptide chains. While these chains are only 124 to 127 residues in length and differ markedly from mammalian chains in their amino acid composition (*7*), Braunitzer has suggested (*5, 8*) a common ancestry, after which various genetic changes, including deletions, have wrought extensive alterations.

As far as the multiplicity of hemoglobins within the homogenate of *Chironomus thummi* is concerned, it was not determined whether this diversity was due to genetic heterozygosity, heterogeneity among individuals, transitions among developmental stages, or the synchronous expression of several loci. We now present evidence, from starch-gel electrophoresis of the hemoglobins from individual larvae of several *Chironomus* species (*9*), that several loci operate synchronously in the formation of hemoglobin polypeptides near the end of larval life.

Hemolymph samples from large last-instar larvae were run on gels similar to that described by Kristjansson (*10*), with Poulik's discontinuous system of buffers (*11*). Gels were stained with benzidine, although staining with amido black usually gave similar patterns. It was found in every species examined that individual larvae contain more than one major hemoglobin. Most species, in fact, have shown consistent patterns with six or more separable forms. *Chironomus atrella* appeared to be exceptional in having only two.

In agreement with the findings of Braunitzer and Braun, *Chironomus thummi* appears to have four major hemoglobins, although there are indications of at least four minor components as well. As seen in Fig. 1, both this and other species characteristically show differences correlating with size and degree of pigmentation. Although in general the intensification of bands parallels the superficial reddening of larvae, especially during the third instar, certain early bands commonly make their appearance well ahead of the remainder. In *Chironomus thummi*, for example, the third major band from the line of origin is an early one. In this sense, there is a sequential

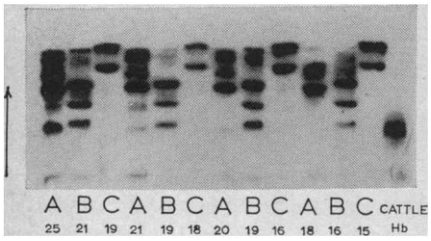

Fig. 1. Starch-gel electrophoresis of hemolymphs from individual third-instar larvae of three *Chironomus* species. (A) *C. tentans* (American). (B) *C. thummi*. (C) *C. atrella*. Numbers designate length in millimeters.

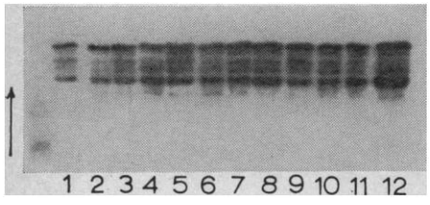

Fig. 2. Electrophoretic pattern from third-instar larvae of *C. tentans* (German), comparable in size and degree of pigmentation. 1–3, 21 mm; 4–6, 22 mm; 7–9, 23 mm; 10–12, 24 mm.

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*).

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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,