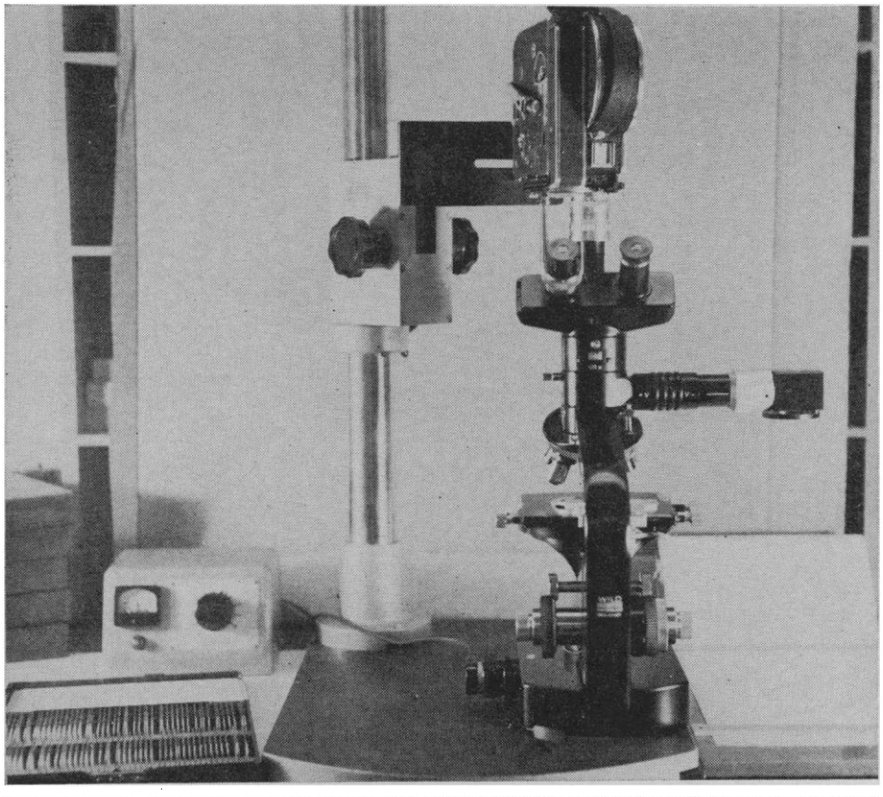
itself is done with the condenser diaphragm fully open.

The optical shuttle is faster and simpler than the drawing technique; alignment can be made more precisely, especially at low magnification. With the wider field of view so made available, a larger area of the microscopic section can be analyzed with a single film. The method has already been valuable to us and may well be useful in other fields.



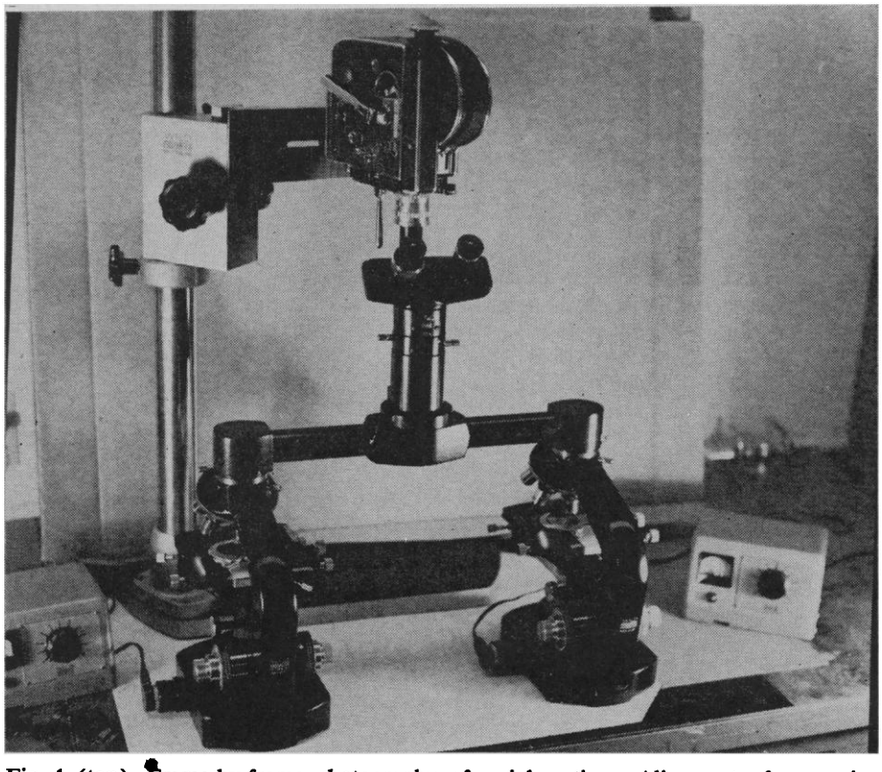


Fig. 1 (top). Frame-by-frame photography of serial sections. Alignment of successive sections is accomplished with the aid of the camera lucida. Fig. 2 (bottom). The optical shuttle assembly.

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2. The method works properly only if comfortable viewing, drawing, and photographing devices can be used simultaneously—only with equipment made by Wild (Heerbrugg, Switzerland) as far as we know, because the camera lucida can be incorporated in the light path. The assembly in Fig. 1 consists of a Wild M20 microscope base with rotating mechanical stage (rather important for ease of alignment of each section), drawing tube, camera tube H, and binocular. Viewing and focusing are done with the binocular; the camera is a Bolex H16REX. These are all standard components.
3. A 12-minute motion picture, representing early investigation of the course of vascular bundles in the stem of the palm *Rhapis*, was presented in the symposium "Long distance transport phenomena," 10th Intern. Botanical Congress, Edinburgh, Scotland, August 1964.
4. The assembly in Fig. 2 consists of two Wild M20 microscope bases with rotating mechanical stages, an inverted discussion tube, camera tube H, and binocular. Separate transformers enable the operator to equalize the light output of the two microscopes. The mounting of the discussion tube in an inverted position is accomplished by two double-male joints and one double-female. We thank the Wild Company which manufactured these pieces especially for us and supplied them free of charge. The special mounting of the discussion tube lengthens the light path above the objectives, and as a result the objectives must be lowered somewhat beyond their normal position. This necessitates use of extension rings between the nose pieces and the 3 × Plane Fluotars. No problem arises, however, with the other low-powered objectives.
5. Part of this work supported by NSF grant GB 2991 to P.B.T.

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Thyrocalcitonin: Cytological Localization by Immunofluorescence

Abstract. *Thyrocalcitonin was detected in the cytoplasm of all epithelial cells of the thyroid gland of the pig, by means of antibody fluorescence. It was present in those cells which normally elaborate thyroglobulin but was not present in the follicular colloid.*

In 1961 Copp *et al.* (*1*) presented indirect evidence that a hypocalcemic principle played a role in calcium homeostasis in the dog. The results of their experiments favored the parathyroid gland as the source of this material, and it was given the name

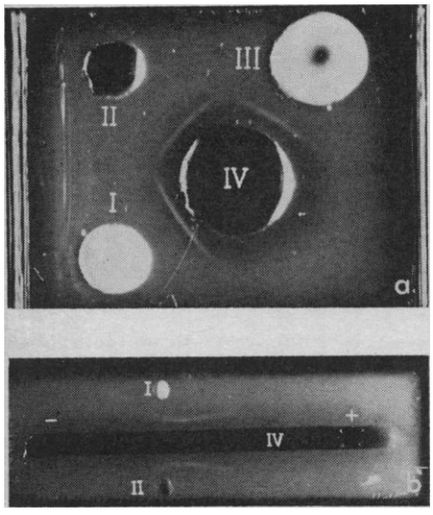

Fig. 1. (*a*) Microimmunodiffusion pattern of reaction between antiserum to TCT and three preparations of TCT, showing pattern of fusion. (*b*) Microimmunoelectrophoresis pattern of reaction between antiserum to TCT and two preparations of TCT, showing single corresponding arcs in anodic position (barbital buffer, *p*H 8.8). (I, crude porcine TCT; II, homogeneous porcine TCT; III, homogenate of porcine thyroid tissue; IV, rabbit antiserum to porcine TCT.)

calcitonin. Shortly thereafter, Hirsch *et al.* (*2*) showed that the thyroid gland of the rat contained a potent hypocalcemic principle which they named thyrocalcitonin (TCT). Experiments similar to those of Copp *et al.* (*1*, *3*) were done in the goat by Foster *et al.* (*4*), and the anatomical separation of the thyroid and parathyroid glands in this species enabled these workers to show that the thyroid gland was the source of the hypocalcemic principle. Subsequently the principle was extracted from the thyroid glands of several species (*2*, *4*, *5*) and partially purified by several methods (*5*). Most recently, a biologically potent, homogeneous, single-chain polypeptide with a molecular weight of 8700 was isolated by Tenenhouse *et al.* (*6*). In addition to meeting recognized criteria of homogeneity, this peptide migrates as a single band on polyacrylamide-disc electrophoresis.

The actual cellular site of TCT synthesis has not yet been defined; but Foster *et al.* (*7*) postulated that the mitochondrion-rich parafollicular cells of the thyroid gland were responsible for TCT secretion.

Our studies were designed to determine the cellular site of TCT synthesis or storage, or both. The fluorescent-antibody technique was used. The TCT was prepared as described (*6*), and an antiserum was developed against a conjugate of TCT and albumin (*8*). This antiserum was immunologically specific (Fig. 1, *a* and *b*) as judged by the results of microimmunodiffusion (*9*) and microimmunoelectrophoresis (*10*) against homogeneous and crude TCT preparations (*6*) and homogenates of porcine thyroid tissue. The globulin fraction of this antiserum was isolated (*11*) and conjugated with lissamine rhodamine B 200 (*12*) to form the fluorescent antibody globulin to TCT. Porcine thyroid, parathyroid, salivary gland, adrenal cortex, and lymph node tissues obtained immediately after the animal's death were prepared and reacted with the conjugated fluorescent antibody globulin to TCT as described (*13*) except that the staining period was 1 hour at room temperature. The tissue sections were examined with a Zeiss fluorescence microscope unit with OSRAM HBO-200 light source, BG12 exciter filter, and OG53 barrier filter, and photographed with High Speed Improved Ektachrome film at 60- to 90-second exposure.

Specific antibody fluorescence was present in the cytoplasm of all discernible thyroid epithelial cells; including those clearly follicular in position as well as those which, in tissue section, appeared to be interfollicular (Fig. 2, *a* and *b*). There was moderate variation in the intensity of fluorescence among the cells. Although the fluorescence appeared to be uniformly distributed throughout the cytoplasm in a few cells, there was a patchy or granular fluorescent distribution in most of the cells. The fluorescence appeared most intense in the perinuclear areas of those cells which were cuboidal. There was no fluorescence in the nuclei of the cells, in the connective tissue, or in the follicular colloid. Specific staining was greatly inhibited by treating the tissue sections with nonconjugated antiserum to TCT before applying fluorescent antibody globulin to TCT (Fig. 2*c*); and it was absent when the fluorescent antibody globulin was absorbed with homogeneous TCT before it was applied to the thyroid tissue (Fig. 2*d*). Specific fluorescence was not observed when the fluorescent antibody globulin was applied to parathyroid, salivary gland, adrenal cortex, and lymph node tissue sections.

These studies show that an antiserum developed against a homogeneous prep-

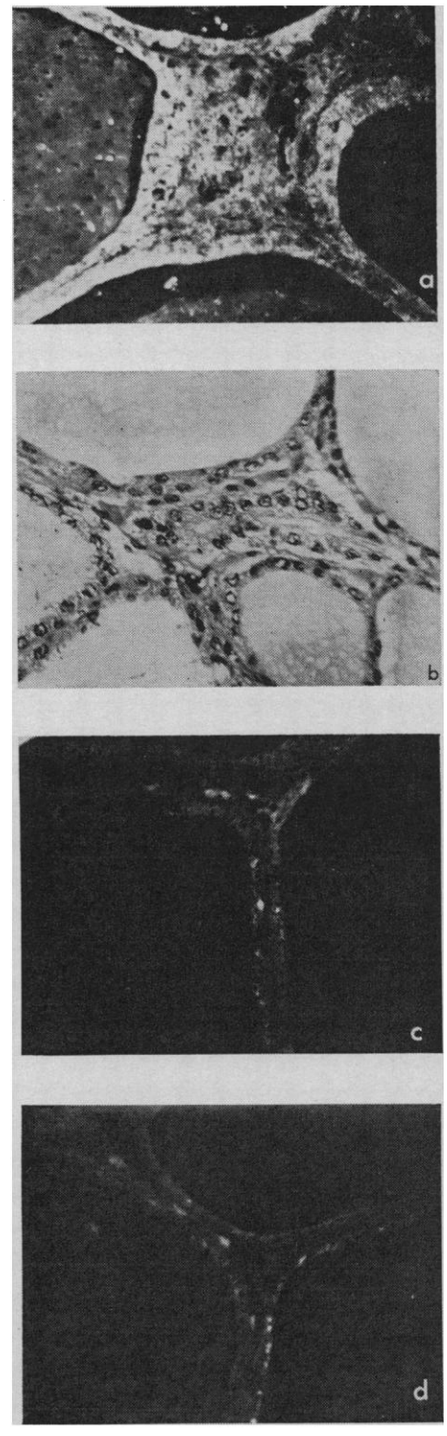

Fig. 2. (*a*) Porcine thyroid gland: fluorescent antibody globulin to TCT stain, showing fluorescence confined to cytoplasm of thyroid epithelial cells (× 400). (*b*) Porcine thyroid gland (section cut from same tissue block as *a*); hematoxylin and eosin stain (× 400). (*c*) Porcine thyroid gland, reacted with nonconjugated antiserum to TCT before application of fluorescent antibody globulin to TCT, showing greatly inhibited fluorescence (× 400). (*d*) Porcine thyroid gland, reacted with fluorescent antiglobulin to TCT which had already been absorbed with homogeneous TCT, showing no fluorescence (× 400).

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