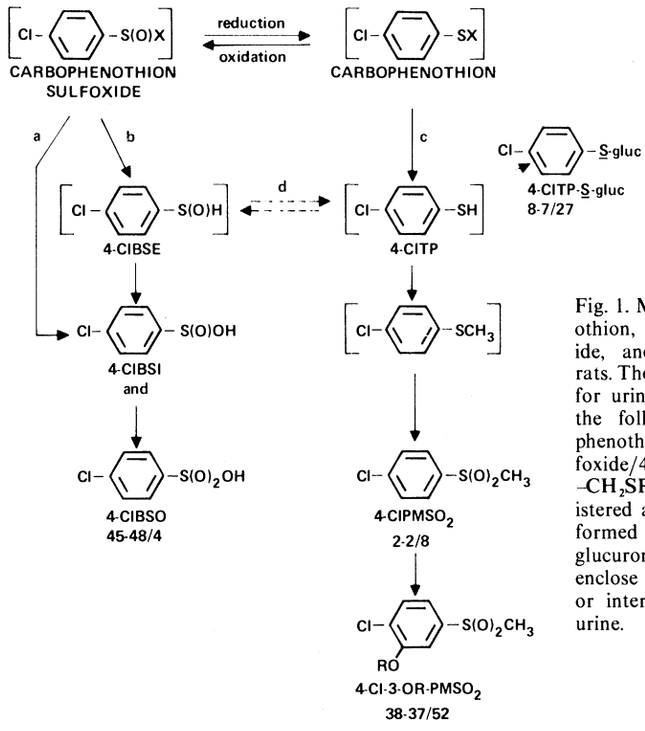

Fig. 1. Metabolism of carbophenothion, carbophenothion sulfoxide, and 4-chlorothiophenol in rats. The numbers are percentages for urinary metabolites given in the following sequence: carbophenothion–carbophenothion sulfoxide/4-chlorothiophenol. X = $-CH_2SP(S)(OC_2H_5)_2$ as administered and $-CH_2SP(O)(OC_2H_5)_2$ formed metabolically; R = H, glucuronide, or sulfate. Brackets enclose administered compound or intermediate not detected in urine.

chlorothiophenyl-*S*-glucosiduronic acid (4-ClTP-*S*-gluc), appear in nearly equal amounts from either precursor. If the 4-ClTP arises from cleavage of carbophenothion (reaction c in Fig. 1), approximately half of the carbophenothion sulfoxide may undergo in vivo reduction to carbophenothion. Although there are possible alternative pathways which might account for the observed metabolites (reaction b, hydrolysis, and then reaction d, disproportionation; or direct disproportionation of carbophenothion sulfoxide to yield carbophenothion), the in vitro enzyme studies support the hypothesis that the enzyme-mediated reduction of the sulfoxide to carbophenothion is prerequisite to the liberation of 4-ClTP.

Detection of carbophenothion in extracts of rat liver 10, 20, and 30 minutes after intraperitoneal administration of carbophenothion sulfoxide (30 mg/kg) provides unequivocal evidence for this conversion in vivo. This evidence was obtained by homogenization of the liver in acetone, extraction of the resultant precipitate with methanol, concentration of the combined acetone-methanol extracts, and ethyl acetate extraction of the aqueous residue. Carbophenothion in the ethyl acetate extracts was confirmed by cochromatography in two different two-dimensional systems with the standard having (i) 2,2,4-trimethylpentane + *p*-dioxane (2 : 1) in the first direction and 2,2,4-trimethylpentane + benzene (3 : 2) in the second direction of development; and (ii) 2,2,4-trimethylpentane + chloroform (3 : 2) in the first direction and hexane + benzene (3 : 2) in the second direction of development. The maximum concentration of carbophenothion (2.2 percent of the total liver ^{14}C; 0.1 percent of the administered ^{14}C) was recovered in the liver extract of a rat killed 30 minutes after treatment. In this extract the ratio of carbophenothion sulfoxide to carbophenothion was 2.7.

Reduction of the sulfoxide moiety of other organophosphorus insecticides in nonmammalian systems has been reported for fensulfothion sulfoxide in the bean plant (*4*) and for disulfoton sulfoxide (*5*) and phorate sulfoxide (*6*) in the soil. Other studies with compounds that do not contain phosphorus have established that sulfoxide reduction occurs in vivo in several mammalian systems, including man (*7*).

Carbophenothion is one of several commercial organophosphorus insecticides with thioether substituents that undergo oxidation to the corresponding sulfoxide and sulfone derivatives. Since these sulfoxidized derivatives are more potent cholinesterase inhibitors than the parent sulfides, the reduction, described here for carbophenothion sulfoxide, may represent a general detoxification pathway in rats and possibly other animals, plants, and environmental systems.

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Cytoplasmic Microtubule Organizing Centers Isolated from *Polytomella agilis*

Abstract. *Basal body rootlets in* Polytomella *function as organizing centers for cytoplasmic microtubules in vivo. A method is described to isolate intact basal body–rootlet complexes. The integrity of the isolated complexes is confirmed by electron microscopy, and the rootlets are shown to be competent as initiation sites for the in vitro polymerization of brain microtubule protein.*

In many developing systems microtubules appear to assemble, at specific times and in characteristic patterns, at preformed intracellular sites. Basal bodies are examples of highly structured assembly sites for the axonemal microtubules of cilia or flagella. Both the pattern and directionality of microtubule assembly in the axoneme appear to result, at least in part, from distal polymerization onto an existing pattern of tubules in the basal body (*1*). In addition, the in vitro assembly of chick brain tubulin onto isolated basal bodies has been shown to occur principally by distal addition of subunits (*2*). The spatial and temporal regulation of cytoplasmic (and spindle) microtubule assembly has also been postulated to reside in intracellular "structures" which function as initial sites of microtubule polymerization (*3*). In

many cases these structures appear as aggregates of an amorphous electron-opaque material with no obvious organization. Although this material serves as the site of initial assembly, thus conferring some directionality, the microtubules may be secondarily ordered into a characteristic pattern by bridge formation between them (*4*). Alternatively, it has been suggested that the intermicrotubule bridges function in stabilizing a microtubule pattern imposed by the organizing center (*5*). Several studies (*5, 6*) have indicated that the amorphous material may be prepatterned on some other cellular structure [a structured organizing center, or in Tucker's terminology (*5*) a template] and this arrangement confers control over the directionality and patterning of microtubule arrays.

In the quadriflagellate alga *Polytomella* the amorphous material mentioned above is associated with basal body rootlets, and large numbers of cytoplasmic microtubules terminate on the rootlets (Fig. 1, a and b). These structured organizing centers have been shown in vivo to initiate microtubule polymerization in a characteristic pattern (*7*). In this report a method is described for isolating intact basal body–rootlet complexes which are competent to initiate the assembly of brain microtubule protein in vitro.

Cultures are grown at 25°C, in the dark, in a complex medium containing 0.1 percent tryptone, 0.2 percent sodium acetate, and 0.2 percent yeast extract, and the cells are harvested in the exponential phase at a density of 2×10^6 to 5×10^6 ml^{-1}.

The cells are washed in fresh medium and deflagellated by vortical motion for 1 minute in a fluted tube (*8*). The bodies are separated from the flagella by low-speed centrifugation and resuspended in a microtubule-stabilizing medium of 50 percent (by volume) glycerol, 10 percent (by volume) dimethylsulfoxide, 5 m*M* $MgCl_2$, and 5 m*M* NaH_2PO_4, *p*H 7.0 (*9*). The suspension is made 0.1 percent (by volume) in Triton X-100 and the bodies are broken in a fluted tube by gentle vortical agitation. This treatment releases intact basal body–rootlet complexes, which are separated from cell debris by low-speed centrifugation (*10*). The complexes are pelleted at 12,000*g* for 10 minutes, and the pellet (P-1) may be used in assembly experiments.

For further purification, P-1 is resuspended in 5 percent sucrose containing 0.2 percent Triton X-100 and the resulting suspension is agitated in a fluted tube. The rootlets of the complexes shorten and may lose some of the associated amorphous material during this treatment. Low-speed centrifugation removes any remaining large debris. The complexes are pelleted at 10,000*g* for 10 minutes, resuspended in 5 percent sucrose, layered on a discontinuous 5, 25, 40, and 60 percent sucrose gradient, and centrifuged at 3,000*g* (Beckman L2-65B ultracentrifuge, SW 56 rotor) for 1 hour. Complexes are collected from the 40 percent layer and pelleted at 10,000*g* for 10 minutes, and the pellet (P-2), which contains intact complexes (Fig. 2), is resuspended in polymerization medium for assembly experiments.

For thin-section electron microscopy basal body–rootlet complexes were pelleted, fixed for 2 hours at 20°C in 2 percent glutaraldehyde (0.1*M* sodium phosphate, *p*H 7.4), and postfixed for 2 hours at 4°C in 1 percent osmium tetroxide (in the same buffer). Intact cells were fixed for 1½ hours at 20°C in 0.5 percent glutaraldehyde (0.05*M* sodium phosphate, *p*H 7.4) and postfixed for 1½ hours at 4°C in 1 percent osmium tetroxide (in the same buffer). For the details of the preparation techniques for electron microscopy see (*7*). For negative stain preparations, one drop of sample was deposited on a grid coated with Formvar and carbon for 1 minute, then stained for 1 minute with one drop of 1 percent aqueous phosphotungstic acid (containing 0.4 percent sucrose at *p*H 7.0) followed by two washes with drops of 0.1*M* sodium phosphate (*p*H 7.0). Excess solution was removed at each step by absorption with filter paper at the edge of the grid.

The four basal bodies in *Polytomella* are arranged in two nearly opposite pairs (Fig. 1a). Two structurally distinct rootlets, one consisting of four microtubules in a three-over-one pattern and the other of a striated fiber subtended by two microtubules, terminate between adjacent basal bodies [for a detailed description see (*7*)]. Large numbers of cytoplasmic microtubules terminate on both types of rootlets (Fig. 1, a and b). The isolated complexes appear structurally intact (Fig. 1c). The four basal bodies are in the same arrangement observed in situ, and all eight rootlets are retained. The cytoplasmic microtubules which normally terminate on the rootlets have disappeared during preparation (Figs. 1c and 2). The isolated complexes have two types of potential sites for brain tubulin assembly: (i) existing microtubule structures such as the basal bodies and rootlet microtubules and (ii) the amorphous material associated with the rootlets (Fig. 1c).

Tubulin is obtained from brains of 16- to 21-day-old chick embryos essentially according to the method of Weisenberg (*11*)

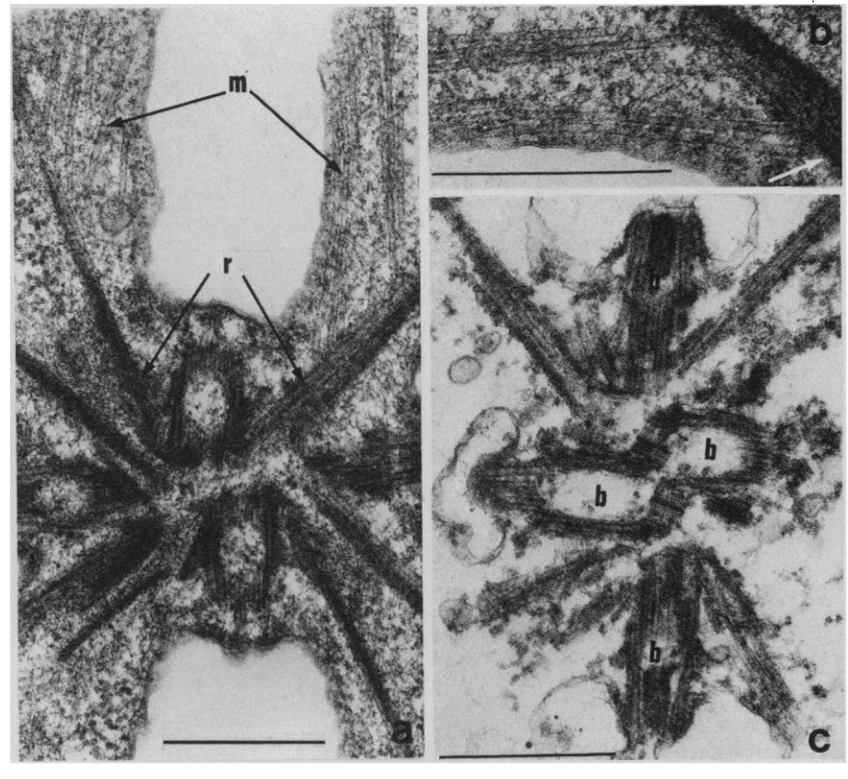


Fig. 1. Thin sections of the basal body–rootlet complex. (a) Cross section through the basal body region of a cell showing the four basal bodies. Rootlets (*r*) terminate between basal bodies and cytoplasmic microtubules (*m*) extend from the rootlets. (b) Higher-magnification view of the attachment of cytoplasmic microtubules (arrow) onto a rootlet. (c) Section of an isolated basal body–rootlet complex showing all four basal bodies (*b*) and attached rootlets. Scale bar, 0.5 μm.

as modified by Snell *et al.* (*2*). Brains are homogenized at 4°C in a polymerization medium containing 100 m*M* PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], 1 m*M* EGTA [ethylenebis-(oxyethylenenitrilo)tetraacetate], and 2.5 m*M* GTP (guanosine triphosphate), *p*H 6.9. The homogenate is centrifuged at 10,000*g* for 10 minutes and then at 39,000*g* for 90 minutes to yield a crude tubulin preparation (S-1). For some experiments tubulin is further purified (an S-2 preparation) by a cycle of temperature-dependent polymerization and depolymerization. The protein concentration (*12*) averages 15 mg/ml in S-1 and 6 mg/ml in S-2, and the tubulin concentration in both is estimated to be approximately 3.8 mg/ml (*2*).

Microtubule assembly onto the basal body–rootlet complexes is carried out by mixing the complexes in polymerization medium with varying amounts of S-1 or S-2 and incubating at 37°C. The reaction is stopped at intervals by adding equal volumes of 2 percent buffered glutaraldehyde, and the polymerization is monitored by negative staining. In a series of experiments, a constant volume of complexes was mixed with tubulin in ratios of 1 : 3, 1 : 1, and 3 : 1 (volume of complexes to volume of S-1 or S-2). At these concentrations of tubulin, initial microtubule assembly onto the complexes is observed after incubation times of 0.5, 1.0, and 5.0 minutes. The number and length of microtubules assembled depend on both the time of incubation and the concentration of tubulin. For example, at a high tubulin concentration a few short microtubules are observed after 1 minute (Fig. 3a), and after 5 minutes of incubation the number and length of microtubules has increased (Fig. 3b). At short incubation times or low tubulin concentrations most microtubules are associated with the complexes. After longer incubation (up to 30 minutes) increasing numbers of free microtubules are observed. These results indicate that the complexes are preferred sites for initiating microtubule assembly.

It is possible that in these preparations only free microtubules are formed, and that during the negative staining these microtubules codeposit with the complexes and therefore appear to be attached. To test this possibility, glutaraldehyde-fixed preparations of complexes and separately polymerized microtubules were mixed and then negatively stained. Some of the free microtubules do deposit with the complexes but they definitely do not appear to be attached. In addition, the complexes from a preparation such as that seen in Fig. 3b can be separated from any free microtubules by low-speed centifugation and assembled microtubules are still observed attached to the complexes (not shown). The preferential assembly onto the complexes has also been examined under conditions in which no free microtubules are formed. Borisy and Olmsted (*13*) have shown that centrifuging a porcine brain extract at high speed removes a particulate fraction that is required for tubule assembly. When complexes are incubated with porcine brain high-speed extracts (230,000*g* for 90 minutes, Beckman L2-65B ultracentrifuge, SW 56 rotor) numerous microtubules assemble onto the complexes (Fig. 4, a and b) and no free microtubules are observed.

There are two basically different types of assembly onto the basal body–rootlet complex. One type is the assembly of tubulin subunits onto the ends of existing microtubules of the basal bodies (not shown) and of the rootlets (Fig. 4b). This resembles the in vitro assembly of subunits onto axonemes (*14*) or isolated basal bodies (*2*). The second type is assembly onto the amorphous material associated with the rootlets (Fig. 4a). The assembly onto existing microtubules (seeded assembly) occurs at lower tubulin concentrations and is more rapid (Fig. 3a) than assembly onto the amorphous material. In vivo, cytoplasmic microtubules initially assemble onto rootlets in an organized array (Fig. 1, a and b), which suggests that the

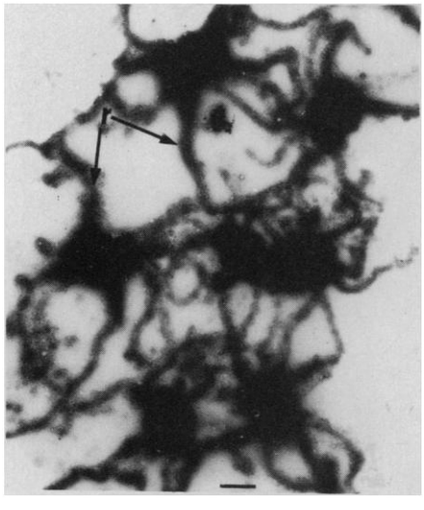
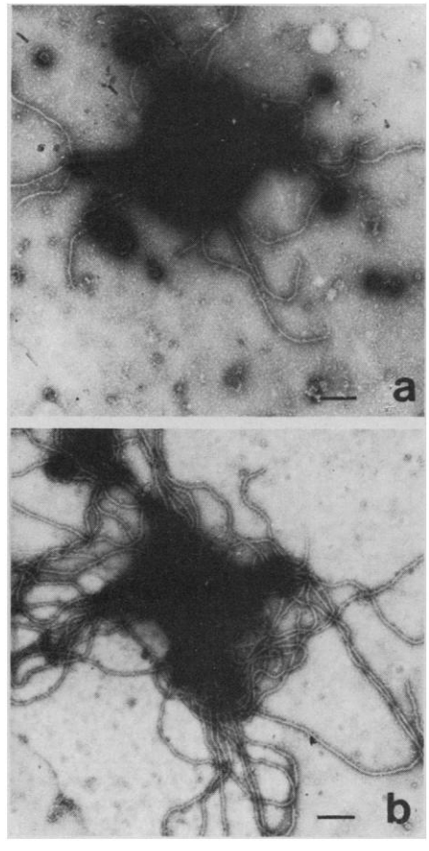


Fig. 2 (above). Isolated basal body–rootlet complexes negatively stained with 1 percent phosphotungstic acid. (Arrows) Rootlets (*r*) extend from the densely stained basal bodies. Scale bar, 1 μm. Fig. 3 (right). Negatively stained basal body–rootlet complexes following incubation at 37°C with chick brain tubulin (one volume of complexes to three volumes of S-2). (a) A few short microtubules have assembled onto the complex after 1.0 minute of incubation. (b) After 5.0 minutes of incubation an increased number of longer microtubules have assembled. Scale bar, 0.5 μm.



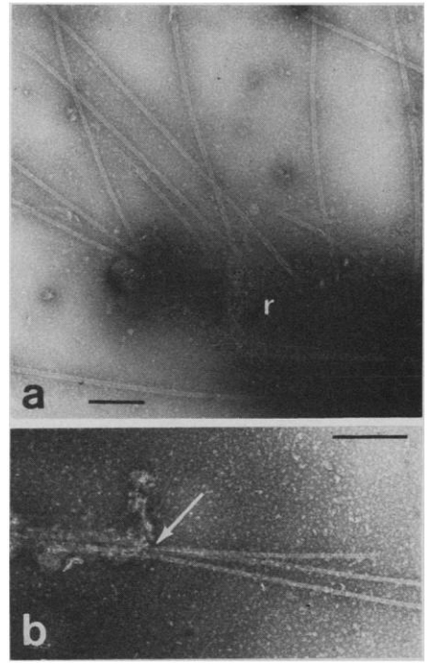


Fig. 4. Details of the two types of assembly onto rootlets incubated at 37°C with high-speed extract of porcine brain. (a) Microtubules assembled onto the amorphous material associated with the rootlet (*r*). (b) Microtubules assembled onto the ends (arrow) of rootlet tubules. Scale bar, 0.5 μm.

amorphous material may include assembly sites that are prepatterned on the rootlets. Such an arrangement would permit the control of patterning and directionality of microtubule assembly. At least some of this capacity is retained in vitro (Fig. 4a). The rootlets do not simply serve as attachment sites for assembled brain microtubules but, in fact, appear to promote tubule polymerization under conditions where little or no free microtubule assembly occurs.

The composition of the amorphous material associated with presumptive microtubule organizing centers in vivo (*3*) is not known. Weisenberg and Rosenfeld (*15*) have reported the isolation of an apparently unstructured aggregate from surf clam eggs which will serve as a microtubule organizing center for the in vitro assembly of microtubules into asters and spindles. It is possible that this aggregate and the amorphous material of other organizing centers may be localized high concentrations of tubulin subunits that can be assembled into microtubules. It seems equally possible that such structures contain other components which influence or are necessary for initiating tubule polymerization. The in vitro polymerization of brain tubulin (6*S*) subunits has been shown to require the presence of disks or rings (*16*) which consist of tubulin and additional protein components (*17*). It is not known if such ring structures are related to assembly of microtubules at specific sites in vivo. The procedures described in this report should permit an analysis of the role of rings and accessory proteins in an in vitro assembly system which, at least in some respects, resembles the in vivo situation.

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The Neural Crest and the Origin of the Insulin-Producing and Other Gastrointestinal Hormone-Producing Cells

Abstract. *It has been proposed that the endocrine cells of the digestive tract derive from the neuroectoderm (neural crest). To test this hypothesis we removed the entire ectoderm, the precursor of the neural crest, of embryonic rats prior to the formation of the neural crest and cultured the mesoendoderm for 11 days. In every case where a pancreas developed, insulin was detected or B cells were observed. Thus, a neural crest origin for these cells is eliminated.*

The neural crest is a temporary embryonic structure which appears along the neural fold concomitant with its closure to form the neural tube. Soon after the formation of the neural crest, the cells migrate to different parts of the embryo to give rise to a variety of otherwise unrelated differentiated cells. Some of these, like the chromaffin adrenomedullary cells and cells of the sympathetic ganglia, synthesize and accumulate high levels of amines as specific products. This peculiarity gives them the property of fluorescing under ultraviolet illumination after fixation with formaldehyde vapor (*1*). A similar reaction also occurs in some cells which can concentrate injected 3,4-dihydroxyphenylalanine (*2*). The cells which exhibit this fluorescence reaction have been termed by Pearse (*3*) APUD (amine precursor uptake and decarboxylation) cells.

Cells that have the histochemical specific characteristics mentioned above have been found in various embryonic and adult tissues, in particular, islet cells and gut epithelium (*4*). With the implicit assumptions that all cells which show the fluorescent histochemical reaction are derived from the neural crest and that the fluorescent cells in the gut are polypeptide hormone (for example, gastrin, glucagon, secretin) producing cells, Pearse proposed that all the polypeptide hormone–producing cells of the digestive tract originate from the neural crest and not from the endodermal cells as was previously thought (*5*). This intriguing theory, which would developmentally link both nervous and hormonal control to a common ectodermal origin, has been tested in only one case: Polak *et al.* (*6*) reported that the parafollicular or C cells which produce calcitonin, a polypeptidic hormone involved in calcium metabolism, are derived from the neural crest. By grafting the neural tube and crest of embryonic quail to chicken embryos, it is possible to follow the migration and fate of neural crest cells of the donor into the recipient tissues, because the nuclei of quail and chicken cells can be distinguished morphologically (*7*). This heterograft technique was combined with immunofluorescent labeling of calcitonin, and quail cells containing calcitonin were detected in the chicken thyroid. Thus, it was concluded that calcitonin-producing cells originate from the neural crest (*6*).

We have tested whether the pancreatic B cells (a typical APUD cell according to Pearse) originate from neural crest cells by culturing the developing embryo in vitro after removal of the ectodermal precursor of the neural crest. If the endocrine B cells were in fact derived from the neural crest, then they should be absent from the pancreas of these ectoderm-deprived embryos. For these experiments, whole 9-day rat embryos prior to four somites (Fig. 1) were removed aseptically from the mothers. At this time the neural groove has not closed to become the neural tube, and the neural crest has not yet formed. After resection of the extraembryonic area, the embryos were incubated in 3 percent trypsin-pancreatin for about 10 to 15 minutes, after which the whole ectodermal sheet was peeled off to completely remove the source of the neural crest cells. Figure 2 demonstrates the effectiveness of this procedure. A cross section of the mesoendoderm (not shown) confirms the absence of the ectodermal component. After several washes in serum to remove the trypsin, the mesoendoderm (Fig. 3) was cultured with Eagle's basal medium in which the amino acids were increased six-