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Is Abnormal Limb Bud Morphology in the Mutant *Talpid*^{2} Chick Embryo a Result of Altered Intercellular Adhesion? Studies Employing Cell Sorting and Fragment Fusion

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ABSTRACT In the past, studies of avian limb morphogenesis emphasized epithelial-mesenchymal tissue interactions and problems of determination of limb symmetry. In contrast, a recent hypothesis, based on studies of the aggregation rates of dissociated cells and on computer modeling, proposes that the paddle shaped polydactylous limb of the *talpid*^{3} mutant is the result of increased intercellular adhesion of limb bud mesoderm cells during limb development. The notion that differences in intercellular adhesion may have profound effects on limb morphogenesis has not been critically explored previously.

The present experimental approach includes studies of cell sorting in aggregates containing both *talpid*^{2} and wild-type cells. In this system, adhesive differences should result in cell sorting. Instead, cell sorting did not occur, indicating by this test at least, that limb bud mesoderm cells of *talpid*^{2} and wild-type embryos are equally adhesive. This conclusion finds support from studies involving tissue spreading in fused fragments of *talpid*^{2} and wild-type limb bud mesenchyme tissue and in studies of kinetics of aggregation of dissociated cells.

The developing avian limb is one of the most extensively studied morphogenetic systems in higher organisms (see for review: Zwilling, '61; Amprino, '65; Goetinck, '66; Saunders and Gasseling, '68). The use of genetic mutants in the study of avian limb development has provided important information for the study of normal limb development (see for review: Abbott, '67). A particularly interesting example of this is the work of Ede and his co-workers with the *talpid*^{3} mutant, which has paddle-shaped polydactylous limbs. The initial defect produced in the limb by the *talpid*^{3} allele occurs in the mesoderm and apparently affects mesenchymal cell movement and coalescence (Ede and Kelly, '64a,b). Ede has proposed that the talpid locus has its effect primarily on the adhesive properties of limb bud mesenchymal cells, with the *talpid*^{3} allele resulting in limb mesodermal cells which are more strongly adhesive than corresponding cells of wild-type embryos (Ede and Agerbak, '68). This hypothesis is of considerable interest because until now, most of the interest in avian limb morphogenesis has centered about problems of morphogenetic cell death (Saunders, '66) and tissue-tissue interactions (i.e., the initial induction of apical ectodermal ridge formation by underlying presumptive limb mesoderm (Saunders, '48; Balinsky, '56, '57); stimulation of mitosis (Searls, '65) and outgrowth (Saunders, '48; Zwilling, '49, '55, 56a,b,c,d) of distal limb bud mesoderm by the apical ectodermal ridge; and the role of limb bud mesoderm in the determination of placement and maintenance of the apical ectodermal ridge (Saunders, '49; Zwilling, '56d, '64; Zilling and Hansborough, '56)). Ede has introduced the problem of adhesive interaction between limb bud mesodermal cells into the general problem of limb morphogenesis.

In experimental attempts to determine the validity of his hypothesis, Ede has demonstrated that trypsin-dissociated *talpid*^{3} limb bud mesodermal cells aggregate

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somewhat more rapidly than cells from wild type embryos. In addition to these possible differences in rates of aggregation of dissociated cells, the *talpid*^{3} aggregates examined after three days in culture appeared to be smoother and smaller than the aggregates of normal cells (Ede and Agerbak, '68). From this evidence it was concluded that the *talpid*^{3} cells are more adhesive and therefore less mobile than wild-type cells, and that these adhesive differences are the basic cause of the abnormal limb form in *talpid*^{3} embryos. This hypothesis has been supported by computer model studies which demonstrate that, at the very least, the model is a feasible one (Ede and Law, '69).

As has been pointed out repeatedly (Steinberg, '64, '70; Armstrong, '66; Phillips and Steinberg, '69), measurement of aggregation rates or aggregate sizes are inadequate as measures of strengths of cellular adhesiveness since these parameters are influenced by a variety of factors in addition to intercellular adhesive strengths. For example, kinetics of adhesion in stirred systems may depend on cell shape (Pethica, '61; Lesseps, '63), the rates at which areas of initial contact between cells can be increased (Garrod and Born, '71, cited in Wolpert, '71), and strength of the plasma membrane itself (Weiss, '61) in addition to the strengths of the bonds made between cells. With this in mind, it was deemed potentially useful to employ different techniques to examine relative strengths of cellular adhesiveness. For this purpose, use was made of the techniques of cell sorting in heterotypic cell aggregates (Moscona and Moscona, '52; Townes and Holtfreter, '55; Trinkhaus and Groves, '55; Moscona, '56; Steinberg, '70) and tissue spreading in heterotypic fused tissue fragments (Steinberg, '70).

It is well known that cells in a heterotypic aggregate (i.e., an aggregate containing cells of more than one cell type) will adhere to each other and, while in this array, will regroup or sort out in a characteristic and reproducible fashion (e.g., Townes and Holtfreter, '55; Moscona, '57; Steinberg, '63a; Burdick, '70; Armstrong, '70, '71). Cell sorting results in the establishment of homogeneous tissues, one of which partially or completely surrounds the other in mixed cell aggregates (Steinberg, '64, '70). In a given combination of tissues, which tissue is external and which is internal is usually quite reproducible. An identical arrangement (i.e., an internal tissue covered completely by an external tissue) is reached if intact fragments of the same tissues used in cell sorting are apposed *in vitro*. In this case, cells of the tissue that normally occupy the periphery of a sorted-out aggregate migrate over the surface of the piece of tissue normally found in the interior until a configuration is attained which is the same as that found following cell sorting (Steinberg, '70).

Although a variety of hypotheses have been proposed to account for cell sorting and the related phenomenon of tissue spreading in fused fragments, two have received the most attention. These are the differential adhesion hypothesis of Steinberg ('63a, '64, '70) and the specific adhesion hypothesis of Moscona ('60, '62, '65) and Roth ('68). Since both hypotheses agree that cell sorting in heterotypic cell aggregates results from adhesive differences between dissimilar cells, cells having markedly different adhesive properties should sort out from one another, regardless of which hypothesis is correct.

In the present examination of the hypothesis of Ede and Agerbak ('68) heterogenetic combinations of *talpid*^{2} and wild-type limb bud mesenchymal tissues are examined with respect to cell sorting and tissue spreading in fused fragments. If Ede and Agerbak are correct in their claim that the *talpid*^{3} limb bud mesoderm cells are more adhesive than mesoderm cells from the limb buds of wild-type chick embryos, one would expect that, in mixed aggregates or in fused fragments of these two tissues, cell rearrangement will occur until the *talpid*^{2} tissue lies internal to the wild-type tissue (fig. 1A). If the hypothesis is incorrect, and the *talpid*^{2} and wild-type cells are equally adhesive, one would expect the two cell types in a mixed aggregate to be randomly arranged (fig. 1B). If adhesive differences are negligible, one would expect *talpid*^{2} and wild-type tissues to form cohering hemispheres in fused fragment culture (Weston and Abercrombie, '66) (fig. 1B).

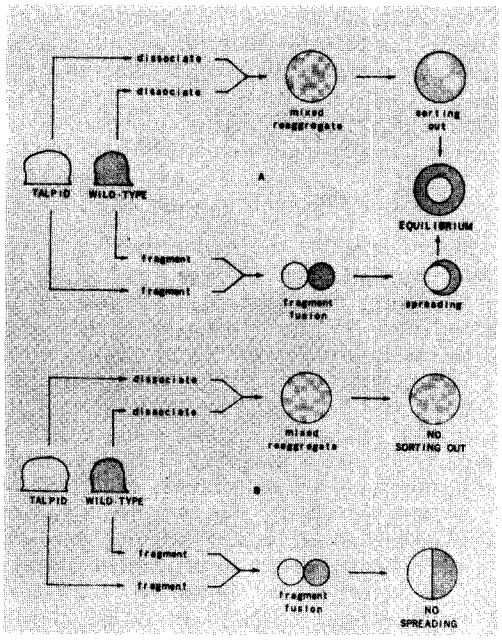


Fig. 1A If talpid limb bud mesoderm cells are more adhesive than wild-type limb bud mesoderm cells one would expect the equilibrium configuration of a mixed aggregate or two fused fragments to be one in which the talpid tissue is surrounded by wild-type tissue.

Fig. 1B If the talpid and wild-type limb bud mesoderm cells are equally adhesive, one would expect the equilibrium configuration of a mixed aggregate to be one in which sorting out has not occurred. One would also expect the equilibrium configuration of two fused fragments to be cohesion without spreading.

Talpid[2] embryos (Abbott et al., '59, '60) were used rather than *talpid*[3] (Ede and Kelly, '64a,b) because of their availability. Limb bud morphogenesis in the two *talpid* mutants is virtually identical since the major developmental differences between the two mutants occur in head formation (Ede and Kelly, '64a,b).

METHODS AND MATERIALS

Chicken stocks

The *talpid*[2] mutant used in these studies has been described by Abbott (Abbott et al., '59, '60). Homozygous *talpid*[2] embryos were obtained from matings of heterozygous *talpid*[2] stock, giving approximately one *talpid*[2]: three normal.[2] *Talpid*[2] eggs were routinely prewarmed overnight at room temperature and then incubated at 37°C for four days simultaneously with eggs of an incrossbred White Leghorn line which served as the normal or wild-type strain. Mutant and normal limb buds are easily distinguishable at four days of incubation (stage 22–23, Hamburger and Hamilton, '51).

Tissue preparation

Isolation of limb bud mesoderm and pigmented retina

Four-day limb buds and seven-day eyes were excised in Hanks Basal Salt Solution (BSS). The limb buds were soaked for seven minutes (37°C) in calcium-magnesium-free Hanks Basal Salt Solution with 2 mM disodium ethylenediaminetetraacetate (CMF-EDTA-BSS) containing 2.5% (w/v) trypsin [3] (pH 7.6), washed twice in BSS containing one drop/10 ml of 1 mg/ml deoxyribonuclease [4] stock solution (DNase) to remove extracellular material (Steinberg, '63b) and then incubated at room temperature for one hour before the epidermis was dissected free of the mesoderm.

The eyes, after removal, were incubated (37°C) for 15 minutes each, first in CMF-BSS, and then in 3% trypsin — 1% (w/v) pangestin [5] dissolved in CMF-BSS (pH 7.6). The eyes were then washed twice in Eagle Minimal Essential Medium [6] containing 10% horse serum,[6] 1% chick embryo extract 50,[7] 100 units/ml of penicillin G [8] and 100 μg/ml of streptomycin sulfate [8] made up in BSS (MEM-EE-HS). The back of the eye was then removed and the pigmented retina was peeled away from the neural retina (Trinkhaus, '63; Whittaker, '63; Armstrong, '70, '71).

Dissociation of limb bud mesoderm and pigmented retina cells

Dissociation was accomplished by an initial incubation (20 minutes for limb buds; 50 minutes for pigmented retina; 37°C) of the tissues in 15 ml screw cap

[2] The authors thank Dr. Ursala Abbott for supplying the *talpid*[2] eggs.
[3] Difco, 1:250.
[4] Worthington Biochemical Corporation.
[5] Difco, 1:75.
[6] GIBCO.
[7] Difco.
[8] Sigma.

tubes containing 0.1% trypsin (w/v) dissolved in CMF-EDTA-BSS (pH 7.6). The tissues were next washed four times, twice in BSS containing DNase, twice in MEM-EE-HS. Actual dissociation of the cells was carried out in MEM-EE-HS with a mechanical test tube stirrer.[9]

Cell concentrations were determined with a hemocytometer. Cell viability was ascertained by exclusion of nigrosin (Kaltenbach et al., '58). Tissue dissociated in the above manner regularly provided cell suspensions in which better than 95% of the cells were single and viable.

Preparation of limb bud mesoderm and heart ventricle tissue fragments

Small pieces of tissue dissected from seven-day heart ventricle and ectoderm-free, four-day limb bud mesoderm were washed twice in BSS and cultured overnight (37°C) in 25 ml Delong culture flasks [10] containing 3 ml of MEM-EE-HS on a rotary water bath shaker [11] at 120 rpm. The initially cuboidal fragments rounded up into spheres under these conditions.

Experimental procedures

Homotypic aggregation

Measured volumes of the initial *talpid*[2] or wild-type limb bud mesoderm suspensions were placed in 25 ml Delong culture flasks containing MEM-EE-HS, to a final concentration of 3×10^6 cells in 3 ml of culture medium. The flasks were incubated on a rotary water bath shaker at 70 rpm and 37°C. Photographs were taken of intact aggregates after 5, 24, 38, and 72 hours of incubation. Photographs were also taken of sectioned aggregates fixed after 72 hours of incubation.

Heterotypic aggregation

Measured volumes of ^{3}H-thymidine labeled wild-type limb bud mesoderm, unlabeled wild-type limb bud mesoderm, *talpid*[2] limb bud mesoderm, and pigmented retina cell suspensions were cultured in binary combinations for three days. The cultures were maintained in 25 ml Delong culture flasks on a rotary water bath shaker at 70 rpm and 37°C with a 1:1 ratio of combinants at a final cell concentration of 3×10^6 cells in 3 ml culture medium (MEM-EE-HS) (1.5×10^6 cells/3 ml/cell type). After three days in culture the aggregates were fixed.

Fragment fusion

Tissue spheres, formed either by overnight aggregation of dissociated cells or by rounding up of chopped fragments of tissue, were cultured in pairs in hanging drops on the undersurfaces of 33 mm plastic Petri dish lids. Under these circumstances, the two aggregates contained in each drop fall to the bottom of the hanging drop, touch, and establish adhesion with each other. A small amount of TWEEN 60 [12] was added to the culture medium (MEM-EE-HS) to reduce surface tension of the hanging drops to minimize disruption of cells at the liquid-air interface. Cultures were incubated in moist chambers (90 mm Petri dishes containing moist absorbant paper) for two to five hours at 37°C. After the fragments had established firm adhesion to one another, they were cultured for two days in MEM-EE-HS in 25 ml Delong culture flasks on a rotary water bath shaker (120 rpm, 37°C).

Autoradiography, histology and photography

Embryos were labeled with ^{3}H-methylthymidine [13] (S.A. 6 C/mM) by cutting small windows in the eggshell and dropping the solution directly onto the embryo with a syringe (Trinkhaus and Gross, '61; Weston, '67). The windows were then covered with cellophane tape. Embryos were inoculated with 10 μC ^{3}H-thymidine in 0.1 ml BSS applied 24 hours, and again 12 hours before dissection. Typically more than 95% of the cells were labeled, as determined by sectioning three-day reaggregates of labeled cells.

All tissue, with the exception of heart-containing aggregates, was fixed in Bouin's fixative, embedded in Paraplast [14]

[9] Vari-Whirl, Van Waters and Rogers, Inc.
[10] Bellco Glassware Co.
[11] Metabolyte Water Bath Shaker, New Brunswick Scientific Co.
[12] Atlas Powder Co.
[13] Calbiochem.
[14] Fisher Scientific.

(62°C), sectioned at 5 μ, and stained with Harris' Hematoxylin and Eosin Y. Aggregates containing heart were fixed in Gendre's fixative, embedded and sectioned as above, and stained with Periodic acid Schiff's and acid fast green. Under these conditions, heart tissue stains red due to large amounts of intracellular glycogen (Steinberg, '62).

Slides containing ^{3}H-labeled material were coated with NTB-2 Kodak Nuclear Track Emulsion (diluted 1:1 with water), dried and stored for two weeks at 4°C in light-tight boxes containing packets of Drierite [15] to absorb moisture, as outlined by Kopriwa and Leblond ('62). These slides were developed in Dektol and stained as above.

Photographs were taken on a Zeiss microscope with a green filter. Panatomic X film was used in all cases.

RESULTS

Cell sorting experiments

The adhesive behavior of *talpid*^{2} and wild-type limb bud mesoderm cells was compared both in cell sorting and fragment fusion experiments. In both cases *talpid*^{2} and wild-type cells were distinguished by ^{3}H-thymidine labeling of one cell type.

In the aggregation experiments, an equal number of two cell types were placed in each culture flask. These mixed suspensions of cells aggregated, and the aggregates were cultured for three days. The aggregates were then fixed, sectioned and studied for the presence or absence of cell sorting. Formation of two homogeneous tissues by cell sorting is taken as evidence for the existence of adhesive differences between two cell types. If similar cells are combined, cell sorting should not occur. As expected, ^{3}H-labeled wild-type limb bud mesoderm cells do not sort out from unlabeled wild-type limb bud mesoderm cells (fig. 2A). Exactly the same behavior is shown by combinations of ^{3}H-labeled wild-type limb bud mesoderm cells and unlabeled *talpid*^{2} limb bud mesoderm cells (fig. 2B). The two kinds of cells remain dispersed at random in the aggregates, rather than forming distinct homogeneous tissues. In combinations of truly heterotypic cells, cell sorting does indeed occur under the culture conditions used here. ^{3}H-labeled wild-type limb bud mesoderm cells and unlabeled pigmented retina cells sort out when placed together in culture, the mesoderm sorting internal to the retina (fig. 2C). Each of the combinations above was repeated ten times resulting in a total of several hundred aggregates.

Fragment fusion experiments

Fusions were made of tissue fragments. Intact tissue pieces (as aggregates of dissociated cells or as pieces of dissected tissue) were placed in contact in tissue culture and cultured for two days. Tissue combinations were then fixed, sectioned, and studied to ascertain whether cells of one tissue migrated over the surface of the second tissue to envelop it. Envelopment of one tissue by the other is taken as evidence for the existence of adhesive differences between the two cell types. If similar tissues are combined, envelopment should not occur. As expected, when ^{3}H-labeled wild-type limb bud mesoderm + unlabeled wild-type limb bud mesoderm combinations cohere, envelopment does not occur (fig. 3A). The same behavior is shown by fragment fusions of ^{3}H-labeled wild-type and unlabeled *talpid*^{2} limb bud mesoderm (fig. 3B). Cohesion between the two tissue fragments to form apposed hemispheres occurred, but envelopment did not occur. These results are consistent with the observations of heterogenetic aggregates containing *talpid*^{2} and ^{3}H-labeled wild-type limb bud mesoderm (fig. 2B). In fragment fusion combinations of truly heterotypic tissues, one tissue surrounds the other under the conditions used above. Heart envelopes wild-type limb bud mesoderm (fig. 3C), and aggregates of trypsin-dissociated pigmented retina cells envelope ^{3}H-labeled wild-type limb bud mesoderm fragments (fig. 3D). This later conformation is the same as that observed in limb bud mesoderm + pigmented retina cell sorting experiments (fig. 2C).

The appearance of slight amounts of silver grain over unlabeled tissue in fig-

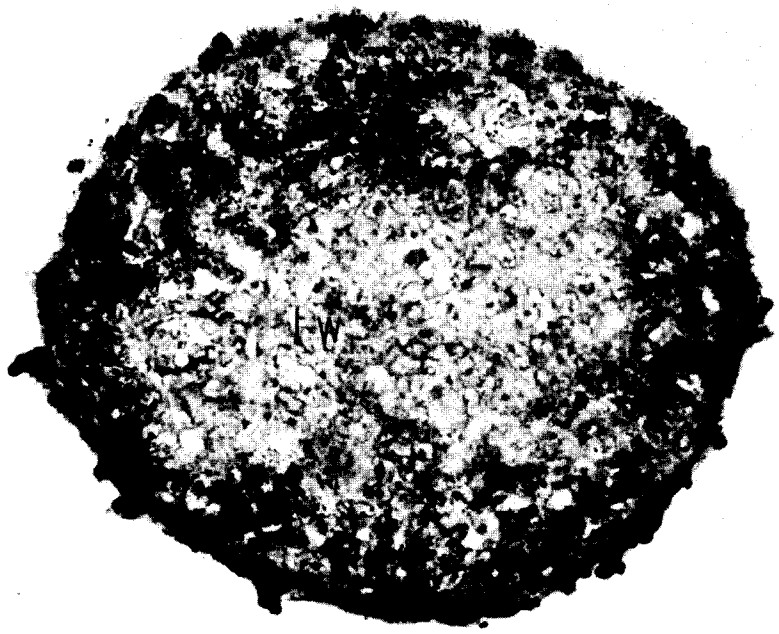
[15] W. A. Hammond Drierite Company.


A
B

ure 3A and B (w and t) has not been explained. The ^{3}H-labeled tissues were all excised in isotope free BSS, washed twice in BSS and then cultured overnight in MEM-EE-HS before they were united with unlabeled tissue. In spite of these precautions, slight amounts of silver grain over unlabeled tissue were always present. The possibility exists that unlabeled cells incorporate labeled precursors released from nuclei of labeled cells which have undergone autolysis in culture (see Saunders, '66). The fragment fusion combinations experiment was repeated eight times resulting in a total of several hundred individual combinants.

Aggregation of dissociated cells in homotypic culture

Inspection of photographs of intact aggregates taken over the three day culture period indicates that *talpid*^{2} and wild-type aggregates are of comparable size and shape throughout the entire incubation period (fig. 4). This is in contrast to Ede and Agerbak ('68), who claim that *talpid*^{3} reaggregated more rapidly and formed



C

Fig. 2A Mixed aggregate of ^{3}H-thymidine labeled- and unlabeled-wild-type limb bud mesoderm. The labeled and unlabeled wild-type cells do not sort out. Three days in culture. × 1700.

Fig. 2B Mixed aggregate of ^{3}H-thymidine labeled wild-type limb bud mesoderm and unlabeled *talpid*^{2} limb bud mesoderm. The *talpid*^{2} and wild-type cells do not sort out. Three days in culture. The apparent morphological differences in the chondrogenic areas of the aggregates pictured in figure 2A,B are not significant. There is normally a range of form in these areas. (Compare fig. 2A,B with figs. 3A,B, 5 and 6). It should also be noted that the decrease in silver grain density at the center of these aggregates is due to the large intercellular distances of the chondroblasts, rather than cell sorting of *talpid*^{2} and wild-type cells. × 1800.

Fig. 2C Mixed aggregate of ^{3}H-thymidine labeled wild-type limb bud mesoderm (lw) and unlabeled pigmented retina. The cells sort out such that the retina is external to the mesoderm. Three days in culture. × 1900.

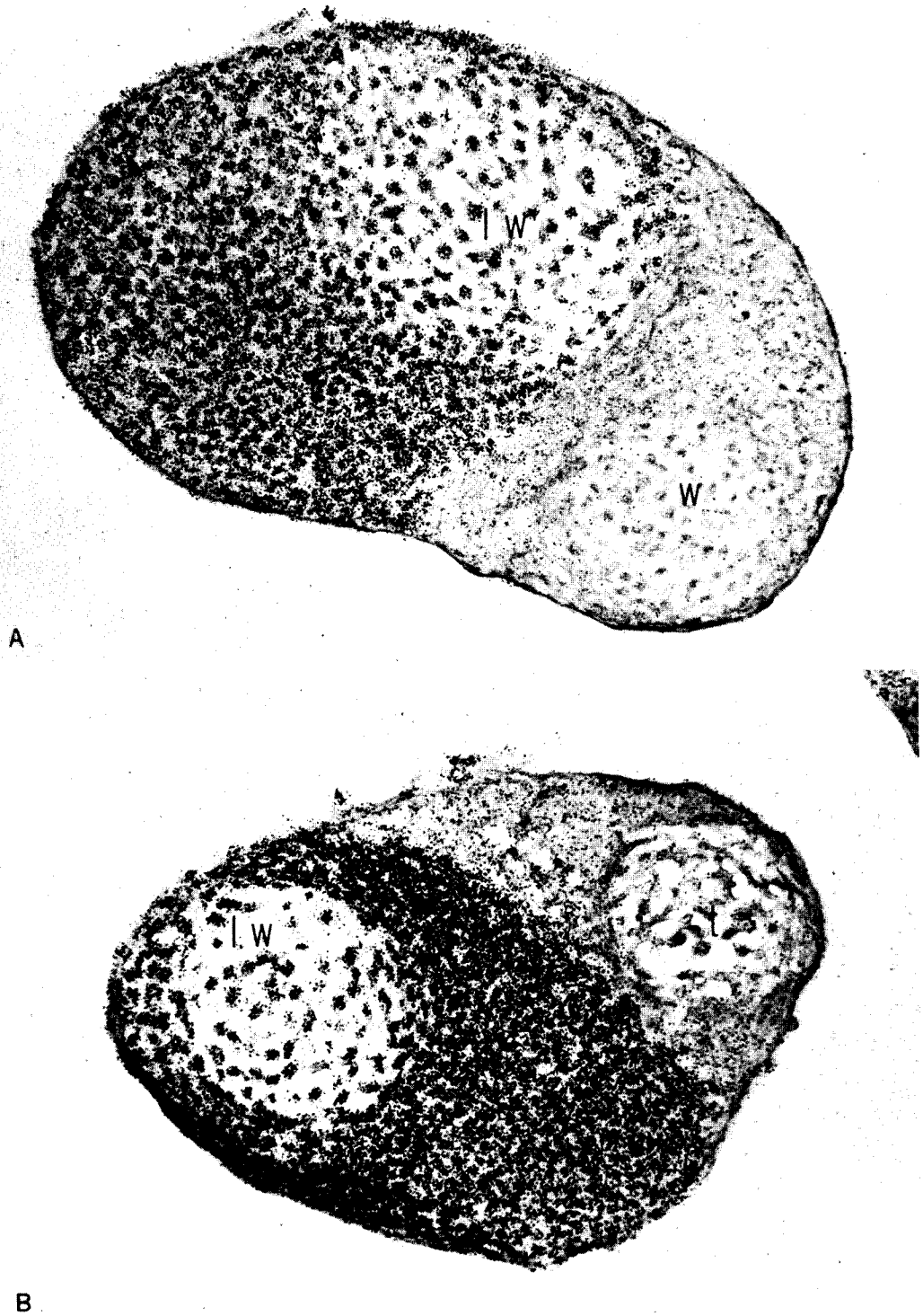
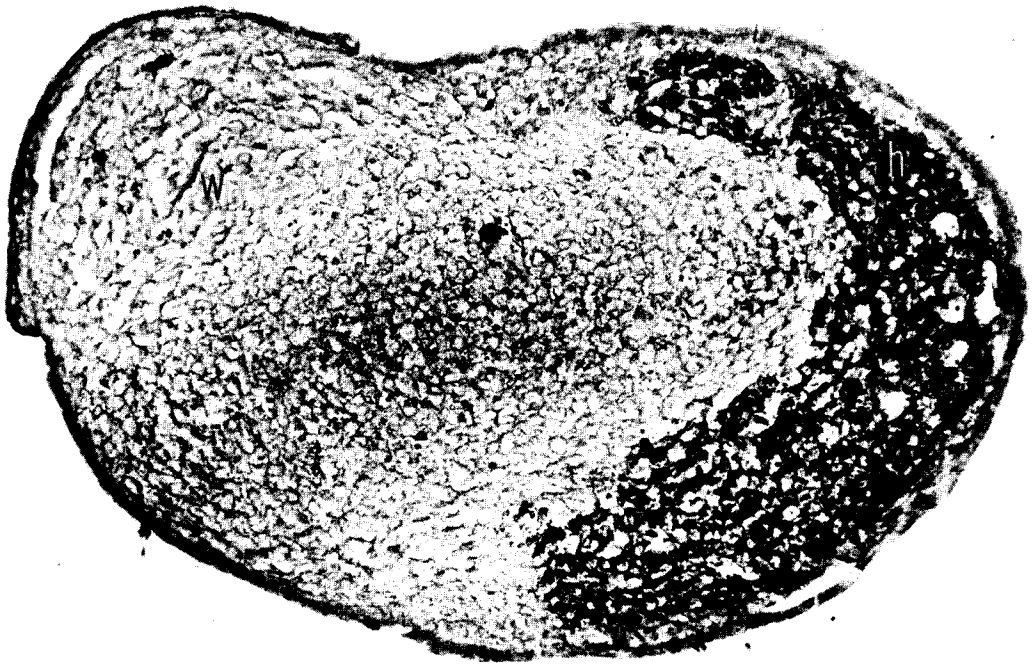
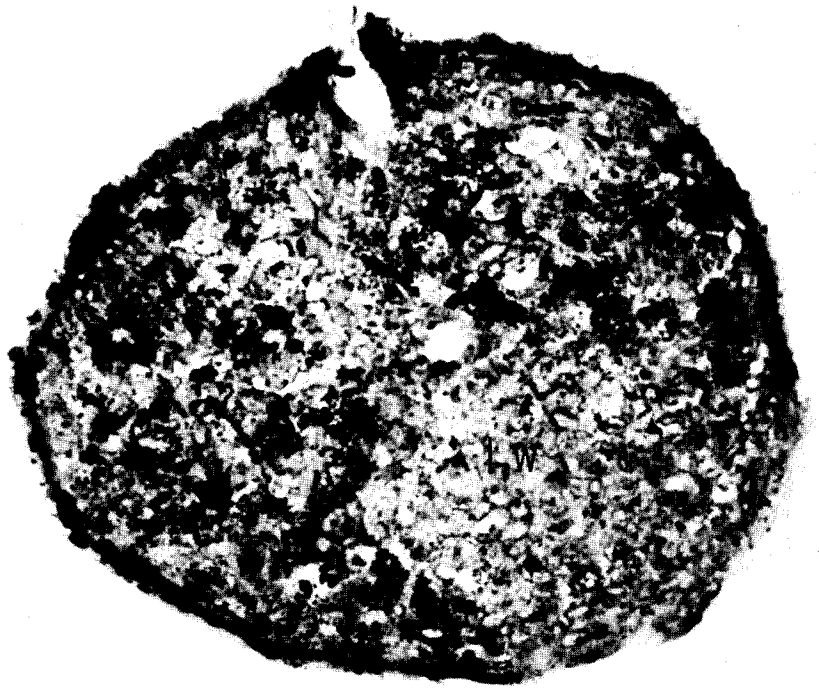


Fig. 3A Fragment fusion of ^{3}H-thymidine labeled wild-type limb bud mesoderm (lw) and unlabeled wild-type bud mesoderm (w). Tissues cohere forming apposed hemispheres. Two days in culture. × 1200.

Fig. 3B Fragment fusion of ^{3}H-thymidine labeled wild-type limb bud mesoderm (lw) and unlabeled *talpid*^{2} limb bud mesoderm (t). Tissues cohere forming apposed hemispheres. Two days in culture. × 1100.



C



D

Fig. 3C Fragment fusion of heart ventricle (h) and wild-type limb bud mesoderm (w). The heart has begun to surround the limb mesoderm. Two days in culture. × 1000.

Fig. 3D Fragment fusion of pigmented retina aggregate and ^{3}H-thymidine labeled wild-type limb bud mesoderm (lw). The limb bud mesoderm is surrounded by the retina. Two days in culture. × 2000.

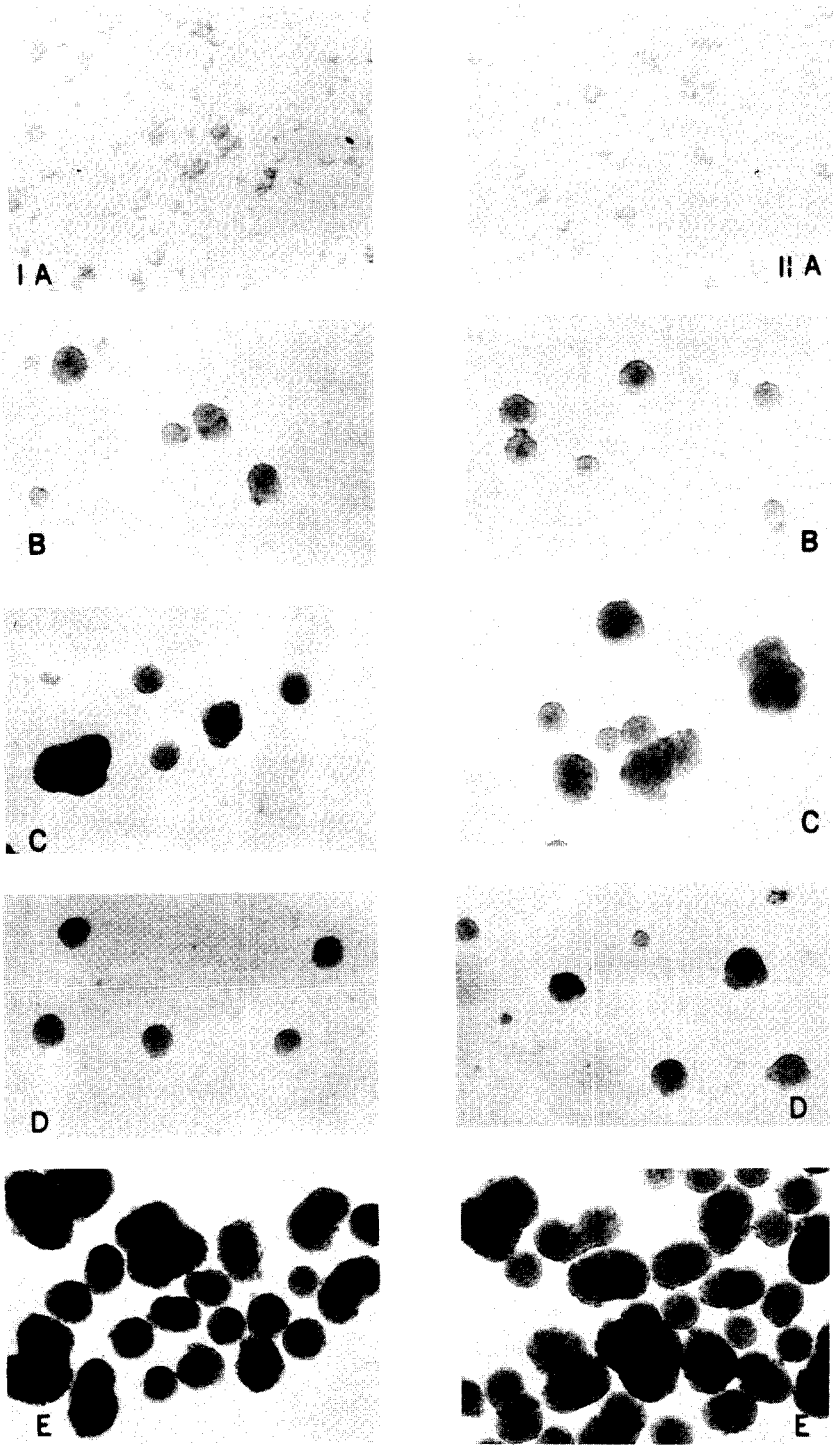


Fig. 4,I Wild-type limb bud mesoderm cell aggregation over a three day period: A. After five hours (tissue photographed in culture flask); B. After 24 hours (tissue photographed in culture flask); C and D. After 48 hours (tissue photographed in culture flask); E. After 72 hours (tissue photographed in spot plate). × 85.

Fig. 4,II *Talpid*[2] limb bud mesoderm cell reaggregation over a three day period: A–E at same times as 4,I A–E. × 85.

smaller, smoother aggregates than wild-type tissue. Aggregation during this period progressed as described initially by Moscona ('61). A large number of small aggregates fuse during the incubation period, resulting in the formation of a small number of large aggregates. A wide variation in size and shape can occur, however, within one culture flask (fig. 4IC,D, IIC,D).

Sectioned preparations of *talpid*^{2} and wild-type aggregates did not reveal the difference in aggregate morphology reported by Ede and Agerbak (fig. 5). All single aggregates were found to be nearly spherical, bounded by a layer of uniformly flattened, closely packed cells that surrounded a loosely packed chondrogenic interior. The chondroblasts generally appeared to be randomly arranged in both *talpid*^{2} and wild-type tissue, but sections of some aggregates exhibited chondroblasts which were aligned in short parallel rows. Compound aggregates (aggregates formed by cohesion of several smaller aggregates) have the form of partially fused spheres and conform to the description of a normal wild-type limb bud mesoderm aggregate given by Ede and Agerbak ('68) (fig. 6). In contrast to the results reported by Ede and Agerbak, we find these aggregates equally frequent in wild-type and *talpid*^{2} cultures.

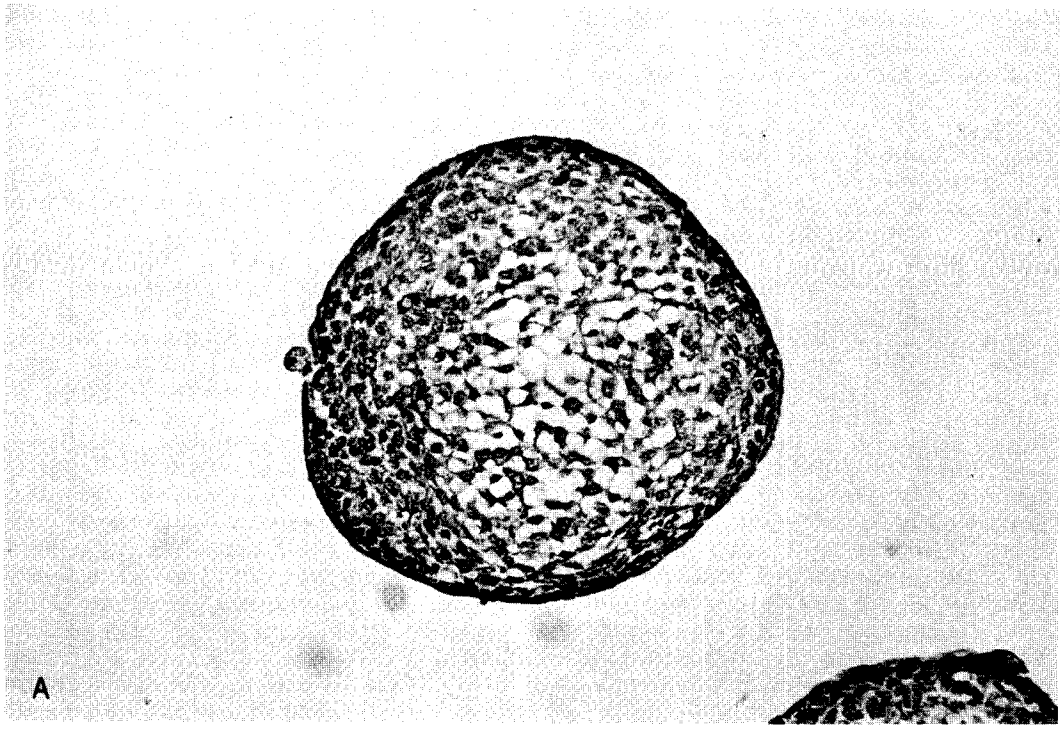
DISCUSSION AND CONCLUSIONS

In testing the hypothesis that the *talpid* gene acts to modify limb morphology by increasing intercellular adhesive strength (Ede and Agerbak, '68), we have employed the techniques of fragment fusion and cell sorting. In the present study, no evidence has appeared to support the suggestion that adhesive differences exist between limb bud mesoderm cells of *talpid*^{2} and wild-type chick embryos.

The results presented here demonstrate that in binary combinations of *talpid*^{2} and wild-type limb bud mesoderm tissue, cell sorting by genotype does not occur in mixed aggregates, and envelopment of one tissue by the other did not occur in fragment fusions. In the aggregates from mixed cell suspensions, the *talpid*^{2} and wild-type cells remained randomly distributed. In fused fragments the tissues formed apposed cohering hemispheres with no sign of envelopment. This leads one to conclude that cells of limb bud mesoderm from four-day wild-type and *talpid*^{2} chick embryos are equally adhesive. Substantiating these findings are the noted similarities in shape and size of *talpid*^{2} and wild-type limb bud mesoderm aggregates observed in culture over a three-day period.

Although cell sorting of limb bud mesoderm by genotype was not observed, the development of histotypically different tissue regions within a single fragment or aggregate was routinely observed. Limb bud mesoderm is, as yet, undifferentiated at the developmental stages used at the beginning of the experimental period (four days incubation, stage 22–23) (Hamilton, '52; Searls, '65; Zwilling, '68). The recognizable differentiation of limb bud chondroblasts has occurred in intact chick embryos (Searls, '65) and in cultured fragments and aggregates (figs. 2A,B, 3A,B, 6) by the end of the experimental period (3 additional days). As in the embryo, these chondroblasts are always found in the central portion of the cultured tissue mass. The phenomena of histotypic localization in aggregates of limb bud mesoderm has been described by Moscona and Moscona ('52) and has since been noted in fragments of limb bud mesoderm (Zwilling, '66). The development of this pattern of tissue localization *in vitro* appears to involve either cell sorting (i.e., chondrogenic cells move to the interior while myogenic cells move to the periphery) or selective differentiation (i.e., cells in the interior of the tissue mass preferentially form cartilage while peripheral cells form muscle and connective tissue). Although some work has been done in this area (Hampé, '60; Searls, '67, '71; Zwilling, '66; Flower, '70), which of the two hypothesis (if either) is correct for the *in vitro* system remains unclear. Searls ('67) has demonstrated that the localization of the two tissues *in vivo* depends on differentiation *in situ* rather than cell sorting.

Criticism can be brought to bear on this work from at least two areas. For one, the *talpid*^{2} mutant employed in the present study is not identical to the *talpid*^{3} mutant used originally by Ede and Agerbak. Both *talpid* alleles show pleitropic



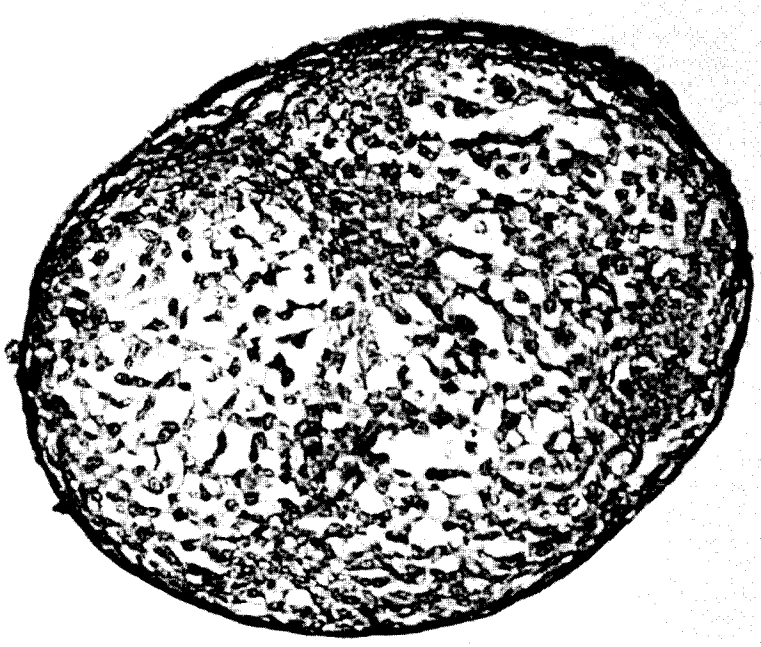


Fig. 5 Sectioned limb bud mesoderm aggregates. Chondrogenic interior is surrounded by tightly layered mesoderm and epithelium. Three days in culture. A. Wild-type. B. *Talpid*[2]. × 1300.

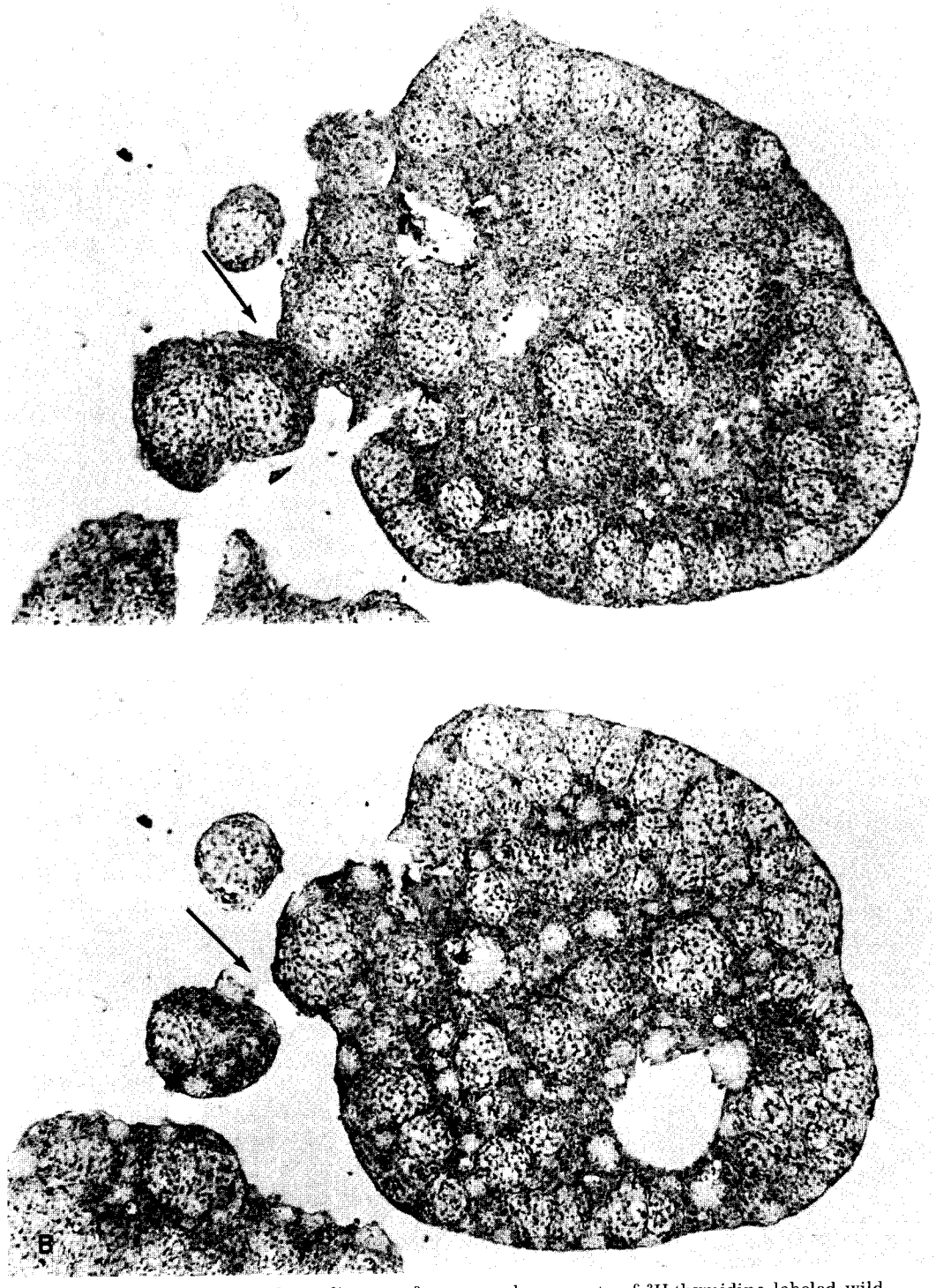


Fig. 6 Two sections, 25 μ distant, of compound aggregate of ^{3}H-thymidine labeled wild-type limb bud mesoderm. Note particularly the fusion of apparently separate tissue and smooth periphery. Three days in culture. × 440.

effects. Abnormalities appear in the development both of the limbs and the head. However, the alleles appear to be identical with respect to the morphogenetic performance of limb tissue (differences appear only in head formation, Ede and Kelly, '64a,b; Abbott, '67), thus it is felt that this criticism is not too serious.

A second criticism arises from questions of the ability of the techniques of cell sorting and fragment fusion to reveal small differences in cellular adhesion of dissimilar cell types. Perhaps the cell aggregation system used by Ede and Agerbak is more sensitive in this respect. The differences in aggregation rate of *talpid*^{3} and wild-type cells that they report may be valid reflections of adhesive differences that are too small to be revealed by cell sorting. With the present state of our knowledge it is impossible to answer this criticism satisfactorily.

In some respects, however, the cell sorting system is superior to the cell aggregation system when one attempts to relate cell behavior *in vitro* to actual morphogenetic events occurring in the embryo. A tissue mass more closely approximates the *in vivo* situation than do colliding cells in suspension culture. As in the embryo, the prime consideration in tissue spreading and cell sorting experiments is the final arrangement of cells in coherent tissue masses. Thus, if altered adhesiveness of talpid limb bud mesodermal cells is of actual importance in generation of altered limb structure (by way of an effect on limb bud cell coalescence) as suggested by Ede, one would expect it to be revealed by cell sorting and tissue spreading. The assay system used here would be expected to be as sensitive to adhesive differences as the limb bud itself. In other words, if differences in adhesiveness are too small to be detected by cell sorting, then it seems unlikely that they would have a major effect on morphogenesis *in vivo*. Finally, in the present study, the kinds of differences in aggregation of dissociated talpid and wild-type cells reported by Ede and Agerbak were not observed.

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