Participation of cartilage grafts in amphibian limb regeneration

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SUMMARY

Irradiated axolotl arms bearing grafts of pure cartilage obtained from the non-irradiated humerus of other specimens show a variable response to amputation of the hand. Some arms grow spikes with little morphogenesis, others produce one or more digits, and some regenerate small and defective but recognizable hands. Identically irradiated control arms which carry no grafts or grafts of irradiated tissues show none of these features. The cartilage grafts must therefore be responsible for the limited amount of regeneration observed. The origin of the regenerated tissues could not be independently determined in this experiment, although grafts were made reciprocally between white and dark genotypes, as all the regenerates were either very pale or completely unpigmented. A previous demonstration that grafted tissue does not reactivate irradiated host cells, however, permits the conclusion that the grafted cartilage provides all the mesodermal tissues of these regenerates.

The grafts consisted exclusively of chondrocytes, while the regenerates contained cartilage, muscle, blood vessels, nerve sheaths and general connective tissue. It is concluded that chondrocytes dedifferentiate into pluripotent blastemal mesenchyme cells which can redifferentiate into all other internal tissues of the limb. The concepts of modulation and neo-blasts (undifferentiated reserve cells) thus have no basis in amphibian limb regeneration.

INTRODUCTION

During the regeneration of amphibian limbs, internal tissues adjacent to the cut end of the limb-stump dedifferentiate and proliferate to form a mesenchymal blastema which is the source of all the mesodermal tissues in the regenerate. Chondrocytes of larval limbs, for example, dedifferentiate by escaping from the cartilage matrix to become indistinguishable from other cells in the blastemal mesenchyme. The term dedifferentiate has a valid descriptive meaning, as used here, without implying anything about the variety of tissues into which particular blastemal cells eventually redifferentiate. That question strictly concerns the degree of determination in apparently undifferentiated mesenchymal cells. The term differentiation seems to have usurped most of the meaning originally attached to determination, however, causing considerable confusion (expressed by Weiss, 1973). Weiss had earlier coined the term modulation to cover the possibility that blastemal cells can only redifferentiate into the tissue from

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which they originated; and added a new meaning to dedifferentiation, which now commonly implies a certain loss of determination, permitting histological transformation or metaplasia.

None of the experiments, devised to decide whether modulation or transformation best explains limb regeneration, seems to have gained acceptance, from the celebrated disarticulation experiments (Bischler, 1926) to the present day (reviewed by Thornton, 1968; Hay, 1968). Blastemal cells are certainly too much alike for direct observation to settle the question. An experimental approach using marked grafts at least reduces this ambiguity, but most grafts are likely to include connective tissue which might contain undifferentiated (undetermined) reserve cells among the fibroblasts. Only scrupulously cleaned limb cartilage provides a pure tissue likely to participate in regeneration.

Several recent studies have traced cells derived from radioactively labelled grafts among regenerating tissues (Oberpriller, 1967; Steen, 1968; Foret, 1970). These studies showed that a variety of grafted tissues could contribute cells to regenerating skeletal cartilage, but they did not trace grafted chondrocytes into any other tissue. The only pure tissue employed, radioactive triploid cartilage, was only observed to provide some chondrocytes of the regenerate (Steen, 1968). All the other grafts could have included reserve cells, and hence even the production of cartilage by cells derived from intestinal blastemata (Oberpriller, 1967) or muscle (Steen, 1968; Foret, 1970) could not prove the occurrence of histological transformation. Apart from the difficulty of detecting radioactivity after more than four or five divisions of the grafted cells, these studies suffered from a common limitation in that the grafts were embedded in equally competent host tissue and thus provided only a minor proportion of the regenerate.

The regenerative potency of a graft might well be more fully expressed if the demand on it is increased by prior irradiation of the host limb. Eggert (1966) performed such an experiment, by grafting pure scapular cartilage into the irradiated limbs of adult newts, and detected only modulation - blastemata appeared after amputation but these only grew into spikes containing irregular masses of cartilage. Desselle (1968) implanted radioactive grafts which were mainly, but not exclusively, cartilage into irradiated limbs of *Triturus cristatus* and traced labelled cells into regions of the blastema expected to form muscle, during the surprisingly normal course of regeneration.

Umanski (1937) and Trampusch (1951, 1958) had previously grafted skeletal elements and other tissues heterotopically into irradiated axolotl limbs, which were subsequently amputated and which frequently then regenerated. Umanski sought to determine the origin of the regenerate according to its anatomical organ specificity. This criterion does not really establish the origin of regenerated tissues, however, but rather whether the graft or the host controls subsequent growth and morphogenesis. Thus, from virtually identical experiments and results, Umanski concluded that grafts provide the regenerating tissues and Trampusch concluded that grafts organized the irradiated host tissue into an
appropriate structure: neither conclusion was reliable. Umanski (1938) perceived this objection and proceeded to confirm his conclusion by means of coloured skin grafts, as discussed later; whereas Trampusch (1972) seems to have recently adopted Umanski's original argument. Neither investigator was able to meet the objection that their regenerates were derived from reserve cells, as all their grafts were invariably contaminated by connective tissue.

The validity of using irradiation in this type of experiment has also been denied, on the hypothesis that a graft might restore regenerative competence to the irradiated host cells (Trampusch, 1958; Vorontsova & Liosner, 1960; Rose & Rose, 1965; Desselle, 1968; Polezhaev, 1972). We believe we have refuted this hypothesis by grafting genetically marked tissue into irradiated arms, reciprocally between dark and white axolots, and consistently obtaining graft-specific regenerates. A detailed analysis of the control of pigmentation, with its application and limitations for this purpose, is already on record (Wallace & Wallace, 1973). The grafts submitted to that analysis consisted of brachial nerves and thus certainly included connective tissue. We have now applied the same experimental design to grafts of pure limb cartilage and obtained clear cases of limb regeneration, which convince us that chondrocytes dedifferentiate in all senses of the term.

MATERIALS AND METHODS

The experiments were performed on young axolotls, *Ambystoma mexicanum*. A first series employed the progeny of a single mating between two dark heterozygotes \( (D/d) \) of the same colony. Ten dark and ten white specimens were anaesthetized when 70-80 mm long, and irradiated with 2 krad (i.e. 20 J/kg) 250 kVp X-rays while partly shielded with lead plates. A more detailed description of the techniques involved has been given previously (Wallace, 1972). Fig. 1A shows the shielding pattern employed for these experimental specimens, arranged so that the entire left arm and both shoulders were exposed to the full dose, while the rest of the body including the right forearm was shielded and received only about 50 rad. Shielded areas are considered to be effectively non-irradiated, as doses up to 250 rad do not influence regeneration perceptibly while 500 rad allows regeneration to occur after some delay (Maden, unpublished).

Cartilage was obtained from the humerus of non-irradiated 80 mm specimens, using both arms of five dark and five white specimens. The entire humerus was excised before snapping off and discarding the epiphyses, to which most of the tendons and ligaments are attached. All visible loose sheets of connective tissue, with blood vessels and pigment, were stripped off the diaphysis which was then placed in half-strength Barth's solution containing penicillin. Ossification had begun in these diaphyses: the thick perichondrial sheath was sometimes penetrated by a blood vessel in the middle of the shaft where the cartilage was eroded,
leaving cones of cartilage at each end of the diaphysis. These cartilage cones were readily isolated by peeling back the perichondrium (Fig. 3A–C). The less damaged cone of each pair was promptly implanted into the irradiated left forearm of a host of different genotype. Surplus cartilage cones were fixed in neutral formalin, preserved in 70% ethanol, and later examined as whole mounts and sections to assess the purity of the grafts. As a control, both arms of six white specimens were irradiated using the shielding pattern in Fig. 1B. Irradiated cartilage was transplanted into one arm, and irradiated muscle into the other, of each specimen. These grafts were prepared from the upper arms of dark specimens which had also been exposed to 2 krad X-rays.

A second series of experiments was conducted the following year, essentially as a repetition of the first but with the following modifications. All operations were performed on considerably younger specimens, 50 mm graft-donors and 55–60 mm hosts all from a single mating between $D/d$ and $d/d$ genotypes. The irradiation dose and shielding pattern duplicated those of the first series, but were performed with a different X-ray source (Pantak Ltd) operating at 300 kV and 12 mA with a dose rate of 650 rad/min at a distance 165 mm from the casing aperture. Cartilage was prepared by the same technique as described above, but only from the left humerus of each donor. The operation was more difficult with these smaller specimens, but the cartilage could be extracted as a single rod from the diaphysis as no central erosion had yet occurred. Only the proximal part of each cartilage rod was grafted, without any deliberate orientation, into the irradiated left forearm of a host of the other genotype. Identically
prepared irradiated cartilage was similarly grafted into the irradiated left forearm of control specimens, whose irradiated right arms received no grafts.

In both series of experiments, all transplantations were performed within three days of irradiation. The grafts could still be seen as mounds covered by healed epidermis two days later, when both arms of all specimens were amputated just distal to the graft and in the corresponding position above the wrist of those arms which did not bear a graft. The specimens were kept at room temperatures averaging 18–20 °C for 20 weeks after amputation. All observations are related to the time of amputation and usually consist of camera lucida drawings or records made at weekly intervals.

RESULTS

Series 1 (70–80 mm specimens)

All the right arms which had been amputated through shielded tissue formed blastemata during the following three weeks. One blastema was knocked off during handling; the stump then regressed to the irradiated elbow region without showing any further sign of regeneration. All the other right arms produced three digits within five weeks and later completed the fourth digit. The regenerating hands of dark specimens were clearly pigmented within five weeks.

In contrast to this regular regeneration, the irradiated left arms bearing cartilage grafts only slowly formed apical protrusions. A few of these protrusions later disappeared to leave smooth-ended limb-stumps. Most protrusions grew into small conical blastemata 4–10 weeks after amputation, some of which also relapsed. The remaining cones elongated into stable spikes during the period 9–12 weeks after amputation. These spikes grew appreciably in length and thickness, but none resembled a hand or produced any digits (Fig. 2A). Each spike contained a single cartilage element and a few blood vessels, but none contained melanophores, either in the dermis or surrounding the blood vessels. Sufficient examples were scored (five white and six dark hosts) to conclude that the failure of pigmentation was a consistent feature of the spikes, whether the host or the graft was of the dark phenotype.

The 11 spikes recorded in Table 1, which had apparently ceased to grow after 20 weeks, were then re-amputated to remove half the tissue and to produce a wound stretching back to the host arm. No further growth was detected during the following 10 weeks and all the spikes remained unpigmented.

The control specimens, whose irradiated arms carried grafts of irradiated cartilage or muscle, did not even form blastemata in response to amputation. Their arm-stumps regressed slowly for the next 20 weeks, showing smooth rounded ends except for transient apical tufts of skin.

A comparison of the results, when either normal or irradiated cartilage was grafted into identically irradiated arms (Table 1), shows the production of spikes to be dependent upon the presence of normal cartilage. We suppose that
Fig. 2. Camera lucida outlines of representative limbs 18 weeks after amputation. Pigmented regions are stippled. (A) from series 1, others from series 2. (A) Spike on left arm of a dark host. (B) 2-digit growths produced by both the apical and a dorsal blastema. (C) 3-digit regenerate on the left arm of a dark host. (D) 4-digit regenerate and a large dorsal digit formed by an accessory blastema on the left arm of a white host. (E) Right hand of the same specimen as D, showing one pigment patch and the greater size typical of normal control regenerates.

Table 1. Results of series 1, scored 20 weeks after amputation

<table>
<thead>
<tr>
<th>Host arm</th>
<th>Graft</th>
<th>Number of arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shielded</td>
<td>None</td>
<td>20 20 1* — 19</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Cartilage</td>
<td>20 20 9 11 —</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Irradiated cartilage</td>
<td>6 6 6 — — —</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Irradiated muscle</td>
<td>6 6 6 — — —</td>
</tr>
</tbody>
</table>

* Regression after accidental loss of the blastema, see text.

Even normal cartilage grafts were commonly eroded by the same processes which cause irradiated arms to regress, while more than half such grafts persisted to provide the central cartilage of a spike. The lack of pigmentation in any of the spikes prevents us from confirming or refuting that supposition. We do not regard the spikes as cases of regeneration; they merely indicate that grafted cartilage may be remodelled and may show a limited amount of growth. According to this experiment, therefore, limb cartilage of juvenile axolotls appears to be capable of modulation but shows no sign of transformation. The apparently intransigent determination of cartilage found here duplicates the evidence provided by Eggert (1966) and is generally in accordance with the other studies mentioned in the introduction. The present result need not imply an intrinsic limitation of differentiated cartilage, however, for a variety of...
technical factors could equally well prevent regeneration. The grafted cartilage may have contained insufficient viable cells owing to incipient ossification, for instance, or the graft may have been situated too far from the slowly regressing stump apex to establish a normal blastema. Such considerations encouraged us to repeat the experiment using younger specimens.

Series 2 (50-60 mm specimens)

All the right arms which had been amputated through shielded tissue formed conical blastemata within 2 weeks. One blastema was probably damaged, as it was resorbed in the following week and the arm subsequently regressed to above the irradiated elbow. Two other arms regenerated hands with only three digits, and the remaining 17 arms each produced four digits within 4 weeks after amputation, at which time the regenerates of dark specimens were well pigmented.

Ten of the irradiated left arms showed small dorsal protrusions over the region of the graft, within 2-3 weeks after amputation. These protrusions elongated appreciably during the following four weeks, while the limb apex gradually regressed back to the level of the graft. At that point, either the elongated dorsal blastemata became apical and later formed hands of various sizes with one to four digits, or a separate apical blastema formed and developed up to four digits in addition to the one or two digits produced by the dorsal blastema (Fig. 2B). Eight specimens only formed a single apical blastema more than 6 weeks after amputation. Four of these developed into the most normal regenerates obtained here (Fig. 2C), while the other four produced spikes or relapsed completely. The remaining two experimental arms merely regressed for 20 weeks. It is remarkable that only two of these specimens developed a simple spike resembling the most common result of the first series. The distinction between a spike and a single digit is that the latter has a series of articulated cartilages with a characteristic narrow-waisted shape. Regeneration occurred sporadically in the different specimens of this series, but both the formation of distinct blastemata and of digits were delayed and progressed more slowly than in the normal sequence of regeneration shown by the right arms of the same specimens.

Control specimens showed no sign of regeneration in their irradiated arms, which slowly regressed for 20 weeks after amputation whether or not a graft of irradiated cartilage was present (Table 2). Any operational trauma associated with the transplantation, therefore, clearly did not alleviate the inhibitory effect of irradiation.

A comparison of the results obtained from this second series of operations (Table 2) shows the production of blastemal cones, and subsequent growth of digits or genuine regenerates, by irradiated arms to be dependent upon the presence of a normal cartilage graft. Only a minority of the experimental arms showed definitive regeneration and even those regenerates were defective in
Table 2. Results of series 2, scored 20 weeks after amputation

<table>
<thead>
<tr>
<th>Host arm</th>
<th>Graft</th>
<th>Number of arms</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Operated</td>
<td>Scored</td>
<td>Stump</td>
<td>Spike</td>
<td>1-2</td>
<td>3-4</td>
</tr>
<tr>
<td>Shielded</td>
<td>None</td>
<td>20</td>
<td>20</td>
<td>1*</td>
<td>—</td>
<td>19</td>
</tr>
<tr>
<td>Irradiated</td>
<td>Cartilage</td>
<td>20</td>
<td>20</td>
<td>4</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Irradiated cartilage</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Irradiated</td>
<td>None</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Regression after resorption of a large blastema, see text.
† Ignoring any digits formed by an accessory blastema.

morphology and size (Fig. 2). These results thus represent only a modest improvement of the first series, but imply that limb cartilage has a greater influence on regeneration than had been detected previously.

Pigmentation and anatomy of regenerates

Only seven specimens formed characteristic hands with three or more digits on their irradiated arms; six of these experimental regenerates were on dark hosts. These gained some pigmentation soon after the appearance of digits, caused entirely by superficial melanocytes occupying the skin. The melanocytes remained relatively sparse, and consequently the regenerates were always unmistakably pale in comparison to the contralateral controls of normal regeneration, even when they had grown considerably (Fig. 2C). Only one white host formed a four-digit regenerate, which was even smaller than a single digit produced by an accessory blastema on the same arm (Fig. 2D). Neither this regenerate nor any of the one or two digit growths on white hosts gained any pigmentation within 20 weeks.

Both series of operations thus yielded identical results in respect to the virtual absence of pigmentation in all spikes, digits or regenerates which were produced under the influence of grafted cartilage. The genotype of the newly formed tissues cannot be determined, therefore, as the pigmentation was not consistently like that of either the graft or the host. If a minimum size and structure is required before a regenerate can attract melanophores, however, this test should only be applied to the genuine regenerates. The six cases of dark hosts with pale experimental hands permit a fairly confident conclusion that regenerates are not derived from irradiated host tissues. The one miniature regenerate on a white host which remained unpigmented is not reliable evidence against an origin from the graft.

The largest and most normal regenerates were examined as cleared whole mounts and in sections for the arrangement of skeletal elements and the presence of other tissues, after fixation at 20 weeks. The best cases approached normality
in these features, but were considerably smaller than normal regenerates (Fig. 3E, F). They possessed well-differentiated cartilage with a perichondrial sheath, strips of muscle and generally abundant connective tissue (Fig. 3G). Occasional melanocytes were found in blood vessels in the regenerates on dark hosts and some sheathed nerves were also present.

Purity of grafts and origin of regenerates

Stages in the process of obtaining the cartilage grafts are illustrated in Fig. 3A–C. Duplicates of the grafted pieces have been examined in serial sections for the presence of foreign tissue: no cellular contamination has been detected in seven pieces from the first series and in nine pieces from the second series of operations. Fig. 3D shows an example in which all cells are typical chondrocytes embedded in matrix. The number of chondrocytes contained in such grafts has been roughly estimated from the sections, as 1000–2000 in the larger fragments of the first series and 500–1000 in the second series.

These grafted chondrocytes were the only non-irradiated cells present in the experimental arms, which commonly produced blastemata at the site of the graft and which quite frequently grew spikes, digits or genuine regenerates that clearly must be attributed to some influence of the graft. The pigmentation of the regenerates tends to indicate that they were derived from the grafts, whose influence may be restricted to their ability to provide blastemal cells. Acceptance of that conclusion, which we hope the discussion will justify, eliminates any explanation involving reserve cells and necessarily implies that chondrocytes are capable of genuine dedifferentiation and subsequent transformation into the other mesodermal cell-types found in our experimental regenerates.

DISCUSSION

There are still relatively few accredited examples of histological transformation in animals. The best-known one is the case of Wolffian regeneration of the lens from the pigmented epithelium of the iris, where the whole sequence of transformation from loss of pigment to acquisition of lens proteins is known in considerable detail (Reyer, 1954; Yamada, 1966). The adjacent pigmented epithelial cells of the retina can transform into sensory retinal cells, iris epithelium or lens (Stone, 1959). A direct conversion of endoderm to ectoderm has been reported in isolated fragments of Hydra (Normandin, 1960; Burnett, 1968). Most other cases of regeneration, particularly among invertebrates, have been almost routinely attributed to undifferentiated migratory neoblasts – an interpretation which is still open to question (Hay, 1968). Although amphibian limb regeneration is held to be a strictly localized process, and only involves cellular migration in exceptional circumstances (Wallace, Wessels & Conn, 1971; Conn, Wessels & Wallace, 1971), the ubiquitous connective tissue fibroblasts could include relatively undifferentiated cells which might serve as local
Transformation of chondrocytes

neoblasts. No previous report of regeneration by irradiated limbs bearing non-irradiated grafts has been able to produce convincing evidence that the regenerate was derived exclusively from tissue which was completely devoid of fibroblasts and potential neoblasts. An additional difficulty in assessing these published reports is the frequent reference to poorly formed regenerates, as there is an understandable tendency to classify malformed and trivial growths as examples of regeneration. We claim that our results defy challenge on most of these grounds and almost inevitably lead to the conclusion that chondrocytes are pluripotent: they apparently dedifferentiate in all senses of the term, whereas the concept of modulation gravely underestimates their capabilities. That conclusion could apply equally well to all other cell-types which promote regeneration when grafted into irradiated limbs, but there seems to be little prospect of obtaining such clear evidence of the potencies of other tissues as is provided here for cartilage.

Admittedly, the present demonstration has obvious defects. We do not understand why the first series of grafts only yielded spikes, while the second series produced a variable number of genuine digits. Even those cases which conform to our criterion of regeneration showed only limited growth, and none achieved a normal degree of pigmentation. It is possible that all these defects are related to a single initial failure to place the graft correctly in order to establish a large apical blastema before the onset of differentiation. Grossly defective and small regenerates tend to be pale. Because of the lack of pigmentation, we are forced to rely on previous evidence that irradiated host cells cannot participate in regeneration. The most direct and appealing evidence for this comes from our previous use of the same colour marker; it was found that nerve grafts in irradiated limbs indisputably produced regenerates showing donor-specific coloration (Wallace, 1972; Wallace & Wallace, 1973). The same conclusion is commonly deduced from observations that grafted tissues

**Figure 3**

A–C. Successive stages in preparing cartilage grafts, all stained in methylene blue, cleared, and shown at the same magnification.

(A) Whole humerus of 50 mm specimen, showing large epiphyses and attached fluffy connective tissue.

(B) Diaphysis isolated from a humerus like A.

(C) Cartilage cones isolated by peeling the perichondrium from 2 diaphyses like B.

(D) Section of a cone like C. All the cells present are typical chondrocytes embedded in lacunae of the cartilage matrix.

(E and F) Regenerates from white cartilage grafts in dark irradiated hosts stained in methylene blue, cleared, and shown at the same magnification. Note the normal arrangement of cartilage elements in the digits, and the few patches of melanocytes visible as black dots.

(G) Section of a digit from F. Note the perichondrial sheath around the cartilage (ca), muscle (m) and absence of melanophores from the skin (sk).
or cells dictate the structure of regenerates on irradiated limbs (Umanski, 1937; Skowron & Roguski, 1958; Lagan, 1961; Trampusch, 1972). Accepting this evidence that only non-irradiated cells can contribute to the blastema, we must conclude that the grafted chondrocytes are responsible for all the internal tissues of the regenerates. Six out of seven regenerates in the present experiment conformed to this conclusion, being pale or white in contrast to the dark host arm. Some explanation is required, however, for the one regenerate produced on a white host, which should be derived from the genetically dark graft cells but which remained unpigmented. It seems fairly clear that blastemal cells do not redifferentiate into melanocytes and certainly there were no melanocytes in the grafts, so they would need to migrate from the trunk of the white host in order to reach the regenerate. A few melanocytes normally enter the limbs of most white specimens and sometimes reach the digits, but this is certainly a slow and haphazard process. Since the coloration of wild-type regenerates also seems to be related to their size or structure, then it is not surprising that the single miniature regenerate on a white host did not become pigmented. The few previous attempts to use this genetic marker for tracing the origin of regenerates do not contradict this supposition, as dark regenerates were usually obtained when the grafted tissue actually contained melanocytes. Umanski (1938) apparently obtained consistent evidence that white skin would produce a complete but unpigmented regenerate when grafted on to the irradiated limb of a dark host. Trampusch (1959) did not obtain uniform results from the converse experiment involving dark skin grafts on white arms which may not have been adequately irradiated, but the vast majority of the regenerates he obtained were well pigmented. Skowron & Roguski (1958) injected cell suspensions from dark donors into heavily irradiated white or dark limbs. Subsequent amputation led to extremely defective regenerates which were sometimes, but not always, pigmented. Our own grafts of nerves, muscle and skin from dark donors onto irradiated white hosts have produced regenerates which were always pigmented to some extent, and sometimes produced dark arm-stumps in the absence of regeneration, but perhaps in too few cases for us to assert that pigmentation is a uniform response even when the grafts may have included melanocytes or melanoblasts (Wallace & Wallace, 1973; Maden, unpublished).

A variety of observations, such as the mutual dependence of neurons and their end-organs as well as the loss of specialized structures in cultured cells, testify that cellular differentiation is an equilibrium condition which requires maintenance. We subscribe to the belief, succinctly argued by Gurdon (1973), that the determination of many cells is equally labile. The potencies of such cells can only be expressed as a result of appropriate stimuli, of course, and so usually remain unknown. Both nuclear transplantation and regeneration can be used to test this potency in amphibians at least, and they provide rather complementary results: at least some nuclei of axolotl blastemal cells are pluripotent (Dasgupta, 1970). Nuclear transplantation is a more sensitive
Transformation of chondrocytes

Technique in that embryonic development produces a wider range of differentiated cell-types than occur in one regenerating organ, yet regeneration can reveal some of the potency of intact cells whose nuclei must be equally or more labile. The common failure of development after nuclear transplantation, like the common imperfection of regeneration, is amenable to several interpretations of which technical failure seems the most probable, and thus deserves far less emphasis than given it by Burgess (1967) or Di Berardino & Hoffner (1971). The accumulating examples of nuclear totipotency or pluripotency (Kobel, Brun & Fischberg, 1973), however, support the conviction, based on regenerative metaplasia, that differentiated cells need not be irrevocably determined. In this context, our present results surely point to the conclusion that dedifferentiation effaces the previous tissue-specificity of a cell to restore much of its developmental potency, perhaps sufficiently to transcend the notional limitations of germ layers.

REFERENCES


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