The Developmental Capacity of Nuclei Taken from Differentiating Endoderm Cells of *Xenopus Laevis*

*by J. B. Gurdon*

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**INTRODUCTION**

An important question concerning embryonic differentiation is whether the nuclei of somatic cells in different parts of an embryo come to differ genetically from each other during development. It has become possible to investigate this matter since King & Briggs (1955) have shown that nuclear transplantation is a satisfactory technique for testing the developmental potentialities of embryonic nuclei. These authors (1957, 1960) have used *Rana pipiens* for transplantation experiments with endoderm nuclei, and have found that these nuclei become progressively limited in their developmental capacity after the late blastula stage.

This paper describes some similar experiments carried out with endoderm nuclei of *Xenopus laevis*. The general conclusion that nuclei change as development proceeds is confirmed; there are, however, considerable differences between *Rana* and *Xenopus* in the rate and time of onset of nuclear changes. These differences make it easier to understand the significance of nuclear differentiation during embryonic development.

**TECHNIQUE, DONOR NUCLEI, AND RECIPIENT EGGS**

The technique used in these experiments with *Xenopus* has been modified from that of Briggs & King (1953) and is described elsewhere together with the method of culturing transplant-embryos (Elsdale, Gurdon, & Fischberg, 1960).

Donor cells have been prepared by dissecting out part of the endoderm with the help of needles. Cells are disaggregated by placing the isolated tissue in Barth-Versene solution for between 10 and 20 minutes (Gurdon, 1960b); they are then transferred to standard Barth solution (Barth & Barth, 1959) and kept until required for transplantation.

The developmental stages of donor embryos have been identified according to Nieuwkoop & Faber’s (1956) normal table for *X. laevis*. All the experiments described below have been carried out with vegetal or endoderm donor cells.

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In blastulae and gastrulae donor cells were taken from the centre of the vegetal cell-mass, and in later stages from the floor of the gut lumen. In hatched tadpoles, cells were taken from the floor of the anterior part of the gut; these cells are rather larger and more easily disaggregated than those forming the outer wall of the gut. In hatched tadpoles the presumptive germ-cells do not lie in that part of the gut from which donor cells were taken.

Proof that the nuclei of a transplant-embryo have all been derived from the injected nucleus alone is provided by the use of marked donor nuclei. These are diploid and were obtained from the 1-nucleolated strain of *Xenopus* (Elsdale, Fischberg, & Smith, 1958). The presence of the nuclear marker can be recognized in a squash preparation of part of the donor embryo, since the number of nucleoli per nucleus can be quickly counted under the phase-contrast microscope (Elsdale, Gurdon, & Fischberg, 1960). Since marked donor nuclei have been injected into unmarked and enucleated recipient eggs, all transplant-embryos should have marked nuclei (i.e. with one nucleolus). Transplant-embryos are sometimes obtained which have two or more nucleoli in each nucleus. If the embryo’s ploidy and nucleolar number is known, the origin of its nuclei is at once evident (Elsdale, Gurdon, & Fischberg, 1960). Tetraploids resulting from the doubling of the injected nucleus and triploids resulting from the participation of the egg nucleus both have 2-nucleolated nuclei, but are troublesome to distinguish by the number of chromosomes. For this reason, and because the tetraploid condition appears to have no effect upon the embryonic development of transplant-embryos (Gurdon, 1959), only 1-nucleolated diploids have been counted in the results below.

Recipient eggs were obtained by injecting frogs with gonadotropic hormone. Normal diploid frogs, which did not therefore carry the nuclear marker, were used. These frogs had either been kept in the laboratory since they were imported, or else they had been reared in the laboratory from imported frogs. The quality of eggs laid by recently imported frogs does not seem to differ consistently from that of frogs reared in the laboratory.

**FACTORS AFFECTING TRANSPLANT-EMBRYO DEVELOPMENT**

The way in which transplant-embryos develop is determined by many different factors, which fall into the four categories below. The effects of the last three must be known before it is possible to relate transplant-embryo development to the first. They are: (a) innate qualities of donor nuclei; (b) technical damage to donor nuclei; (c) innate qualities of recipient eggs; (d) technical damage to recipient eggs. Study of variation in the last three of these has shown that they may affect transplant-embryo development in only two ways (Gurdon, 1960b). The first concerns variation in the degree of donor-cell distortion, and the second concerns variation in egg quality (i.e. in the ability of eggs to recover from technical interference).
When donor cells of a similar volume are transplanted with different sized pipettes, this varies the extent to which the donor cell-wall is disrupted. It is found that the degree of donor-cell distortion is directly related to the proportion of total transplantations which form regular late blastulae (Gurdon, 1960b). It seems that, if the donor cell-wall is insufficiently broken, the injected nucleus is unable to combine satisfactorily with the recipient egg cytoplasm. Though the degree of donor-cell distortion has been kept constant as far as possible, small variations in this respect are unavoidable and can cause a variation of up to 20 per cent, in the proportion of late blastulae formed. For this reason it is best to base the main conclusions from transplantation experiments on the development of transplant-embryos expressed as a proportion of regular late blastulae, and not as a proportion of total transplantations.

When nuclei from the same donor embryo are transplanted (with no variation in technique) into eggs of different frogs, it was found that the development of transplant-embryos was appreciably more normal with the recipient eggs of some frogs than with those of others (Gurdon, 1960b). A test in which fertilized eggs were treated just as for nuclear transplantation, except that no nucleus was injected, showed that the standard transplantation treatment may result in abnormal development with the eggs of some frogs, but may cause no harm to those of others. This demonstrates that the eggs of different frogs vary considerably in their ability to withstand the experimental manipulations to which they are subjected. It is unfortunately not possible to recognize the quality of recipient eggs other than by the development to which they give rise. This makes it necessary to do control experiments using donor nuclei of known developmental potentialities, such as undifferentiated blastula nuclei. Control transplantations show in which experiments egg quality was good; only these 'selected' experiments can be regarded as giving a clear indication of innate nuclear qualities. The quality of recipient eggs is usually consistent throughout an ovulation, but it sometimes happens that eggs deteriorate in quality when a frog has already been laying for several hours. For this reason control transplantations are if possible carried out at the beginning and end of each experiment.

Experiments published elsewhere (Gurdon 1960b) have shown that abnormal development of transplant-embryos beyond the blastula stage cannot be caused by any part of the standard technical treatment of donor nuclei. If donor nuclei from several normal blastulae are transplanted into the eggs of one frog, they give very similar results (Text-fig. 4B). This demonstrates that blastula nuclei do not differ in their capacity to give normal transplant-embryo development, and are therefore suitable for controls with which to test the quality of recipient eggs. It is thus evident that transplantation experiments can be analysed in such a way that the only variable affecting transplant-embryo development is the innate quality of donor nuclei.
RESULTS

Table 1 is a summary of all experiments with endoderm nuclei carried out since the technique became standardized; it includes only diploid transplant-embryos from first-transfer experiments and does not contain the results of

<table>
<thead>
<tr>
<th>Donor embryo age group and normal stage numbers of Nieuwkoop (1956)</th>
<th>Total transplantations</th>
<th>Stages of transplant-embryo development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Late blastula</td>
<td>Early gastrula</td>
</tr>
<tr>
<td>Blastulae (7–9)</td>
<td>533 (100%)</td>
<td>172 (32%)</td>
</tr>
<tr>
<td>Gastrulae (10–13)</td>
<td>1,014 (100%)</td>
<td>452 (45%)</td>
</tr>
<tr>
<td>Neurulae (14–22)</td>
<td>469 (100%)</td>
<td>182 (39%)</td>
</tr>
<tr>
<td>Muscular response post-neurulae (23–26)</td>
<td>236 (100%)</td>
<td>77 (34%)</td>
</tr>
<tr>
<td>Heart-beat tadpoles (29–34)</td>
<td>282 (100%)</td>
<td>72 (25%)</td>
</tr>
<tr>
<td>Hatching tadpoles (35–37)</td>
<td>471 (100%)</td>
<td>95 (20%)</td>
</tr>
<tr>
<td>Swimming tadpoles (39–41)</td>
<td>681 (100%)</td>
<td>77 (11.5%)</td>
</tr>
</tbody>
</table>

The percentages represent transplant-embryo survival in terms of total transplantations for each donor.
serial transfers. In the survival curves of Text-fig. 1 there is an appreciable mortality among transplant-embryos from all donors; it happens that a higher proportion of late blastulae was obtained from gastrula and neurula donors than from blastula donors, but this difference is not significant. Apart from this

**Table 2**

*Selected experiments only*

The percentages in the middle of each square represent transplant-embryo survival in terms of total transplantations; the percentages at the bottom of each square indicate transplant-embryo survival in terms of the number of late blastulae for that donor stage.

<table>
<thead>
<tr>
<th>Donor embryo age group and normal stage numbers of Nieuwkoop (1956)</th>
<th>Total transplantations</th>
<th>Stages of transplant-embryo development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastulae (7-9)</td>
<td>327 100%</td>
<td>Late blastula 113 35%  110 34%  106 33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early gastrula 98%  94%  96%  94%  94%  106 33%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late gastrula and neural folds 216 44%  43%  175 35%  85%  81%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swimming tadpole 79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal feeding tadpole 91 28%  81%  81%</td>
</tr>
<tr>
<td>Gasrulae (10-13)</td>
<td>502 100%</td>
<td>Late blastula 221 44%  220 44%  216 43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early gastrula 100%  100%  100%  98%  98%  98%  98%  98%  98%  98%  98%  98%  98%  98%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late gastrula and neural folds 175 35%  175 35%  175 35%  175 35%  175 35%  175 35%  175 35%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swimming tadpole 79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal feeding tadpole 169 34%  169 34%  169 34%  169 34%  169 34%  169 34%  169 34%</td>
</tr>
<tr>
<td>Neurulae (14-22)</td>
<td>140 100%</td>
<td>Late blastula 50 36%  48 35%  40 29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early gastrula 100%  100%  100%  96%  96%  96%  96%  96%  96%  96%  96%  96%  96%  96%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late gastrula and neural folds 79%  79%  79%  79%  79%  79%  79%  79%  79%  79%  79%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swimming tadpole 27 19%  27 19%  27 19%  27 19%  27 19%  27 19%  27 19%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal feeding tadpole 26 18%  26 18%  26 18%  26 18%  26 18%  26 18%  26 18%</td>
</tr>
<tr>
<td>Muscular response post-neurulae (23-26)</td>
<td>152 100%</td>
<td>Late blastula 53 34%  50 32%  40 29%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early gastrula 100%  100%  100%  96%  96%  96%  96%  96%  96%  96%  96%  96%  96%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late gastrula and neural folds 75%  75%  75%  75%  75%  75%  75%  75%  75%  75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swimming tadpole 25 17%  25 17%  25 17%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal feeding tadpole 22 15%  22 15%  22 15%</td>
</tr>
<tr>
<td>Heart-beat and hatching tadpoles (29-34)</td>
<td>174 100%</td>
<td>Late blastula 47 27%  36 21%  28 16%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early gastrula 100%  100%  100%  76%  76%  76%  76%  76%  76%  76%  76%  76%  76%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late gastrula and neural folds 59%  59%  59%  59%  59%  59%  59%  59%  59%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swimming tadpole 47 17%  47 17%  47 17%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal feeding tadpole 13 6%  13 6%  13 6%</td>
</tr>
<tr>
<td>Swimming tadpoles (39-41)</td>
<td>436 100%</td>
<td>Late blastula 89 21%  71 16%  48 11%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Early gastrula 100%  100%  100%  80%  80%  80%  80%  80%  80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Late gastrula and neural folds 54%  54%  54%  54%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Swimming tadpole 19%  19%  19%  19%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normal feeding tadpole 13 3%  13 3%  13 3%</td>
</tr>
</tbody>
</table>

It can be seen that the older the donor embryos, the smaller is the proportion of late blastulae and normal tadpoles obtained from their nuclei. It is not profitable to examine these general trends in any detail, since they include experiments done on bad recipient eggs, many of which developed abnormally irrespective of the type of injected nuclei.

Table 2 contains only the results of 'selected' experiments, in which the recipient eggs were of good quality. There is no clear-cut way of classifying recipient eggs as good or bad, since all degrees of egg quality are found. The best that can be done is to group together all those experiments in which control blastula nuclei have given predominantly normal development. These experiments should provide a clearer indication of trends that have already been seen in Text-fig. 1, but which were then obscured by poor egg quality. In Text-fig. 2
the point at which each survival curve starts shows the percentage of total transplantations which became regular late blastulae.

**Text-fig. 2.** Survival curves for selected experiments only (figures from Table 2). Transplant-embryo survival is expressed as a percentage of total transplantations for each donor age group.

**Text-fig. 3.** Survival curves for selected experiments only (figures from Table 2). Transplant-embryo survival is expressed as a percentage of the number of regular blastula transplant-embryos obtained from each donor age group.
In Text-fig. 3 transplant-embryo survival is shown as a proportion of late blastula transplant-embryos and not as a proportion of total transplantations. This method of presentation avoids the consequences of technical variation in the degree of donor-cell distortion, but only shows transplant-embryo survival beyond the late blastula stage. The survival curves in Text-fig. 3 are therefore believed to be a good representation of the innate qualities of different donor nuclei, since the effects of other factors which might affect these curves have been removed. Some of the ‘selected’ experiments have been shown in diagrammatic form (Text-figs. 4–9) and will be discussed below with the survival curves.

First-transfer experiments

The most normal transplant-embryo development that has been obtained from blastula nuclei is shown in Text-figs. 4B, 6, and 9. In these experiments nuclei from eight different blastulae have been tested. In the second series of Text-fig. 6 all transplant-embryos became normal tadpoles; in the other seven series most embryos were normal, but in each case a few developed abnormally. The cause of these abnormalities is not clear. It may be that the quality of all eggs is rarely good enough to withstand transplantation; it is often found in this laboratory that up to 10 per cent. of naturally fertilized eggs, obtained after hormone injection, develop abnormally; these abnormalities must presumably be attributed to poor egg quality. The small percentage of abnormal development obtained from blastula nuclei certainly does not justify the conclusion that
some of these nuclei are differentiated, or lack the potentiality for normal
development. The present transplantation experiments are therefore believed
to be entirely consistent with the view that blastula nuclei are undifferentiated.
The survival curve for blastula nuclei in Text-fig. 3 will be used for comparison
with the other survival curves for nuclei of later donor stages.

TEXT-FIG. 5. Transplant-embryos derived from nuclei of a mid-gastrula, a swimming tadpole, a heart-
beat tadpole, and a late gastrula. About 40 transplantations were made for each series, and recipient
eggs from the same frog were used throughout.

In this and all following diagrams, microcephalic and oedematous tadpoles are represented diagram-
atically with a small eye and heart oedema in contrast to normal tadpoles (with a large eye). One
microcephalic and two normal tadpoles are shown under the stage 29 donor.

TEXT-FIG. 6. Transplant-embryos from a mid-late blastula, a late blastula, an early gastrula, a hatching
tadpole, and a swimming tadpole, all from recipient eggs of the same frog. Approximately 30 trans-
plantations per series.

Gastrula nuclei have given the results shown in Text-figs. 4A, 5, 6, and 9. In
the experiment depicted in Text-fig. 4A, 14 nuclei were transplanted; 3 eggs
failed to cleave at all, but the remaining 11 cleaved regularly and all formed
normal tadpoles which, had the experiment been continued, would almost
certainly have metamorphosed. Since the results of this experiment did not contain any abnormally cleaved eggs it constitutes particularly clear evidence that the endoderm nuclei from early gastrulae are totipotent and undifferentiated. In the other series with gastrula nuclei a few transplant-embryos are abnormal, though no more than with blastula nuclei. The survival curve for gastrula

![Text-fig. 7. Transplant-embryos from a neural-fold stage, a stage 41 swimming tadpole, a stage 39 swimming tadpole, a heart-beat tadpole, another neural-fold stage, and a muscular-response stage; all into the eggs of one frog, and about 35 transplantations per series.](image)

nuclei in Text-fig. 3 is within 5 per cent. of that for blastula donors at each point, a difference which is certainly not significant. There is no indication of any decrease in nuclear potentialities during gastrulation, since late blastulae and late gastrulae give similar results (compare the second and fifth series of Text-fig. 9).

It is worth drawing attention here to the consistent results that have been obtained when nuclei from several different blastula and gastrula donors are transplanted into the recipient eggs of one frog, as in Text-figs. 4B, 5, 6, and 9. The proportion of total transplantations which become late blastulae is very similar in these experiments, as shown by the number of embryos under each donor; also the proportion of late blastulae which become normal tadpoles is about the same in each case. The similarity in results is most marked among series in which recipient eggs of the same frog were used, that is, among all those shown in one figure. These consistent results would be expected from totipotent, undifferentiated nuclei.

Nuclei from older donor embryos appear to become progressively limited in their development potentiality. This differentiation is reflected not only in the proportion of total transplantations which form regular late blastulae, but
also in the proportion of late blastulae which develop normally. The point at which survival curves commence in Text-fig. 2 becomes lower with increasing age of the donor stage; this illustrates the decline in the proportion of late blastulae formed from older donor nuclei. It was pointed out above that the technique may cause up to 20 per cent. of variation in the proportion of blastula transplant-embryos. Such variation in technique is probably responsible for the fact that a greater proportion of blastula transplant-embryos was obtained from neurula and gastrula donors than from earlier stages. In donors later than the heart-beat stage there is a pronounced reduction in the numbers of blastula transplant-embryos. This difference is too large to be accounted for by technical variation, and must therefore represent a real difference in nuclear potentialities. In most experiments the proportion of blastulae formed decreases

Text-fig. 8. Twenty transplant-embryos were obtained from endoderm nuclei of an original muscular-response stage tadpole. When these embryos had become late blastulae, three were used to provide nuclei for serial transplantations. The three resulting clones differ strongly from each other, but the transplant-embryos within each clone are very similar. About 50 transplantations were made for each of the four series. The serial clones (but not the first transfers) were made with the eggs of one frog.
progressively from the heart-beat stage with increasing age of the donor; this typical condition is seen in Text-figs. 6 and 7, and particularly clearly in Text-fig. 5. However, this trend is not always consistent. It may happen that nuclei from two donor embryos of exactly the same age, when transplanted into eggs of the same frog, will give entirely different results; this can be seen by comparing the 1st and 5th series of Text-fig. 7, and particularly clearly from a comparison of the 3rd and 4th series of Text-fig. 9. One may conclude from these results that
the ability of endoderm nuclei to form regular blastulae after transplantation becomes progressively reduced with increasing age of donor embryos beyond the heart-beat stage; on the other hand, the rate at which this capacity is lost is not always closely correlated with the developmental stage of the donor embryo.

The second respect in which endoderm nuclei appear to become differentiated concerns their decreasing capacity for giving normal transplant-embryo development. Blastula transplant-embryos derived from older donors are much less likely to become late gastrulae and normal tadpoles than transplant-embryos from younger donors (Text-fig. 3). This loss of capacity begins in nuclei from neurula donors. From this stage onwards the developmental potentiality of nuclei becomes increasingly restricted right up to the swimming tadpole stage, which is the latest from which nuclei have been transplanted. Even at this advanced stage, transplant-embryos from some nuclei have developed into normal tadpoles, though the proportion of such undifferentiated nuclei is very small.

As before, there is great variation in the extent to which this loss of capacity has proceeded in donor embryos of the same age. In Text-fig. 5 nuclei from a swimming tadpole have given wholly abnormal development, but in Text-fig. 7 two swimming tadpoles have given mostly normal development; the proportion of abnormalities is markedly different in the two swimming tadpoles of Text-fig. 9. Thus, though there is a clear tendency for more abnormal transplant-embryo development to be obtained from the nuclei of later donor stages, there is no exact correlation between the age of the donor embryo and the developmental potentialities of its endoderm nuclei.

Serial-transfer experiments

Serial nuclear transfers involve exactly the same technique as first-transfer experiments. The only difference is that the donor embryo is not derived from a normally fertilized egg, but is itself a transplant-embryo obtained by nuclear transplantation (King & Briggs, 1956). Reasons have been given above for believing that *Xenopus* endoderm nuclei do not differentiate until after the late gastrula stage; thus, if a transplant-embryo is used for serial transplantation before it has developed beyond a blastula or gastrula, all its endoderm nuclei should have the same potentiality as each other. If a blastula transplant-embryo derived from a normal nucleus is used as a donor, serial transplantation of its nuclei should yield a high proportion of normal development. On the other hand, if the original nucleus was differentiated so that it would have developed abnormally, then serial transplantation from it should (if the abnormality is inherited) provide many embryos (a clone) all suffering from the same kind of abnormality.

Two points can be investigated by serial transplantation of nuclei from transplant-embryos which have not developed beyond the blastula or gastrula stage. First, such experiments show whether the lack of potentiality of differentiated
nuclei is inherited or not, and, secondly, whether the differentiation of nuclei is reversible.

The extent to which nuclear changes are inherited is apparent from a comparison of one clone with another. If they are consistently different from each other this can only be attributed to heritable differences between the original donor nuclei. In Text-fig. 8 nuclei were taken from an original muscular response tadpole, and three transplant-embryos were used at the blastula stage to provide donor nuclei for serial clones. In the left-hand clone the great majority of embryos were normal; the proportion of abnormal embryos is very small and not more than is obtained with first-transfer blastula nuclei. This clone therefore shows that the original donor nucleus was undifferentiated. In the middle clone every transplant-embryo was arrested as a late blastula. The third clone on the right contains a range of abnormalities, but none of the tadpoles shown was quite normal, all being microcephalic and oedematous. The variation within this clone is discussed below. The three clones in this experiment are consistently different from each other, and so demonstrate clearly that the abnormalities in the first-transfer series are heritable.

In this experiment three blastula transplant-embryos were used to provide serial donor nuclei. It was not, of course, known how these three blastulae would have developed if they had been allowed to differentiate further. However, an indication of the way they might have developed can be derived from the development of the other 17 first-transfer embryos. The three serial donor embryos were chosen at random from 20 blastula transplant-embryos, and would therefore be expected to have developed in the same kind of way as did the 17 which were left. The potentialities of the 3 serial donors turned out to be consistent with the 17 first-transfer embryos. This result suggests that all the abnormalities present in the first-transfer group are heritable in the way that the abnormalities of the three embryos used for serial transplantation have been found to be.

The second kind of information which serial transplantation can give concerns the reversibility of nuclear differentiation. This is shown by the variation within a clone as opposed to the variation between clones. Clones from original blastula donors are shown in Text-fig. 9. Since the great majority of embryos in the first transfer series were normal, nuclei from embryos selected at random for serial donors would also be expected to be totipotent in the majority of instances. The first and second clones of Text-fig. 9 are predominantly normal; the clone from the 6th series contains more abnormal embryos than would be expected, but this series was carried out on the last eggs laid in one frog’s ovulation, and some of these may have been immature. The first two clones of Text-fig. 9 and the first of Text-fig. 8 show that totipotent nuclei have no obvious tendency to age or to become abnormal after continued transplantation. These series also confirm that technique and egg quality cannot explain the abnormalities found in other clones. There is very little variation in clones from undifferentiated nuclei,
and no variation in those with differentiated nuclei (2nd clone of Text-fig. 8); however, clones in which no embryos are quite normal (though some are nearly so) are very variable. This is seen in the third clone of Text-fig. 8, and in the two clones from the fourth series of Text-fig. 9; in both cases the original donor was advanced and would therefore have contained differentiated nuclei. This variation could be explained in two ways. It might be that the potentiality of the original donor was like that of the worst or best embryo in the clone derived from it, and that after serial transplantation some nuclei increase or decrease their potentialities; or it might be that embryos obtained from abnormal nuclei are much more sensitive to technique, &c., than embryos with normal nuclei. In connexion with the latter possibility, haploid embryos, which have abnormal nuclei, differentiate very variably (Gurdon 1960a); though this variation might be due to genetic differences from one haploid nucleus to another, it might also be due to the effect of various environmental factors on nuclei suffering from the same abnormality (haploidy). Sufficient experiments have not yet been carried out to show whether variation in abnormal transplant-embryo clones represents a change in nuclear potentiality or not. Until other possibilities have been excluded, this limited variation cannot be regarded as evidence that nuclear differentiation is reversible.

CONCLUSIONS CONCERNING THE POTENTIALITIES OF ENDODERM NUCLEI IN XENOPUS

Nuclear differentiation in relation to the developmental stages of donor embryos

The nuclei of differentiating endoderm cells become progressively limited in their developmental capacity. This differentiation is evident not only from the proportion of transplantations which result in late blastulae, but also from the proportion of blastula transplant-embryos which develop normally. The proportion of late blastulae obtained from the nuclei of embryos up to the muscular response stage does not vary, but becomes progressively reduced from nuclei of heart-beat and later stages. Although variation in technique can affect the proportion of late blastulae, it cannot account for the great difference in this respect between nuclei from young and advanced donor embryos. The majority of transplant-embryos derived from blastula and gastrula nuclei develop normally as far as swimming tadpoles; however, the more advanced the donor, the more transplant-embryos develop abnormally, and the more severe are the abnormalities from which they suffer.

These results indicate that the endoderm nuclei of Xenopus only begin to become differentiated in embryos older than late gastrulae. From the neurula stage onwards there is a progressive increase in the proportion of differentiated nuclei, but even in swimming tadpoles there remain a few endoderm nuclei with entirely unrestricted developmental potentialities.

These conclusions have been based on the results of selected experiments. It was, however, pointed out (p. 509) that the same conclusions can be drawn,
though less obviously, from all experiments. Fischberg, Gurdon, & Elsdale (1958) reached similar general conclusions from some earlier experiments with _Xenopus_.

**Limited application of results**

Attention must now be drawn to certain limitations which apply to these results. Only endoderm nuclei have been investigated, but comparable changes will probably be found to take place in the nuclei of other differentiating tissues (Briggs & King, 1957).

The potentialities of nuclei have not been tested for all developmental stages up to that in which the endoderm is differentiated. No transplantations have been made from nuclei of earlier stages than a mid-blastulae, but there is no reason to believe that such nuclei would give more normal development than was obtained from late blastulae and gastrula nuclei. The latest donor stage from which nuclei were transplanted was a swimming tadpole in which the gut had just formed two right-angled bends. In order to transplant from later stages, other substances than Versene would be required for cell disaggregation, and it is not certain that these would be harmless. The potentialities of endoderm nuclei have therefore been tested only between Nieuwkoop’s stages 8 and 41, but some gut cells remain undifferentiated until stage 46, several hours later.

The development of transplant-embryos has only been followed up to the formation of normal tadpoles. Transplanted nuclei have therefore been tested only for their ability to bring about embryonic development up to the tadpole stage. Transplant-embryos which become normal tadpoles can be reared into sexually mature frogs (Gurdon, Elsdale, & Fischberg, 1958); the study of these frogs gives additional information about the nuclei from which they were derived, and will be dealt with elsewhere (Gurdon, in preparation).

**The part of a nucleus in which differentiation takes place**

The changes undergone by differentiating endoderm nuclei were described above as 'innate nuclear qualities'. This term refers to the conclusions based on transplant-embryo development when all effects of the transplantation technique and egg quality have been removed from the results. The evidence for nuclear differentiation is of two kinds, concerning first the proportion of total transplants which form late blastulae, and, secondly, the proportion of late blastulae which develop normally. Nuclei from late donor stages such as hatching tadpoles undergo mitosis rather seldom (see below), but after transplantation are required suddenly to enter a phase of frequent mitoses (cleavage). Those nuclei which are differentiated so as not to be able to do this—that is, those which cannot form regular late blastulae—have not been used for serial transplantation. Thus nothing is known about the heritability or eventual reversibility of this kind of nuclear differentiation, which seems unlikely to be connected with the chromosomal parts of the nucleus.
The other kind of nuclear differentiation which affects the ability of late blastula transplant-embryos to develop normally has been shown by serial transplantation to be both heritable and, to a large extent at least, irreversible. These two characteristics are associated with gene-controlled factors. Moreover, it is shown below that there is no correlation between these nuclear changes and certain general, reversible nuclear qualities, such as size and phase of mitosis. Most nuclear components other than the chromosomes are changed at each mitosis; these nuclear changes, which are heritable, cannot therefore be associated with the nuclear membrane, nuclear sap, or nucleolus. Thus there are strong reasons for believing, as in Rana (King & Briggs, 1956), that the changes in endoderm nuclei demonstrated by the development of late blastula transplant-embryos have taken place in the chromosomes.

The smallest endoderm nuclei to have been transplanted (stage 41) are at least as large and as well protected by cytoplasm as nuclei from the animal hemisphere of a blastula; but, as animal and vegetal blastula nuclei give equally good results, nuclear changes cannot be associated with the size of nuclei (Gurdon, 1960b). The proportions of endoderm nuclei ‘in mitosis’ (nuclear membrane disappeared) has been counted in embryos at different stages of development, reared at 21° C. One hundred endoderm nuclei of the kind used as donors for nuclear transplantation were counted for each donor stage. The proportion of nuclei in mitosis was 17 per cent. in a blastula, 7 per cent. in a late gastrula, 5 per cent. in a neural-folds embryo, 2 per cent. in a heart-beat embryo, and 1 per cent. in a stage 41 swimming tadpole. These figures give only a rough indication of the frequency with which nuclei in mitosis were used for transplantation; they show, however, that the transplantation of mitotic nuclei, whatever effect this may have, is too infrequent to affect the evidence for nuclear differentiation.

The significance of nuclear changes

There is no obvious correlation between nuclear differentiation and the morphogenetic development of tissues. This is shown below (p. 521) by comparison of nuclear differentiation in Rana and Xenopus. Very little seems to be known about the state of determination of endoderm tissue in Xenopus. Though the endoderm of Amphibia is described as ‘regionally determined histologically some time before gastrulation’ by Kemp (1951), Okada (1957) has shown that pharyngeal endoderm from a neurula can form many different endoderm structures. As it is not known what other tissues endoderm can form when grafted into various regions of an embryo, it does not seem possible to compare tissue determination and nuclear differentiation. It seems possible, however, that nuclear differentiation may be connected with cell differentiation. In Xenopus the nuclei used for transplantation have been taken from the most undifferentiated endoderm cells of each donor stage. As the gut becomes formed, many of the peripheral endoderm cells become differentiated—that is, they lose their more or less spherical shape, and their nuclei often become elongated;
these cells also become very strongly connected to each other, and it is for this reason that nuclei have not been transplanted from fully differentiated cells. What has in effect been done in these experiments is to transplant nuclei from undifferentiated endoderm cells, and to show that the nearer the time of cellular differentiation, the more nuclei become differentiated. These results are consistent with the considerable variation that has been found in the differentiation of nuclei from embryos of late donor stages (p. 516). Some donor cells were probably nearer this differentiated state than others. It is therefore suggested that nuclear differentiation may play a significant part in the processes which take place when individual cells attain their final functional state. This hypothesis can only be tested when a method is found by which differentiated cells can be harmlessly dissociated.

COMPARISON OF NUCLEAR DIFFERENTIATION IN XENOPUS LAEVIS AND RANA PIPiens

Briggs & King (1957, 1960), using R. pipiens, have transplanted endoderm nuclei from donors of various ages between late blastulae and tail-bud embryos. The survival of transplant-embryos derived from R. pipiens nuclei is shown in

<table>
<thead>
<tr>
<th>Donor stage</th>
<th>Species</th>
<th>Total transplantations</th>
<th>Stages in transplant-embryo development</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Late blastulae</td>
<td>Early gastrulae</td>
</tr>
<tr>
<td>Late blastula or early gastrula animal cells, (undifferentiated nuclei)</td>
<td>Rana</td>
<td>92</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Xenopus</td>
<td>327</td>
<td>100%</td>
</tr>
<tr>
<td>Late gastrula endoderm</td>
<td>Rana</td>
<td>155</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>Xenopus</td>
<td>502</td>
<td>100%</td>
</tr>
<tr>
<td>Neurula endoderm</td>
<td>Rana</td>
<td>98</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Xenopus</td>
<td>140</td>
<td>100%</td>
</tr>
<tr>
<td>Tail-bud endoderm</td>
<td>Rana</td>
<td>130</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Xenopus</td>
<td>152</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3 and has been expressed as a percentage of late blastula transplant-embryos for each donor stage; these figures are therefore comparable to those taken from Table 2 for Xenopus. The survival of transplant-embryos in Rana and Xenopus is compared in Table 3 and Text-fig. 10.
Transplant-embryos derived from blastula nuclei of *Rana* are mostly normal; there is, however, a sharp decline in the developmental potentialities of endoderm nuclei from gastrulae and later stages. Thus in *Rana* the differentiation of endoderm nuclei starts at the beginning of gastrulation and continues from that stage onwards; by the tail-bud stage all endoderm nuclei are differentiated, since none has given rise to normal transplant-embryo development (Briggs & King, 1957).

![Text-fig. 10. Survival curves for endoderm nuclei of *Rana* (dotted lines) and *Xenopus* (solid lines); the numbers have been taken from Table 3. Each curve shows the survival in terms of the late blastula transplant-embryos of one donor stage.](image)

In *Xenopus*, on the other hand, the differentiation of endoderm nuclei does not begin until the neurula stage, and then only takes place very gradually from that stage onwards; even in swimming tadpoles some endoderm nuclei remain undifferentiated. In both *Rana* and *Xenopus* a similar proportion of normal transplant-embryos are obtained from undifferentiated blastula nuclei, but in other respects the rate and time of onset of endodermal nuclear differentiation are very different for the two species.

The possibility that transplant-embryo survival is affected by differences in
the technique for the two species seems improbable (Elsdale, Gurdon, & Fischberg, 1960). The only significant difference in technique concerns the method of enucleation of recipient eggs. In *Rana* the egg nucleus is removed, while in *Xenopus* it is inactivated by irradiation and allowed to degenerate in the egg cytoplasm (Gurdon, 1960a). In *Xenopus* there is no reason to suppose that the dying egg nucleus has any effect on either the injected nucleus or the egg cytoplasm, but this question cannot be finally settled until it is possible to enucleate the eggs of both species by the same method.

The *Xenopus* transplantations described in Text-fig. 10 are selected results, while the results for *Rana* are the average of all experiments with endoderm nuclei. The use of selected results for *Xenopus* does not, however, affect the conclusions, since a similar comparison with *Rana* is obtained if the total results for *Xenopus* (Table 1) are used instead of selected results. It appears that Briggs & King, with *Rana*, have not found much variation corresponding to the eggs of individual frogs. They have used frogs which have been taken from their natural conditions, and this might account for the eggs of *Rana* being more consistent in quality than those of *Xenopus*. It could, on the other hand, be that the much greater size of *Rana* eggs renders them less susceptible to damage by experimental manipulation, so that all *Rana* eggs are resistant to the technique, while this is only true of some eggs in the case of *Xenopus*. Whichever of these possibilities is correct, it cannot invalidate the very marked difference in nuclear differentiation between the two species.

Both *Rana* and *Xenopus* pass through very similar stages of morphological differentiation. Cleavage, gastrulation, and elongation of the embryo are proportionately faster in *Xenopus*, but this is largely due to the higher temperature at which *Xenopus* develops. The rate of embryonic development does not appear to affect the rate of nuclear differentiation, since nuclei from *Xenopus* embryos which were reared at 18° C. and 26° C. gave a similar proportion of normal and abnormal transplant-embryos. These experiments lead to the conclusion that no consistent relationship exists between the rate of nuclear differentiation and that of morphological tissue differentiation. This confirms the conclusion (p. 520) that there is no exact correlation between the differentiation of a tissue and that of its cell nuclei.

**SUMMARY**

1. The developmental potentiality of embryonic endoderm nuclei in *X. laevis* is shown to change as the tissue to which they belong becomes differentiated. This change has been demonstrated by taking nuclei from endoderm tissue in different stages of differentiation, and transplanting them into enucleated unfertilized eggs; the development of the resulting transplant-embryos indicates the developmental capacity of their nuclei.

2. Since the proportion of total transplantations which become late blastulae is affected by the technique, the main conclusions have been drawn from the
further development of late blastula transplant-embryos. The quality of recipient eggs, which is always variable, may also affect transplant-embryo development, and conclusions have therefore been mainly derived from selected experiments in which control donor nuclei have shown that egg quality was good. Reasons have been given for believing that non-hereditary nuclear qualities, such as their size and stage in mitosis, do not affect the conclusions drawn from transplant-embryo development; their development is therefore solely dependent on the specific genetic qualities of the donor nuclei used.

3. No decline in the developmental potentiality of endoderm nuclei was found in blastulae and gastrulae. However, the capacity of nuclei to form normal tadpoles decreased progressively from after gastrulation until the beginning of torsion in the gut of swimming tadpoles; at this late stage there was still a small proportion of undifferentiated nuclei from which normal tadpoles have been obtained. Thus nuclear differentiation affects an increasing proportion of nuclei to an increasing extent, as the endoderm becomes differentiated. Serial transplantation has shown that the changes involved in nuclear differentiation are heritable and, at least to a large extent, irreversible.

4. These results are compared with those of Briggs & King who transplanted nuclei from endoderm cells of *R. pipiens*. In both *Xenopus* and *Rana*, nuclei are undifferentiated at the late blastula stage, but after this the rate and time of onset of nuclear differentiation is very different for the endoderm of the two species. These differences show that there is no exact correlation between nuclear differentiation and tissue differentiation. It is suggested that nuclear differentiation may be concerned in the processes which take place when individual cells become differentiated into their final functional state.

**Résumé**

La capacité de développement de noyaux prélevés sur les cellules endodermiques en voie de différenciation de *Xenopus laevis*

1. La capacité de développement des noyaux endodermiques de l'embryon de *Xenopus laevis* se modifie quand le tissu auquel ils appartiennent se différencie. Ce changement est démontré par le prélèvement des noyaux du tissu endodermique à différents stades de la différenciation et par leur transplantation dans des œufs fécondés énucléés; le développement des embryons-transplants qui en résultent manifeste la capacité de développement de leurs noyaux.

2. Puisque la proportion de transplantations totales qui donnent des blastulas âgées est affectée par cette technique, les conclusions principales ont été tirées du développement ultérieur des blastulas âgées issues des embryons-transplants. La qualité des œufs servant d'hôtes, qui est toujours variable, peut aussi affecter le développement de l'embryon-transplant, et des conclusions ont donc été tirées principalement d'expériences sélectionnées, dans lesquelles les donneurs de noyaux témoins ont montré que la qualité de l'œuf était bonne. On donne des
raisons pour lesquelles il y a lieu de penser que des propriétés non héréditaires
des noyaux, telles que leur taille et leur stade mitotique, ne modifient pas les
conclusions que l'on peut tirer du développement des embryons-transplants.
Leur développement ne dépend donc que des propriétés génétiques spécifiques
des noyaux donneurs utilisés.

3. Il n'a été trouvé aucune diminution du pouvoir de développement des
noyaux endodermiques dans les blastulas et les gastrulas. Cependant, le
pouvoir qu'ont les noyaux de former des têtards normaux décroît progressi- 
ment depuis la gastrulation jusqu'au début de la torsion de l'intestin chez les
têtards nageants; à ce dernier stade, il reste encore une petite proportion de
noyaux indifférenciés, à partir desquels on a obtenu des têtards normaux. Ainsi
la différenciation nucléaire atteint un nombre croissant de noyaux, quand
l'endoderme vient à se différencier. Des transplantations séries ont montré que
les changements impliqués dans la différenciation nucléaire sont transmissibles
et, au moins pour une large part, irréversibles.

4. Ces résultats sont comparés à ceux de Briggs & King, qui ont transplanté
des noyaux de cellules endodermiques de *Rana pipiens*. Chez *Xenopus* comme
chez *Rana*, les noyaux sont indifférenciés au dernier stade de la blastula, mais
après cela la vitesse et l'installation de la différenciation nucléaire sont très
différentes pour l'endoderme des deux espèces. Ces différences montrent qu'il
n'y a pas de lien étroit entre la différenciation nucléaire et la différenciation
tissulaire. On suggère que la différenciation nucléaire jouerait un rôle dans les
processus qui se placent au moment où les cellules individuelles acquièrent leur
différenciation finale et fonctionnelle.

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