The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

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WITH ONE PLATE

INTRODUCTION

AN important problem in embryology is whether the differentiation of cells depends upon a stable restriction of the genetic information contained in their nuclei. The technique of nuclear transplantation has shown to what extent the nuclei of differentiating cells can promote the formation of different cell types (e.g. King & Briggs, 1956; Gurdon, 1960*c*). Yet no experiments have so far been published on the transplantation of nuclei from fully differentiated normal cells. This is partly because it is difficult to obtain meaningful results from such experiments. The small amount of cytoplasm in differentiated cells renders their nuclei susceptible to damage through exposure to the saline medium, and this makes it difficult to assess the significance of the abnormalities resulting from their transplantation. It is, however, very desirable to know the developmental capacity of such nuclei, since any nuclear changes which are necessarily involved in cellular differentiation must have already taken place in cells of this kind.

The experiments described below are some attempts to transplant nuclei from fully differentiated cells. Many of these nuclei gave abnormal results after transplantation, and several different kinds of experiments have been carried out to determine the cause and significance of these abnormalities.

The donor cells used for these experiments were intestinal epithelium cells of feeding tadpoles. This is the final stage of differentiation of many of the endoderm cells whose nuclei have already been studied by means of nuclear transplantation experiments in *Xenopus*. The results to be described here may therefore be regarded as an extension of those previously obtained from differentiating endoderm cells (Gurdon, 1960*c*).

MATERIAL AND METHODS

The animals used for these experiments belong to the subspecies *Xenopus laevis laevis*. The transplantation technique has been carried out as described previously (Elsdale *et al.*, 1960), except that the donor tissue was exposed to

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the dissociating Versene solution (5×10^{-4} M) for 30–40 minutes. The *Xenopus* nuclear marker was used (Elsdale *et al.*, 1960), and marked donor nuclei were transplanted into unmarked recipient eggs. Among the transplant-embryos described below, all those which developed beyond the blastula stage contained marked nuclei, thus proving that they were derived from the transplanted nucleus and not from the egg nucleus. The nuclear marker can only be seen in embryos which have passed the blastula stage.

Donor cells

The differentiated cells used to provide donor nuclei were intestinal epithelium cells from the mid-intestine of feeding tadpoles (stages 46–48 of Nieuwkoop & Faber, 1956). These cells (plate) have the following features characteristic of their differentiated state: a tall columnar shape with basally situated nuclei; pigment granules inside the surface exposed to the gut lumen; and, most important, the striated border typical of cells having an absorptive function. Some of these cells still contain a few yolk platelets, but these are rapidly absorbed at about this stage. All the epithelium cells in the part of the intestine used for these experiments are of this kind except for less than 1 per cent. which are typical gland cells; there are no undifferentiated cells in the epithelium at this stage. The epithelium cells are larger than the other cell types present in the mid-intestine, and so can be easily recognized in the dissociated cell preparations.

Controls

Owing to the variable quality of the *Xenopus* recipient eggs laid in the laboratory (Gurdon, 1960*b*), the transplantation of intestinal epithelium cell nuclei has been accompanied by control transplantations of blastula or gastrula nuclei. Since no change in developmental capacity has been detected in *Xenopus* nuclei until after the late gastrula stage, either blastula or gastrula nuclei have been used as controls according to convenience.

RESULTS

Six experiments involving the transplantation of intestinal epithelium cell nuclei (referred to as intestine nuclei) gave similar results, and these have been combined in Table 1. In each experiment control transfers from blastulae or gastrulae (referred to as embryonic nuclei) were interspersed with transfers of intestine nuclei.

Normal tadpoles

Altogether 10 normal feeding tadpoles have been obtained from the transplantation of intestinal epithelium cell nuclei. These tadpoles have diploid nuclei carrying the nuclear marker referred to above. They therefore provide a clear demonstration that at least a few differentiated intestine cells contain nuclei

TABLE 1

The development resulting from the transplantation of nuclei from differentiated and embryonic cells of Xenopus laevis

Donor stage (Nieuwkoop & Faber, 1956)	*Total transfers*	*No cleavage*	*Total transfers resulting in cleavage*	*Development resulting from transplanted nuclei*								
				Abortive cleavage	*Partial cleavage*	*Complete blastulae*	*Arrested blastulae*	*Abnormal gastrulae*	*Abnormal post-neuralae*	*Stunted tadpoles*	*Died as swimming tadpoles*	*Normal feeding tadpoles*
Intestinal epithelium cell nuclei (stage 46–48)	726	347	379	175	156	48	18	8	5	6	1	10
	100%	48%	52%	24%	21·5%	6·5%	—	—	—	—	—	1·5%
Blastula or gastrula endoderm nuclei (stage 8–12)	279	66	213	8	32	173	4	17	19	27	6	100
	100%	24%	76%	3%	11%	62%	—	—	—	—	—	36%

which are capable of giving rise to all the cell types necessary for the formation of a feeding tadpole.

These normal tadpoles constitute only 1½ per cent. of the 726 transplanted intestine nuclei, and all the remaining transfers resulted in various degrees of abnormality ranging from a complete lack of cleavage to nearly normal tadpoles (Table 1). Some experiments have been carried out in order to determine the significance of these abnormalities and, in particular, whether the abnormalities are due to a limited developmental capacity of the transplanted nuclei or to other factors such as variation in technique. The two methods used were first, the cytological analysis of eggs fixed soon after they had been injected with nuclei, and secondly, the serial transplantation of nuclei from abnormal transplant-embryos.

The cytological analysis of eggs fixed soon after receiving transplanted nuclei

The procedure followed in this analysis was to transplant nuclei from one donor embryo into eggs laid by one frog. Soon after transplantation some of the eggs were taken at random and fixed while the remainder were allowed to develop as far as they were able. The fixed eggs were then serially sectioned and stained. Subsequent microscopic examination of the sections often revealed abnormalities of the transplanted nucleus and achromatic apparatus. The eggs which were not fixed served as exact controls since they were laid by the same frog as the fixed eggs and contained transplanted nuclei from the same donor embryo. These showed how the sectioned eggs would have developed if they had not been fixed. In this way a certain cytological abnormality could be associated with a particular developmental abnormality, thus indicating the cause of the latter. This analysis was carried out on eggs with transplanted intestine nuclei as well as on those with transplanted embryonic nuclei.

The total lack of cleavage following nuclear transfer

Forty-eight per cent. of the intestine nuclei and 24 per cent. of the embryonic nuclei failed to promote cleavage of any kind after transplantation (Table 1). The following results show that this can be attributed to a failure in the technique such that the transplanted nucleus was not effectively exposed to the recipient egg cytoplasm. The eggs fixed about 40 minutes after receiving transplanted nuclei fell into two distinct categories. First there were those with distinct regions of cytoplasm; these had an almost yolk-free area in the animal half of the egg, containing the developing transplanted nucleus, and close to it the dying irradiated egg nucleus (Gurdon, 1960*a*, fig. 1). This is the typical condition of irradiated recipient eggs which have been fertilized or in which a successful nuclear transfer has been made. The other fixed eggs revealed an entirely different situation. These had a relatively homogeneous cytoplasm just as in newly laid unfertilized eggs, and the irradiated egg nucleus was found in the vegetal half.

There was no yolk-free area in the animal half of the egg and the transplanted nucleus was either entirely absent or else could be seen inside an intact donor cell.

The total absence of the transplanted nucleus from a recipient egg is probably due to the donor cell sticking to the injection pipette and so being withdrawn from the egg with the pipette. This would not be observed in the course of an experiment unless looked for carefully. The presence of a whole donor cell in the egg clearly results from a failure to break the wall of the donor cell when it is sucked into the injection pipette. The successful breaking of the cell wall depends upon the extent to which the cell is distorted in the pipette. A very close correlation has been found between the degree of donor cell distortion and the proportion of transfers which result in normal cleavage (Gurdon, 1960*b*). It was found that if the cell wall is very little distorted, the great majority of transfers fail to cleave, while strong distortion results in many developmental abnormalities probably through exposure of the nucleus to the saline medium. It is very difficult to distort intestine cells to an ideal degree, and in order to avoid damage to the nuclei these cells were distorted rather little. Transplanted intestine nuclei would therefore be expected to result in a total lack of cleavage much more often than the nuclei of the larger embryonic cells.

TABLE 2

The cytological analysis of eggs fixed 60–80 *minutes after transplantation*

	Number of eggs fixed	*Eggs with no developing nucleus*	*Chromosomes clumped at first mitosis*	*3–4 polar spindle at first mitosis*	*Normal at first mitosis*
(*a*) Tadpole nuclei from intestinal epithelium cells	70	22 out of 70 31·5%	3 out of 11* 27%	4 out of 11* 36%	—
(*b*) Nuclei from blastulae and gastrulae	59	8 out of 59 13·5%	0 out of 30* 0%	4 out of 30* 13%	20 out of 30* 67%

The cleavage of control transfers which were allowed to develop as far as they were able

	Number of transfers	*Uncleaved*	*Abortive cleavage*	*Partial blastulae*	*Complete blastulae*
(*c*) Tadpole nuclei from intestinal epithelium cells	60	18 30%	16 26·5%	21 35%	5 8·5%
(*d*) Nuclei from blastulae and gastrulae	95	12 12·5%	2 2%	14 15%	67 70·5%

* Only these eggs were fixed at the time of the first nuclear division.

The results of the cytological analysis of fixed eggs are compared with the development of their controls in Table 2. There is a very close correspondence between the proportion of developing eggs which failed to cleave and the

proportion of fixed eggs in which the transplanted nucleus was either lacking or was present in an intact donor cell. This applies to the results of transplanting intestine as well as embryonic nuclei, and justifies the conclusion that the total lack of cleavage following nuclear transplantation can be attributed to the technical difficulty described above. The developmental capacity of nuclei which fail to promote any cleavage at all after transplantation has not therefore been tested.

Abortive Cleavage

This term refers to eggs which consist only of abnormal blastomeres and uncleaved regions of cytoplasm. The blastomeres are of irregular size and shape, and contain no normal nuclei though sometimes asters and chromatin can be seen. Such eggs usually die after a few irregular cleavages. Many of the eggs which receive intestine nuclei develop in this way, but very few of those with embryonic nuclei do so (Table 1). Useful information regarding the cause of this abnormality is provided by the cytological examination of eggs fixed during metaphase of the first division of the transplanted nucleus. Only 11 eggs with intestine nuclei were found to have been fixed at exactly this time, and in 3 of these the chromosomes were clumped and pycnotic. In some cases the spindle also seemed abnormal. It is clear that these eggs could not have cleaved normally; the chromosomes would probably have broken up into pycnotic lumps and have been distributed to abnormal blastomeres. As shown in Table 2, the percentage of fixed eggs with clumped chromosomes was very close to the percentage of control transfers which resulted in abortive cleavage. It can be concluded that abortive cleavage results from the incapacity of the transplanted nucleus to divide normally at its first division.

It is not known why some nuclei divide abnormally after transplantation, but it is possibly because their chromosomes have not replicated by the time they enter mitosis. The nuclei of intestinal epithelium cells divide infrequently and have a relatively long interphase period between mitoses. Since chromosome replication takes place during interphase, some nuclei would by chance be transplanted when at the beginning of interphase and so would have unreplicated chromosomes. The time at which a transplanted nucleus enters division is determined by the egg cytoplasm, and except in nuclei which become tetraploid, this division takes place at about 80 minutes after transplantation. Thus the situation may arise in which an intestine nucleus is forced to enter mitosis even though its chromosomes are unreplicated. This would be expected to lead to the abnormal chromosome condition described above. Embryonic nuclei, on the other hand, divide at frequent intervals during cleavage. The interphase period in which their chromosomes are unreplicated is short, and embryonic nuclei are therefore generally transplanted with already replicated chromosomes. This would explain why intestine nuclei give abortive cleavage much more often than embryonic nuclei. An hypothesis of this general kind has also been

suggested by Briggs, King, & Di Berardino (1960) to account for abnormal cleavage in their experiments.

It can be concluded that transplanted nuclei which promote abortive cleavage do so through their inability to divide normally. This prevents them showing the range of cell types that they are genetically capable of giving rise to.

Partial cleavage

A blastula is described as partially cleaved when part of it consists of normal blastomeres, and the rest is uncleaved or abortively cleaved. Blastulae of this kind usually die before gastrulation commences, but if the uncleaved portion is very small they may form abnormal gastrulae. Transplanted intestine nuclei result in partial cleavage more commonly than embryonic nuclei (Table 1). Eleven eggs with transplanted intestine nuclei were fixed during the first nuclear division, and 4 of these had apparently normal chromosomes but an abnormal achromatic apparatus with 3- or 4-polar spindles (Table 2). At least some chromosomes were present on each of the three or four metaphase plates. It is possible that a normal set of chromosomes might be distributed to one of the poles of such a spindle, leaving an aneuploid number of chromosomes at the other poles. In this way a partial blastula could be formed with the aneuploid blastomeres giving rise to the abnormally cleaved part of the egg. Whatever the cause of this condition, there is agreement between the proportion of fixed eggs with an abnormal mitotic apparatus but apparently normal chromosomes, and the proportion of the developing controls which became partial blastulae (Table 2). The significance of partial cleavage has been directly determined by the serial transplantation experiments described below.

Developmental abnormalities following the transplantation of nuclei from a foreign genus

Experiments involving the transfer of nuclei from different genera to eggs of the same species show that genetically very different nuclei may give rise to the same percentages of abnormal cleavage. These experiments therefore show that the frequency of cleavage abnormalities does not necessarily represent the degree of genetic difference between transplanted nuclei. Blastula nuclei from *Hymenochirus curtipes* and *X. laevis* were transplanted to recipient eggs of *X. laevis*, and the results are given in Table 3. The genetic difference between *Hymenochirus* and *Xenopus* is demonstrated by the early arrest in development of all *Xenopus* eggs which received *Hymenochirus* nuclei in contrast to the normal development of many of the *Xenopus* to *Xenopus* transfers. However, in spite of this, the percentage of transfers which resulted in partial, abortive, or entirely deficient cleavage was the same in both cases. These results show that the post-blastula development of transplant-embryos can indicate a genetic difference between the nuclear and cytoplasmic species, while this is not necessarily so of cleavage. Since genetically very different nuclei give the same frequency and

severity of abnormal cleavage, this provides an additional reason for believing that the abnormal cleavage resulting from transplanted intestine nuclei does not indicate any genetic difference between the nuclei of intestine and embryonic cells.

TABLE 3

The transplantation of nuclei from Hymenochirus *and* Xenopus *into recipient eggs of* Xenopus

Donor nuclei	*Total number of trans-plantations*	*Uncleaved*	*Abortive cleavage*	*Partial blastulae*	*Complete blastulae*	*Neural folds*	*Normal swimming tadpoles*
Hymenochirus curtipes early gastrula	169 100%	62 37%	1 1%	22 12%	84 50%	0 —	0 —
Xenopus laevis early gastrula	78 100%	22 28%	3 4%	11 14%	42 54%	35 —	20 —

The transplantation of nuclei from abnormal nuclear transplant-embryos

Information on the cause and significance of partial blastulae and of abnormal post-blastulae has been obtained by means of serial nuclear transfers. The basic design of the experiments was as follows. Nuclear transfers were made using original intestine or embryonic donor cells. When the resulting 'first-transfer' embryos had differentiated as far as they were able, some of their endoderm nuclei were used for serial transfers, giving rise to the 'first serial-transfer' generation. As a result of experience, the best differentiation that will be achieved by an abnormal transplant-embryo can be judged to within narrow limits, before developmental arrest takes place and cell death sets in. For instance, partial blastulae in which an appreciable part of the surface area is uncleaved, never develop beyond the late blastula or very early gastrula stage. Similarly, it has been found that embryos in which part of the yolk-plug protrudes during gastrulation will never form normal late gastrulae or neurulae, and that embryos which do not elongate properly will remain as stunted post-neurulae with a belly oedema. The furthest differentiation to be expected can with practice be judged to within much narrower limits than these. By this type of experiment the differentiation of the most normal serial-transfer embryos can be directly compared with that of the first-transfer embryo from whose nuclei they were derived.

Original donor nuclei were taken from 31 abnormal first-transfer embryos (11 from original gastrula nuclei and 20 from feeding tadpole intestine nuclei). These abnormal embryos were selected arbitrarily and are a random sample of the partial blastulae and abnormal post-blastulae included in Table 1. The nuclei from each first-transfer embryo gave rise to a wide range of abnormal embryos, and sometimes to normal tadpoles, as shown in Table 4. The differentiation of each first-transfer embryo is compared with that of the serial-transfer embryos derived from its nuclei in Text-figs. 1 and 2. In these diagrams the

stage of differentiation attained by each first-transfer embryo is shown by a solid line; the dotted continuation of this line represents the most normal differentiation achieved by any of the resulting serial-transfer embryos. It can be seen

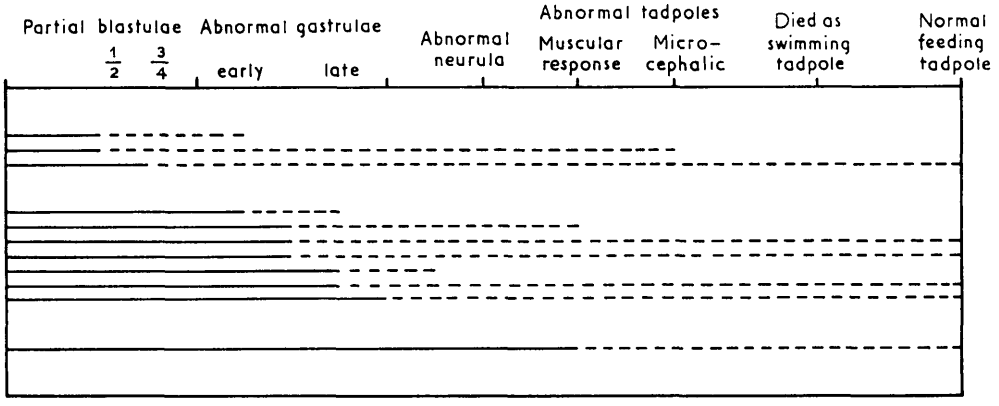

FIG. 1. Serial nuclear transfers from abnormal first-transfer embryos. Original gastrula donor nuclei (embryonic nuclei). Furthest differentiation attained by each first-transfer embryo (solid line) and by the most normal of the serial-transfer embryos derived from its nuclei (dotted line).

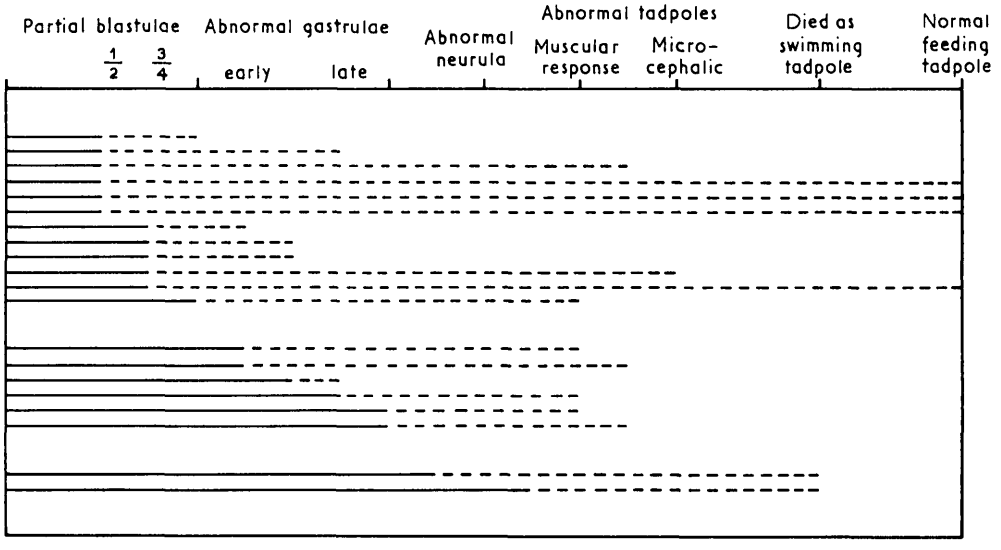

FIG. 2. Serial nuclear transfers from abnormal first-transfer embryos. Original tadpole intestine nuclei. Furthest differentiation attained by each first-transfer embryo (solid line) and by the most normal of the serial-transfer embryos derived from its nuclei (dotted line).

that in all 31 cases some of the serial-transfer embryos differentiated more normally than the first-transfer embryo from whose nuclei they were derived. It is interesting that the nuclei of partial blastulae can sometimes promote the

development of a normal or nearly normal tadpole. This is of some importance since a large proportion of transplanted intestine nuclei result in partial blastulae.

TABLE 4

Some typical examples of the development promoted by nuclei from abnormal nuclear transplant-embryos

Original donor	*Abnormal transplant-embryos used as donors*	*Total transfers*	*Partial and complete blastulae*	*Complete blastulae*	*Post-neurulae*	*Heartbeat tadpoles*	*Normal feeding tadpoles*
Gastrula endoderm cells; stages 11–13 of Nieuwkoop & Faber, 1956	½ cleaved blastula	36	8	2 (died as gastrulae)	—	—	—
	½ cleaved blastula	36	13	11	7	4	—
	¾ cleaved blastula	36	13	6	5	4	4
	Total exogastrula	35	—	9 (3 developed further than the donor)	—	—	—
	Abnormal mid gastrula	26	—	24	18	8	4
	Abnormal late gastrula	32	—	12	8	3	3
	CONTROL: Normal gastrula	57	—	14	4	3	3
Intestinal cells of feeding tadpoles; stages 46–48 of Nieuwkoop & Faber, 1956	½ cleaved blastula	36	4	4	3	3	—
	½ cleaved blastula	36	10	10	4	4	2
	¾ cleaved blastula	36	15	6 (died as gastrulae)	—	—	—
	Abnormal mid gastrula	48	—	17	4	—	—
	Abnormal late gastrula	54	—	17	4	1	—
	Abnormal early neurula	72	—	20	12	9	4
	CONTROL: Normal neurula	58	—	14	4	4	3

There are two possible explanations for these results. First, the developmental capacity of a nucleus might increase as a result of nuclear transplantation so that serial-transfer embryos are more normal than first-transfer embryos. Second, the abnormality of the first-transfer embryo might be due to some non-genetic cause such as poor egg quality. In the latter case the developmental capacity of the transplanted nucleus would not increase, but would not always be fully expressed owing to the effect of factors such as poor egg quality.

Evidence has already been obtained from different kinds of experiments that

the developmental capacity of a nucleus does not increase as a result of transplantation (Gurdon, 1960*c*). Confirmation of this has been obtained in the present experiments by making further serial-transfers in the same way as described above. Nuclei from an abnormal first-transfer embryo gave rise to the embryos of the first serial-transfer generation; the most normal of these embryos was then used to provide nuclei for a further transfer generation. In each case the most normal embryo of one transfer generation was used to provide nuclei for the next. If the developmental capacity of nuclei increases as a result of transplantation, this kind of selective serial-transfer experiment should lead to more normal development in each successive transfer generation. About ten such experiments have been carried out, and in some of these, four serial-transfer generations were made from the nuclei of one abnormal first-transfer embryo. In every experiment, the development of the embryos in each serial-transfer generation was about the same, and the later transfer generations did not contain more normal embryos than the first serial-transfer generation. These results show that the developmental capacity of a nucleus does not increase as a result of serial transplantation. It can therefore be concluded from these experiments that the minimum developmental capacity of a nucleus is shown by the most normal transplant-embryo to which it gives rise. Thus the presence of a feeding tadpole among any of the transplant-embryos derived from a nucleus shows that the nucleus had the genetic information required for this before transplantation, even though the first-transfer embryo as well as most of the serial-transfer embryos may have been abnormal.

The more normal development of the first serial-transfer embryos compared to the first-transfer embryos can be satisfactorily explained by attributing the abnormalities of the first-transfer embryos to a non-genetic cause. It is known that the quality of eggs laid by *Xenopus* in the laboratory is variable, and that poor egg quality sometimes causes abnormal development of transplant-embryos (Gurdon, 1960*b*). If the quality of some recipient eggs is variable, only a certain proportion of the transplant-embryos resulting from these eggs will develop as normally as the developmental capacity of their nuclei will allow. The effect of making serial transfers is to transplant the mitotic products of a nucleus into several different recipient eggs, so that at least some of these are of good quality and therefore demonstrate the real developmental capacity of the original somatic nucleus. So long as a sufficient number of transfers are made in the first serial-transfer generation for at least some eggs to be of good quality, the later serial-transfer generations would not be expected to contain more normal embryos than the first-transfer generation. As pointed out above, the most normal embryo that can be obtained from an original donor nucleus is generally contained in the first serial-transfer generation. The effects of poor egg quality therefore account for all the results reported here.

It has now been shown that the developmental capacity of a nucleus and of its mitotic products does not increase from one transfer generation to the next,

but that the serial-transfer embryos may differentiate more normally than the first-transfer embryo derived from the same original donor nucleus. These two observations together show that the abnormality of many transplant-embryos must be due to non-genetic factors such as poor egg quality. This is true of all abnormal transplant-embryos which contain some nuclei capable of giving rise to more normal differentiation than they showed themselves, and this includes all 31 first-transfer embryos used in these experiments.

The developmental capacity of original donor nuclei from which abnormal first-transfer embryos were obtained

It has been established above that the developmental capacity of a somatic nucleus is generally shown by the most normal embryos of the first serial-transfer generation. The right-hand end of each dotted line in Text-figs. 1 and 2 therefore represents the developmental capacity of one original somatic nucleus. The developmental capacity of the original intestine nuclei used in these experiments can now be compared with that of the original embryonic nuclei. The capacity of embryonic and intestine nuclei to give rise to normal feeding tadpoles is shown by the number of dotted lines which reach the right-hand extremity of Text-figs. 1 and 2. It can be seen that 6 out of 11 (55 per cent.) of the original embryonic nuclei and 4 out of 20 (20 per cent.) of the original intestine nuclei were able to promote the differentiation of a normal feeding tadpole.

The following conclusions can be drawn for these results: (i) Of the original intestine nuclei which gave rise to partial blastulae and abnormal post-blastulae after first-transfer, 20 per cent. contained the genetic information required for the formation of feeding tadpoles. The equivalent figure for original gastrula nuclei was 55 per cent. The numbers on which these figures are based are too small to show whether they indicate a significant difference in developmental capacity between intestine and embryonic nuclei. (ii) Of the original intestine nuclei which gave rise to partial blastulae and abnormal gastrulae after first-transfer, 12 out of 18 (67 per cent.) were capable of giving rise to *muscular-response stage tadpoles* with functional nerve and muscle cells. The comparable figure for original gastrula nuclei was 7 out of 10 (70 per cent.). Both of these values are obtained from the results given in Text-figs. 1 and 2.

DISCUSSION

The genetic information contained in the nuclei of differentiated cells

The genetic information contained in a nucleus before transplantation is shown by the range of cell types that its mitotic products can form after its transplantation so long as the genetic information carried by a nucleus does not change as a result of transplantation itself. It will be assumed in this discussion that the developmental capacity of a nucleus and of its daughter nuclei

does not increase as a result of transplantation. There is no evidence that any increase does take place, and the evidence that it does not comes from the serial transplantation of nuclei between different species and subspecies (Gurdon, 1962*b*) and of nuclei from abnormal transplant-embryos within the same subspecies (p. 632). Thus, if a transplanted nucleus supports the development of a feeding tadpole, this is regarded as showing that it possessed the genetic information required for this when it was present in the donor cell, and did not acquire this information only after transplantation. This applies to the results of serial transfers just as much as to the results of first transfers, since apart from variation in donor stage, exactly the same procedure is involved in both kinds of experiment. Thus the genetic information contained in a nucleus is regarded as equal to or greater than that required to form the most normal of the transplant-embryos derived from it, whether by first or serial transfer. This is so even when the first-transfer embryo is abnormal and when only a few of the serial-transfers form feeding tadpoles.

The minimum genetic information contained in the nuclei of intestinal epithelium cells can be determined by combining the results of first transfers and serial transfers. The first transfers showed that $1\frac{1}{2}$ per cent. of the intestine nuclei can give rise to feeding tadpoles. Serial transfers have shown that a further $5\frac{1}{2}$ per cent. of the intestine nuclei also have this capacity. This figure of $5\frac{1}{2}$ per cent. represents the proportion of intestine nuclei which gave partial blastulae and abnormal post-blastulae after first-transfer, but which could give rise to some feeding tadpoles after serial transfer. This figure is calculated as follows. It was found that 27 per cent. of the original intestine nuclei formed partial blastulae and abnormal embryos after first transfer (Table 1). About 20 per cent. of these, or $5\frac{1}{2}$ per cent. of the original intestine nuclei, gave rise to feeding tadpoles after serial transfer (p. 633). Combining the results of first and serial transfers, at least 7 per cent. of intestinal epithelium cell nuclei contain the genetic information required to form all cell types of a feeding tadpole, except perhaps for the germ cells whose presence has not yet been looked for.

This figure of 7 per cent. is expressed in terms of the total number of transplanted intestine nuclei. However, the developmental capacity of many of these nuclei was not in effect tested, and the percentage of intestine nuclei capable of promoting the formation of feeding tadpoles is increased if this is taken into account. The cytological examination of fixed eggs showed that the total lack of cleavage after transplantation can be wholly attributed to technical difficulties. The transfers which result in no cleavage are a random sample of the total transfers made and can be discounted from the results. The intestine nuclei capable of promoting the formation of feeding tadpoles then constitute 13 per cent. of the remaining successful transfers (Table 5).

It can be inferred from the cytological analysis of fixed eggs that abortive cleavage results from nuclei which happen to have been taken for transplantation at an unsuitable stage in their mitotic cycle. If this is so, then the nuclei

which give rise to abortive cleavage are a random sample of those transplanted, and can be excluded from the total number of transfers. The remaining nuclei are those which were transplanted successfully as well as at a suitable stage in their mitotic cycle for their developmental capacity to be tested; the intestine nuclei capable of supporting the development of feeding tadpoles then constitute 24 per cent. of these (Table 5).

TABLE 5

Summary of conclusions reached regarding the developmental capacity of nuclei taken from differentiated and embryonic cells of Xenopus laevis

Donor nuclei	*Developmental capacity of nuclei. Stages of Nieuwkoop & Faber, 1956*	*Results of first-transfers only as percentage of total transfers*	*Combined results of first and serial transfers**		
			As percentage of total transfers	*As percentage of total transfers, less those resulting in no cleavage*	*As percentage of total transfers, less those resulting in no cleavage or abortive cleavage*
Intestinal epithelium cell nuclei	Capable of forming feeding tadpoles; stage 50	1·5% (10)	7% (49)	13% (49)	24% (49)
Blastula or gastrula cell nuclei		36% (100)	57% (158)	74% (158)	77% (158)
Intestinal epithelium cell nuclei	Capable of forming muscular response tadpoles; stage 26	2·3% (17)	20% (142)	37% (142)	70% (142)
Blastula or gastrula cell nuclei		48% (133)	65% (181)	85% (181)	88% (181)

The figures in brackets represent the number of individuals.

* The figures for serial transfers were calculated as described on p. 634.

The main conclusions from these results are the following, though the evidence for each is not equally strong.

(i) It has been clearly shown that about 7 per cent. of the total number of transplanted intestine nuclei have the genetic information required to form normal feeding tadpoles. This figure represents the combined results of first and serial transfers expressed as a percentage of total transfers.

(ii) Thirteen per cent. of the eggs receiving *successfully* transplanted intestine nuclei can give rise to feeding tadpoles. This figure represents the combined results of first and serial transfers expressed as a percentage of those transfers which resulted in some kind of cleavage. There is good evidence that the transfers which result in no cleavage do so for technical reasons and are a random sample of the total transfers.

(iii) The formation of normal feeding tadpoles can be promoted by 24 per cent. of the intestine nuclei which were transplanted successfully as well as at

a suitable stage in their mitotic cycle. This figure is the combined results of first and serial transfers expressed as a percentage of the total transfers excluding those which resulted in no cleavage or abortive cleavage.

If the capacity of a transplanted nucleus to give rise to muscular response tadpoles with functional nerve- and muscle-cells is considered, then a greater percentage of intestine nuclei fall into the three categories above. These percentages as well as the equivalent figures for embryonic nuclei are given in Table 5.

The differentiation of cells and the developmental capacity of their nuclei

The results so far obtained from nuclear transplantation experiments in Amphibia have contributed in two ways to the question of whether stable nuclear changes are causally connected with cellular differentiation. Some experiments have shown that different kinds of cells may have unchanged nuclei, while others have demonstrated a stable restriction of developmental capacity in the nuclei of differentiating cells.

The following results have demonstrated the wide range of genetic information contained in the nuclei of cells which are approaching, or which have actually attained, the differentiated state. The experiments described above are of this kind; they show that at least 7 per cent. of the nuclei of intestinal epithelium cells can promote the formation of normal feeding tadpoles, and that at least 20 per cent. can promote the formation of muscular response tadpoles with functional muscle- and nerve-cells (Table 5). Evidence of this kind has also been described by King & McKinnell (1960). From 142 eggs of *Rana pipiens* injected with 10–20 adenocarcinoma cell nuclei they obtained one post-neurula embryo showing a certain degree of tissue differentiation.

It can be argued that some cells may become differentiated under the influence of nuclei from neighbouring cells, and hence that a few cells in a differentiated tissue may have nuclei capable of forming normal tadpoles while the majority of cells have nuclei which do not possess the capacity to form other cell types. Such an argument seems to be excluded by the experiments with intestine nuclei in *Xenopus*, since it was found that at least 20 per cent., and probably 70 per cent. (Table 5), of these nuclei could give rise to muscle- and nerve-cells after transplantation. These experiments therefore show that a nucleus can be responsible for the formation of an intestinal epithelium cell and at the same time possess the capacity to form other kinds of differentiated cells.

Other experiments have clearly shown that some of the nuclei derived from somatic cells have undergone a stable change restricting their developmental capacity. The clearest evidence for these changes comes from serial nuclear transplantation experiments in *R. pipiens* (King & Briggs, 1956) and in *Xenopus* (Gurdon, 1960*c*). These experiments have not shown that the nuclear changes took place during the normal development of the donor embryos from which the nuclei were taken. If this should prove to be the case, it still remains to be

determined whether these nuclear changes are necessary for cellular differentiation to take place, or whether they are only a result of this.

Until the significance of stable nuclear changes is known, the results of nuclear transplantation experiments seem to be consistent with the view that stable changes restricting the developmental capacity of nuclei are not essential for cellular differentiation to take place. This conclusion can now be related to different theories of differentiation.

Cellular differentiation is most probably initiated by the effect of the cytoplasmic environment on a nucleus, so that the nucleus provides specific genetic information which promotes the formation of a particular cell type (recent discussion by Fischberg & Blackler, 1961). Three possible ways in which this could happen are the following. First, nuclei might undergo a progressive loss of genetic material, so that cellular differentiation would result from the genetic material that is retained in different nuclei. Secondly, an inactivation of certain parts of the genetic material might take place, so that specific genetic information would be provided by the non-inactivated parts of a genome. This kind of inactivation would be stable under the normal conditions of cell mitosis. A theory of differentiation along these lines is suggested by various reports of stable nuclear changes in somatic cells (e.g. Brink, 1960). The third possibility is that the genetic information provided by a nucleus is entirely dependent on its cytoplasmic environment at any one time; in this case a nucleus would never undergo any stable changes having a qualitative effect on its function. This kind of system is suggested by the reversible appearance of puffs in the polytene chromosomes of insects (e.g. Breuer & Pavan, 1955) and by cases of metaplasia (e.g. Reyer, 1954). The first of these three possibilities is rendered very improbable by the results of the experiments reported in this article; these have shown that a nucleus may be responsible for the differentiation of one cell type while still possessing the capacity to form all other types of somatic cell in a feeding tadpole. It has previously been found that most of the normal feeding tadpoles resulting from transplanted nuclei of *Xenopus* will eventually form adult frogs (Gurdon, 1962*a*). However, the possibility still exists that intestine nuclei may have undergone stable changes restricting their capacity to form adult frogs and normal germ cells, since intestine nuclei have not yet been tested in these respects. These results are therefore consistent with any theory of cell differentiation which does not require that the nucleus of a differentiated cell has lost the genetic information required for the formation of other differentiated somatic cell types.

SUMMARY

1. Nuclei from differentiated intestinal epithelium cells of feeding tadpoles and from control blastulae of *Xenopus* have been transplanted into enucleated recipient eggs. The differentiated state of the intestinal epithelium cells was shown by their possession of a striated border.

2. The cleavage and embryonic development resulting from the intestinal epithelium cell nuclei was much more abnormal than that resulting from control blastula transfers.

3. 1½ per cent. (10 out of 726) of the first transfers of intestine nuclei resulted in normal feeding tadpoles.

4. The serial transplantation of nuclei and of their mitotic products showed that some of the intestine nuclei which promoted abnormal development after first transfer could nevertheless promote the formation of normal feeding tadpoles after serial transfer. The combined results of first transfers and of serial transfers demonstrated that at least 7 per cent. of the intestine nuclei possessed the genetic information required for the formation of normal feeding tadpoles.

5. The cytological examination of eggs fixed soon after receiving transplanted nuclei indicated that the lack of cleavage and abortive cleavage following transplantation result from nuclei which were not effectively exposed to the recipient egg cytoplasm or which were transplanted at an unsuitable stage in their mitotic cycle. If these cases are excluded from the results, the intestine nuclei capable of promoting the formation of feeding tadpoles then constitute 24 per cent. of the remaining successful transfers.

6. A similar interpretation of the experimental results shows that 70 per cent. of the successfully transplanted intestine nuclei have the genetic information required to form muscular response stage tadpoles with functional muscle- and nerve-cells.

7. These results show that a nucleus can promote the formation of a differentiated intestine cell and at the same time contain the genetic information necessary for the formation of all other types of differentiated somatic cell in a normal feeding tadpole. It is concluded that the differentiation of a cell cannot be dependent upon the incapacity of its nucleus to give rise to other types of differentiated cell.

RÉSUMÉ

Potentialités de développement de noyaux issus de cellules de l'épithélium intestinal de têtards se nourrissant

1. Des noyaux de cellules différenciées de l'épithélium intestinal et de blastulas témoins ont été transplantés dans des œufs énucléés chez le *Xénope*. On reconnaît l'état différencié des cellules de l'épithélium intestinal à la présence d'une bordure striée.

2. La segmentation et le développement embryonnaire obtenus à partir de noyaux de cellules de l'épithélium intestinal sont beaucoup plus anormaux que ceux obtenus à partir de noyaux de blastulas.

3. 1½ pour cent (10 sur 726) des transplantations simples ont donné des têtards normaux se nourrissant.

4. La transplantation en série de noyaux et de leurs descendants a montré que des noyaux d'intestin ne fournissant qu'un développement anormal lors

de la première transplantation sont néanmoins capables de réaliser le développement d'un têtard normal à la prise de nourriture après une série de transplantations. Les résultats combinés des transplantations simples et des transplantations en série ont montré qu'au moins 7 pour cent des noyaux de l'intestin possèdent l'information génétique nécessaire au développement d'un têtard normal se nourrissant.

5. L'étude histologique d'œufs fixés sitôt après la transplantation d'un noyau a montré que l'absence de segmentation et l'apparition de segmentation abortive après transplantation de noyau provenaient de cas où le noyau ne s'était pas vraiment trouvé au sein du cytoplasme de l'œuf hôte, ou bien de noyaux ayant été transplantés au cours d'une phase défavorable du cycle mitotique. En tenant compte de ces cas dans les résultats, les noyaux d'intestin capables d'édifier un têtard normal se nourrissant représentent 24 pour cent des cas où la transplantation a été effectuée avec succès.

6. Une interprétation semblable des résultats expérimentaux montre que 70 pour cent des noyaux d'intestin transplantés avec succès possèdent l'information génétique permettant d'obtenir un embryon au stade de la réponse musculaire, possédant des cellules musculaires et nerveuses fonctionnelles.

7. Ces résultats prouvent qu'un noyau est capable de participer à la formation d'une cellule intestinale différenciée, tout en possédant cependant l'information génétique nécessaire à l'édification d'un têtard normal. On peut en conclure que la différenciation d'une cellule n'est pas pour son noyau l'inaptitude à fournir tout autre type de cellule différenciée.

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EXPLANATION OF PLATE

Sections of the mid-intestine of a feeding tadpole of *X. laevis*. Owing to the coiling of the intestine the sections are only transversely cut in some places. By serial sections it could be seen that some part of each cell reaches the gut lumen and constitutes part of the striated border. The striated border and underlying pigment granules can be most clearly seen in fig. C.

Figs. A and B. Stage 46 of Nieuwkoop & Faber (1956).

Fig. C. Stage 47. Most of the donor cells used in these experiments were taken from tadpoles at this stage.

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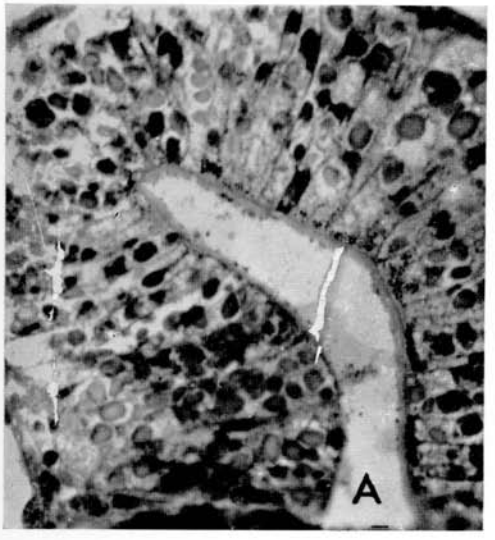
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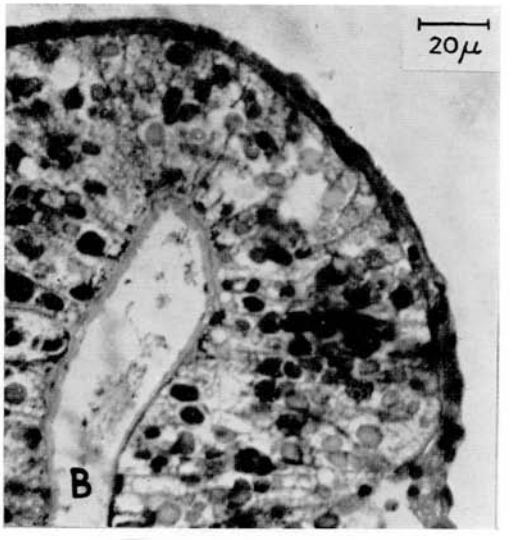
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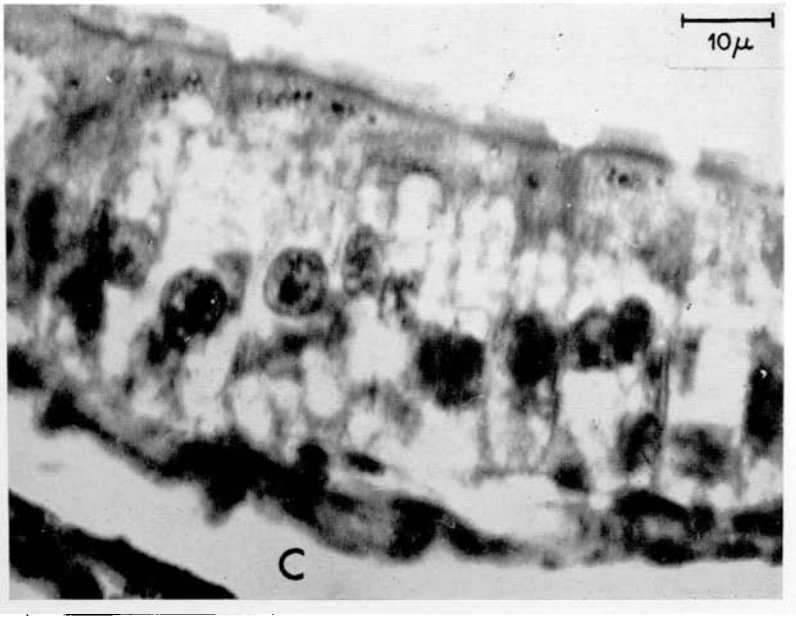
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