X-ray Inactivation of Nuclei as a Method for Studying their Function in the Early Development of Fishes

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INTRODUCTION

It is generally accepted at present that during cleavage in echinoderms, amphibians, and fishes, the nuclei do not have specific functions in regulating development, their role being at this time restricted to participation in the processes of cleavage (Schechtman & Nishihara, 1955). Eggs devoid of nuclei sometimes begin cleavage which may proceed up to the stage of the late blastula. Exirpation or inactivation of nuclei may be achieved through the separation of the nuclear region of the egg by means of centrifugation (Harvey, 1940); through extirpation of the female nucleus followed by fertilization with sperm inactivated by a heavy dose of radiation (Briggs, Green, & King, 1951); through spontaneous degeneration of the male nucleus during artificial androgenesis (Stauffer, 1945); and by means of other techniques.

Exposure of early cleavage stages in amphibians (Mangold & Peters, 1956; Sanides, 1956) and fishes (Neyfakh, 1956a) to heavy doses of ionizing radiation also leads to arrest of development at the late blastula stage. Such arrest at late blastula or early gastrula is observed in many lethal hybrids of echinoderms, amphibians (see the survey of Moore, 1955), and fishes (Korovina, 1939). This syndrome has acquired the special name of ‘arrested blastula’.

One may say that the conditions necessary for the development of the egg from its fertilization up to the ‘arrested blastula’ are already created during ovogenesis (Mazia, 1952) through the activity of the egg’s own nucleus and, perhaps, of the nuclei of follicular cells. Thus, beginning with Boveri (1918), the view developed that in ontogeny the nuclei begin their specific developmental function only just before the onset of gastrulation. This viewpoint was recently supported by the fact that the synthesis of specific proteins of the male parent (detected by the appearance of antigenic properties) may be observed in the hybrids of sea-urchins only after the onset of gastrulation (Harding, Harding, & Perlman, 1954; Harding, Harding & Bamberger, 1955).

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It is, however, hardly possible to identify the manifestation of nuclear activity at the onset of gastrulation with the real beginning of nuclear activity: obviously some time has to elapse between the beginning of the activity and its observed results. The methods of investigation referred to above do not allow one to investigate this point, as the extirpation or inactivation of the nucleus is performed almost at the beginning of development, so that the experimenter cannot obtain information about the processes going on between the stage of treatment and that of observation.

The work presented here is an attempt to use selective damage to the genetical nuclear apparatus by heavy doses of X-radiation to investigate nuclear function in the developing embryo.

The nuclei of eggs and early embryos are known to be more highly radiosensitive than the cytoplasm; in a number of cases this has been quantitatively determined. Androgenetic diploid embryos of Bombyx mori develop normally after irradiation of eggs with a dose exceeding 500,000 r. before fertilization (Astaurov, 1947). One α-particle entering the nucleus of the Habrobracon egg has in the statistical sense the same effect (63 per cent. mortality) as 2 × 10⁷ α-particles entering the cytoplasm; when evaluated in equal volumes this corresponds to a nuclear sensitivity 3,000 times higher than that of the cytoplasm (Borstel & Rogers, 1958). The cytoplasmic sensitivity of fish eggs becomes noticeable after irradiation with doses of the order of 60–80 thousands of röntgens (Neyfakh, 1956b), while radiation-induced damage in the nucleus, manifested in decreased embryo survival, becomes evident after treatment with a dose as small as 100 r. Chromosome aberration can be found after a still smaller dose, namely, 25 r. In some cases one may suppose that the damage to the nucleus is determined indirectly, through the action of irradiated cytoplasm. But even if this effect plays some part, it does not affect the main conclusion that the disturbance of development and activity of the embryo is determined by the radiation effect upon the nucleus (Astaurov, 1958).

It seems, therefore, practicable to find a dose-range within which the radiation destruction of the nucleus will be practically complete, whereas that of the cytoplasm will not yet be significant. Under these conditions the radiation effect may be compared to the simultaneous extirpation of all the nuclei from the embryo. Embryonic development after such a procedure can proceed only up to the time determined by the preceding nuclear activity and may show itself as a temporary ability of the cytoplasm to undergo independent development.

MATERIAL AND METHOD

The work was carried out on embryos of the pond-loach (groundling), Misgurnus fossilis L. This species has until recently been undeservedly little used in embryological and radio-embryological studies. The possibility of working the

1 This expression, and analogous ones used below, is intended to mean not only that the nucleus is more injured than the cytoplasm, but also that nuclear damage is more often fatal to the cell than equivalent damage to the cytoplasm.
EARLY FISH DEVELOPMENT

Year round, the simplicity with which the adult fish may be kept, the ease with which mature gametes may be obtained, their abundance, and the tolerance of the eggs when incubated, permit one to class the loach with such embryological material as the frog, chick, or sea-urchin.

Text-fig. 1. Stages of normal development of the loach. Temperature 21.5°. A, 2 hours, 4 blastomeres; B, 4 hours, 32 blastomeres; C, 5 hours, morula; D, 7 hours, mid-blastula; E, 9 hours, late blastula; F, 11 hours, early gastrula; G, 15 hours, mid-gastrula; H, 18 hours, end of gastrulation; I, 28 hours, 13 somites.
Mature gonad products were obtained 30–36 hours (temperature 18°C) after injection of a chorionic gonadotropic hormone preparation (200 rat units, preparation of the Institute of Experimental Endocrinology, Moscow). Artificially inseminated eggs with a fertilization rate of 70–98 per cent. were incubated in Petri dishes, up to 250 eggs per dish of 9 cm. in diameter at a temperature of 21–22°C, which is the upper optimal limit.

Developmental stages are shown in part in Text-fig. 1. Each stage is referred to by the number of hours of development required to reach it at 21.5°C. Even if the actual temperature of incubation is lower, and development consequently slower, the same method of referring to the stages is used. Thus the early gastrula stage ‘13 hours’, begins after 13, 19, and 26 hours at temperatures of 21.5°C, 18°, and 13°, respectively.

Irradiation was carried out in a small volume of water (closely packed eggs were just covered with water) with the X-ray apparatus RUP–1, using 185 kV., 15 mA., no filter, HVL–3 mm. Cu., distance of 75–120 mm., and dose rate of 2–5 kr./min. Exposure time, depending on the dose, was from 1 to 20 minutes.

As criteria of the radiation effect, the amount of development and the time of survival were chosen. The amount of development was expressed as the maximal stage reached (‘arrested stage’, designated by hours of normal development at 21.5°C). The later development of irradiated embryos always proceeds slowly and the actual time taken was disregarded. Text-fig. 2 shows the time course of development for normal and irradiated embryos and the way in which the ‘arrested stage’ is determined. Individual fluctuations in development are small when heavy doses are applied. The error in assigning the developmental stage to
normal embryos does not exceed $\frac{3}{4}-1$ hour, but is somewhat more in irradiated ones. Determination of the developmental stage in malformed embryos at post-gastrulation stages is to a certain degree subjective. When comparing two embryos, however, the more developed is readily distinguished from the earlier arrested. Survival data, supplementing the data on development, provide more precise quantitative expression than stage determination for malformed embryos.

As the survival criterion, the time in hours at standard temperature when an 80 per cent. mortality is reached was chosen (Text-fig. 3). This time differed little (1–2 hours) from that of total mortality, but it could be determined more precisely. Counting of dead embryos and assessment of stage were carried out every 1–2 hours. The error in the determination of the moment of 80 per cent. mortality of the embryos is at most half this interval.

**RESULTS**

*Dose dependence at various developmental stages*

In order to select radiation doses meeting the aim of this work, the eggs were exposed to irradiation with doses ranging from 1.5 to 100 kr. at different developmental stages. The results expressed in the maximal stage reached ('arrested stage') and in the 20 per cent. survival time are presented in Text-figs. 4 and 5.

The curves representing the dependence of development on dose are more or less similar with different irradiated stages. The steep exponential drop with small doses is succeeded by complete, or almost complete, parallelism to the abscissa. With very heavy doses the slope of the curves somewhat increases again. The dose at which the inflexion of the curve takes place is less when
TEXT-FIG. 4. Dependence of the arrested stage on the irradiation dose.

TEXT-FIG. 5. Dependence of the time of survival of 20 per cent. of embryos on the irradiation dose.
irradiating earlier (at the 3-hour stage, 6 kr.; at the 5-hour stage, 10 kr.) than when irradiating later (at the 13- and 15-hour stages, 20 kr.). When irradiating at the 7-hour stage the inflexion is less marked and a certain slope is retained throughout the curve. When irradiating at the 17-hour stage there is no plateau though the slope is small with large doses.

Survival curves have the same pattern in general. Irradiation at the earliest time (up to 3 hours) is, however, an exception: here a paradoxical effect is observed, namely, a slightly increased survival time with heavier doses. This is explained by some delay in cleavage, so that the more strongly irradiated embryos attain the stage when death occurs later than those irradiated with lower doses. This cleavage delay is marked in sea-urchins (Henshaw, 1940) and other invertebrates, less marked in amphibians (Kheissina, 1956), and slight in fishes.

The strong dependence on dose seen in the left part of the curves can only be explained by damage to the genetical structure of the nucleus. The plateau formation within the dose range of 6–20 to 60 kr. means that with these doses radio-sensitive structures are maximally damaged. Thus 20 kr. may serve as the lower dose limit meeting the requirements of the present work. The upper limit cannot be defined precisely enough, but 60 kr. is probably the dose at which cytoplasmic effects become significant at later developmental stages. At the end of gastrulation (17 hours) application of heavy doses cannot give precise results comparable to those obtained at earlier stages because of the appearance of some dependence on dose within the range chosen.

**Dependence of development and survival on the stage of exposure to irradiation**

Data on irradiation at different developmental stages are set out in Text-figs. 6 and 7. The later the stage exposed to irradiation, the higher (along the ordinate) lie the points corresponding to the moment of irradiation, arrest of development, and 20 per cent. survival. The curve connecting the points of arrest of development shows the dependence of the stage of developmental arrest on the stage of irradiation: the curve connecting the points representing 20 per cent. survival similarly shows the dependence of survival on stage of irradiation.

Text-fig. 6 shows the results of one experiment. The different curves correspond to different irradiation doses. The lower part of the curves, concerned with the first few hours of development, has been described in more detail elsewhere (Neyfakh & Rott, 1958) and will be considered here only briefly. The various periods into which the curves fall were carefully studied in many experiments, and in all cases the results did not differ essentially. Text-fig. 7 shows some curves obtained in analogous experiments to demonstrate the reproducibility of the pattern of the curves.

The curve relating development and stage of irradiation may be divided into four periods.

The first period covers irradiation from the beginning of development to the stage of the early large-celled blastula (from 0 to 6 hours). The development of all
TEXT-FIG. 6. Effect of high irradiation doses (20, 40, and 60 kr.) on different developmental stages. Each point on the line labelled 'moment of irradiation' represents the irradiation of one series of embryos. The stage at irradiation may be determined by the projection of this point either on the ordinate or on the abscissa. The development of the irradiated embryo proceeds from the moment of irradiation up to the arrested stage and the amount of development may be determined as the horizontal distance between the 'moment of irradiation' and the corresponding point on one of the curves labelled 'arrest of development'. The different curves correspond to different dosages. The embryo arrested in its development survives for a time. Eighty per cent. of the embryos are dead at the time (the actual time, not the stage) indicated by the corresponding point on one of the curves labelled 'time of 20 per cent. survival'.
the embryos exposed to irradiation during this period is arrested at the stage of late blastula ("arrested blastula"). No differences were found between doses within the range from 20 to 60 kr., as in the experiment of Text-fig. 6, or from 6 to 80 kr. in other experiments. There is no dependence on the stage of irradiation within this period.

The second period covers irradiation from the early to the late blastula (from 6 to 8½ hours). Its range may vary in different experiments by half an hour of standard development. The arrest of development during this period, unlike the preceding one, depends sharply on the stage of irradiation. An embryo exposed to radiation 1 hour later than another halts its development at a stage equivalent to...
to 2–4 hours later. During this period some dependence on dose is observed (cf. the curve for the 7th hour, Text-fig. 4), though as a whole the curves for all the three doses of Text-fig. 6 (20, 40, 60 kr.) closely follow each other.

The third period covers irradiation from the late blastula to the mid-gastrula (from 8½ to 14–15 hours). As in the first period, no dependence on stage of irradiation was observed within this period. But the anlage of the axial organs, normally appearing by this time, is lacking in irradiated embryos. Some dependence on dose during this period is manifested in the fact that after 20 to 40 kr. the yolk becomes entirely covered, whereas after 60 kr. development stops at the 16-hour stage, before the yolk is covered.

The fourth period starts with the mid-gastrula. The criterion of the beginning of this period, a small bulge produced by the first formation of axial organs, cannot unfortunately be defined with precision. The effects of irradiation of later stages were followed in the experiment of Text-fig. 6 beyond the end of gastrulation (to the 20-hour stage). During this period a marked dependence on stage of irradiation was again observed. Some dependence on the dose also begins to appear, so that the designation of a stage as the maximal one reached begins to lose its significance; though with all the doses applied (20–60 kr.), through the whole period, there is no doubt of the objective dependence of development on the stage of irradiation. Development after irradiating at the stage of the first somites, though it cannot be estimated precisely enough, proceeds up to hatching, when the death of the majority of embryos prevents further observations.

Survival curves, in general, follow rather closely the developmental curves. As with the latter, four periods may be distinguished, of which the first and third are characterized by independence of stage of irradiation, whereas the second and fourth are characterized by strong dependence. A dependence on dose is observed to a small degree in the third period, and to a greater degree in the fourth period.

The essential peculiarity of the 20 per cent. survival curve is the fact that all its transitions from one period to the next occur at an irradiation stage 1–1½ hours younger than those of the curve of arrest of development. This independence shows that the processes concerned in arrest of development and in mortality, though closely related, are not quite identical.

**Mortality dynamics**

Increased mortality of the embryos at 11–14 hours of development (at standard temperature) after irradiation in the first period, and at 22–26 hours after irradiation in the second period, may suggest that there are non-specific ‘critical stages’ at these times. Such an idea can be tested by analysis of the curves showing the dependence of mortality on time. Mortality of the embryo in ‘critical stages’, if such exist with respect to radiation, must increase with time relatively independently of the stage of irradiation and of the dose, and must be determined only by the developmental stage of the embryo.
Text-fig. 8 shows mortality curves obtained when varying the stage of irradiation with doses of 20–40 kr. From the 11th hour of development a mortality may be achieved which begins and ends at almost any moment of development. Thereby, the curves which are completely included within the suggested 'critical stages' do not differ from others which either start in these periods, end in them, or lie beyond them.

**DISCUSSION**

*Effect of heavy doses of ionizing radiation*

Analysis of the dependence of radiation effect on dose (Text-figs. 4 and 5) show that the early fish embryo represents, with respect to radiation, a system of two components with different sensitivity or different role. Damage to the first component (the radio-sensitive one) occurs at doses of the order of 6–20 kr., whereas damage to the second component (the radio-resistant one) begins at doses of the order of 60–100 kr. All the data available in the literature suggest that it is the cell nuclei that are likely to be the radio-sensitive component and the cytoplasm the radio-resistant one. After irradiation of the eggs or early embryos with heavy doses arrest of development takes place at the late blastula stage, which is typical for nuclear injuries. Only by increasing the dose up to 80–100 kr. can one obtain an earlier or even immediate effect (arrest of develop-
Periodical increases of radio-sensitivity in the course of cleavage precisely coincide with the rhythm of the cleavage divisions (Neyfakh, 1956a) and correspond to telophase (Belyayeva & Pokrovskaya, 1959). It is obvious that here, as in other material (Luther, 1938; Henshaw, 1940) the change in radio-sensitivity is determined by the changes in chromosome structure; the chromosomes are, therefore, the primary objects of damage.

The increase and decrease of the mortality rate of embryos when irradiated at different phases of the mitotic cycle coincide with an increase and decrease of chromosome aberration frequency, which directly proves the injury to the genetic apparatus.

When applying larger radiation doses, of the order employed in the present work, the injuries to the nucleus become even more profound. Karyokinesis is depressed (though cytokinesis is retained in early cleavage), and atypical and pycnotic forms of nuclei appear. A Feulgen-positive substance is found outside the nuclei and even outside the cells (Belyayeva & Pokrovskaya, 1958). An analogous appearance of Feulgen-negative nuclei was described in amphibians (Kheissina, 1956) and in Habrobracon (Borstel, 1955).

Radiation injury of the cytoplasm may be ascribed to the doses of 60–100 kr. only tentatively. The precise form of this effect, its dependence on dose and on developmental stage, have been studied but little. It would therefore be more correct to confine oneself to the statement that up to a dose of 60 kr. the primary radiation injury to the cytoplasm is of a restricted character, usually undetectable in development.

Analysis of the dose-effect curves permit us to state that with doses of 6–20 kr., in which range there is a complete or almost complete independence of effect on dose, the injury of the radio-sensitive component is the maximum possible for ionizing radiation. So far as it is development and survival which serve as the criteria of the radiation effect, it may be rigorously stated that with the above doses the activity of the radio-sensitive component in the regulation of development and viability is completely inhibited.

An important question arises as to how soon the inactivation of the nucleus takes place after irradiation. If the time required for inactivation of the nucleus depends to some extent on the radiation dose (within the range of heavy doses used) one may expect that during the period of nuclear activity a certain dependence on dose will be observed, due to the fact that a smaller dose (e.g. 20 kr.) inactivates the nucleus somewhat later than a heavier one (e.g. 40 kr.). Text-figs. 6 and 4 (for the 7th hour) show indeed that such a dependence exists. Since, however, this dose-dependence, even with a wide dose range, does not exceed 2–2½ hours, which corresponds to 40–50 minutes of nuclear function during this period, it appears that the time required for inactivation of the nucleus after irradiation is not large and indeed hardly significantly exceeds the error of the method, which has been evaluated as 1½–1 hour.
The role of the nucleus in the effect of radiation on development

Inactivation of the nuclear genetic apparatus does not lead to the immediate arrest of development. For some time after irradiation the development proceeds at the normal tempo, then this tempo slows down and at last development is arrested (cf. Text-fig. 2). Depending on the irradiation stage, the embryo can pass through stages corresponding to 3–10 hours of normal development. This is possible because development itself is realized by radio-resistant cytoplasmic structures, which may exist independently for some time. Nevertheless, the connexion with the nucleus is here much closer than, for example, in *Acetabularia*

or amoeba which may exist without the nucleus for a long time, retaining the power of protein, and, in *Acetabularia*, even of ribonucleic acid, synthesis (Brachet, 1952; Chantrenne, 1958).

The nucleus may be regarded from two aspects: as the site of the formation of non-specific substances necessary for the realization of the developmental processes in the cytoplasm, and as the source of specific hereditary information by means of which the genotype controls the development of phenotypical character (Brachet, 1952; Mazia, 1952; Stern, 1955; Chantrenne, 1958). One may conceive that the nucleus excretes a limited amount of substances consumed in the course of development, or that a certain structural organization arises which is able to ensure only the immediately succeeding developmental processes. In both cases the fate of the embryo after irradiation is determined by the 'stock' present in the cytoplasm at the moment of irradiation.

Analysing the prolongation of development after irradiation at different stages...
(Text-fig. 9) one may see that the amount of this 'stock' is different at different moments of development. This leads to the conclusion that the nucleus functions either periodically or with varying intensity. Analysis of the data presented in Text-figs. 6 and 7 suggests a solution of the problem as to which it is. Let us analyse the first and third developmental periods (0–6 and 8½–14 hours) in which the stage of arrest of development is independent of the stage of irradiation. The cytoplasm of the embryo irradiated within such a period is able to develop without the nucleus up to the stage of arrest. This ability of the cytoplasm is not altered during the whole period; the later the nucleus is inactivated by irradiation within this period the smaller is the 'stock' remaining within the cytoplasm.

This implies that during the first and third periods the nucleus does not increase the ability of the cytoplasm to undergo independent development, does not synthesize the hypothetical substances or structures which are consumed in the course of the development. In other words, in the course of normal ontogeny development is realized during these periods without the constant control of the nucleus on account of nuclear products produced before the onset of the period.

Strictly speaking, one may claim that if irradiation at different moments of a given period leads to arrest of development at one and the same stage, the part of the cell affected by radiation does not during this period influence at all the ability of the rest of the cell to develop. But this certainly does not imply that the nucleus is in a kind of anabiosis during these periods of inactivity. It may participate in various preparative metabolic processes not connected directly with development.

During the second and fourth periods (6–8½ and beyond 14 hours) the ability of the cytoplasm to develop independently increases. Inactivation of the nucleus at every later moment (within the period) finds the embryo in a different state: its cytoplasm contains a larger amount of nuclear 'information' about further development than at the preceding irradiation. Strictly speaking, one may assert that if the irradiation at successively later moments of a given period leads to arrest at ever later developmental stages, the structure affected by irradiation does, during this period, significantly affect the ability of the non-damaged part of the cell to develop. Thus, during the second and fourth periods the nucleus actively produces a 'stock' of substances or structure which is necessary for further developmental processes.

Possibilities of other interpretations

One might try to interpret the data obtained in terms of radio-sensitivity. Radio-sensitivity depends upon the degree of injury to cellular structures and on the role of these structures in the activity of the cell. The changes of cell sensitivity which depend on the phase of mitotic cycle, and the changes in embryo-sensitivity which depend on the ratio of cells undergoing division, cannot play any role in these experiments, in which the injury is so large that dose dependence no longer exists. The changes in the embryo's response to irradiation
(Text-fig. 9) must, therefore, be explained by the role of structures affected by irradiation, i.e. of the nuclei, in development.

In many experiments on the effect of injurious agents, of high temperature in particular, arrest of development and increase of mortality were observed at stages close to those characteristic of heavy radiation (late blastula and end of gastrulation; cf. the survey of Trifonova, 1949). The possibility arises therefore of the non-specific character of the phenomena observed. The data available in the literature supplemented by the observation of the author on the effect of high temperature (33°C for 1 hour) on loach development led to the following conclusions:

1. Arrest of development at the late blastula takes place only in some embryos, whereas others may either be arrested before this stage or may develop further. This is more striking in the case of mortality which, to a significant degree, takes place at the moment of treatment or immediately after it, and then at all succeeding stages with a relative increase at the late blastula and early gastrula.

2. The effect of high temperature significantly injures the nuclear apparatus of embryonic cells. This is shown by the differential temperature sensitivity of different phases of the maturation divisions and of cleavage detected in sea-urchins, silkworms (Ostryakova-Varshaver, 1952), and loach (Vakhrameyeva & Neyfakh, 1959). Thereby nuclear disturbances arise (Lieder, 1954) which are very similar to chromosome aberrations of radiation origin. Even ultrasound leads primarily to disturbance of mitosis (Bonhomme & Pourhadi, 1957).

Thus one may suppose that the similarity between such injurious agents as high temperature and heavy doses of ionizing radiation is due to the fact that these agents all injure the nucleus to a significant degree. Denaturation injuries of other parts of the cell besides the nucleus complicate the injury pattern induced by high temperatures, so that its similarity to the effect of radiation is only relative.

The role of the nucleus in the early development of the loach

Inactivation of the genetic apparatus of nuclei at different developmental stages allows one to determine the periods of active function of the nucleus. A scheme of the time and duration of periods of nuclear functioning and the stages determined by these periods is shown, for the early development of loach, in Text-fig. 10.

The first developmental period—from fertilization to late blastula—is determined during ovogenesis by the activity of the egg nucleus and perhaps by that of the follicular cell nuclei (Mazia, 1952). It is natural that during this period conditions are created which are necessary not merely for cleavage and blastulation: yolk synthesis, membrane formation, &c., are needed for the whole of embryonic development. However, the independent development of the cytoplasm may be realized only up to the stage of the late blastula.
The embryo's nuclei begin their specific function in the early blastula and continue it for $2\frac{1}{2}$–3 hours up to the stage of late blastula. As a result the embryo becomes able to develop from the late blastula up to the end of gastrulation. In the middle of gastrulation a new period of nuclear activity begins, which ensures development at post-gastrula stages.

**TEXT-FIG. 10.** Scheme of the relations of nucleus to cytoplasm in the early development of the loach. The lower scale gives the hours and stages of normal development. The lines connecting the upper and lower horizontal lines indicate which particular stage of embryonic development is influenced by nuclear activity occurring at a given stage. Above the upper line the period at which the nucleus determines the given developmental stages is shown.

The scheme suggested does not conform to the idea that nuclear activity begins in the late blastula, or to the idea that the further activity of the nucleus is continuous. As mentioned in the introduction, however, such ideas are based on inadequate methods for investigating the very beginning of nuclear function; even such a criterion as ribonucleic acid synthesis in the nucleolus (Brachet, 1952) may be secondary to the processes occurring directly in chromosomes. The idea of a temporal succession of developmental processes beginning in the nucleus and ending in morphological transformations figured in the literature long ago. Thus Voss (1933) separated physiological activity (nuclear function) from morphological activity (cytoplasmic function), suggesting a scheme according to which gastrulation is preceded by a period of nuclear function beginning at the late blastula. It was shown later that the appearance of antigens precedes the morphological stages characterized by these antigenic properties (Clayton, 1953). It seems that the supposition that genetical nuclear function begins at an even earlier period does not contradict these ideas.

Several investigations have linked together an increased metabolism with the onset of gastrulation. Nuclear defects in lethal hybrids result in disturbance of normal metabolism precisely at this stage: the cessation of deoxyribonucleic acid synthesis (Chen, 1954; Gregg & Løvtrup, 1955; Løvtrup & Werdinius, 1957), the cessation of the increase in oxygen consumption, and perhaps other phenomena. This does not contradict the scheme suggested, as the onset of nuclear function may be overlooked against the background of the metabolic transformations which start later. Very interesting, however, seem the data recently obtained on
protein metabolism in sea-urchin embryos and on ribonuclease activity in early amphibian embryos. Synthesis of structural proteins, which is connected with the preliminary breakdown of reserve yolk proteins in the eggs of sea-urchins, has three maxima: at the early and late blastula, and at the mid-gastrula (Kavanau, 1954). In frogs, ribonuclease activity greatly increases at the early blastula and then at the mid-gastrula (Finamore, 1955). Though we cannot expect to find a complete analogy between the activity phases of nuclear function of echinoderms, amphibians, and fishes, it is evident that at the early blastula and mid-gastrula, just when an increased nuclear activity seems to be found in fishes, important metabolic transformations take place in echinoderms and amphibians.

It has been shown (Neyfakh & Rott, 1958) that in the loach and sturgeon (*Acipenser stellatus*) the synchrony of cleavage divisions is fundamentally disturbed at the early or mid-blastula stage, just when nuclear activity as detected by radiation effects begins. The mitotic coefficient during this period of normal development sharply decreases. This too underlines the important changes taking place in the early or mid-blastula.

In conclusion, the periodicity of nuclear activity raises many problems. Does the character of the activity differ between one period and another, as nuclear morphology alone to some extent suggests? Is there qualitative change in activity during any one period? What occurs in the nucleus between the periods of activity: is it then, for instance (besides undertaking the replication required for mitosis), accumulating the raw material for its next active period and receiving information from the cytoplasm which conditions the nature of the succeeding activity? Is there a periodicity at later stages of development, interruption of activity occurring, for instance, during the critical transitions implied in Waddington's (1957) epigenetic landscape? These are some of the immediate questions for which adequate methods of investigation need to be developed.

**SUMMARY**

1. The effect of high radiation doses (20–60 kr.) on different stages of the early development of the loach (*Misgurnus fossilis* L.) was studied. Analysis of dose dependence shows that a radio-sensitive component (presumed to be the nuclei) is affected maximally by such doses, whereas a radio-resistant component (presumed to be the cytoplasm) is not affected significantly. The use of such radiation doses is, therefore, a means of investigating the role of nuclei in development.

2. Irradiation with these doses at any time from fertilization to early blastula leads to the arrest of development at one stage—the late blastula—and to death some hours later. Irradiation at any time during the period from late blastula to mid-gastrula similarly leads to arrest of development at one stage—the end of gastrulation. Irradiation during the period from early to late blastula results in the arrest of development at different stages of gastrulation depending on the moment of irradiation. Irradiation after the mid-gastrula results in the arrest of
development at different stages of organogenesis, depending also on the moment of irradiation.

3. The results cannot be explained by changes of nuclear sensitivity, nor by non-specific critical periods in development. They can be explained by changes in the role of nuclei in development, by periods of specific dependence on the function of nuclei.

4. The independence of the stage of developmental arrest on the stage of irradiation, when irradiating from fertilization up to early blastula and from late blastula up to mid-gastrula, shows the relative inactivity of nuclei during these periods of normal development. The strong dependence of the stage of development reached at arrest on the stage of irradiation, when irradiating the early to late blastula and after the mid-gastrula stages, demonstrates the activity of nuclei in development during these periods.

5. The conclusion may be drawn that in the early development of the loach the nuclei function periodically, each period of activity ensuring a subsequent episode of development. Nuclear activity during ovogenesis ensures the development of the egg up to the stage of the late blastula; nuclear activity during the early and mid-blastula stages ensures gastrulation. Development of axial organs and further organogenesis is ensured by nuclear function beginning from the mid-gastrula stage.

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