Dimensions of the Notochord and Somites in Embryos of *Xenopus laevis* Treated with Thiocyanate

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As a result of previous research, Ranzi & Tamini (1939) pointed out that the notochord of Amphibian embryos of several species treated with sodium thiocyanate (NaSCN) solutions is larger than the notochord of control embryos. Later, several substances were identified which cause enlargement of the notochord: full data and a complete review of the literature on this subject are given by Ranzi & Citterio (1954) and Ranzi (1957). A careful statistical study on the number of nuclei in *Rana esculenta* embryos was carried out by Corti (1950). In embryos which had been treated for 24 hours with 0.05 M NaSCN, from the late blastula stage onwards, she found 1233 ± 28 nuclei at the tail-bud stage, whereas the controls reared in spring-water had only 1107 ± 24 nuclei: these two sets of data are significantly different (standard error of the difference ± 37).

Enlargement and induction of notochord by NaSCN treatment have been reported by Badínez, Carrasco, & Manríquez (1954). Notochord can be obtained from ventral explants and from explants of presumptive mesoderm by means of urea, which acts like NaSCN (Leone, 1952), or by means of NaSCN (Ôgi, 1957a). However, Ôgi (1957b) counted the nuclei of a few embryos of *Bufo vulgaris* and *R. nigromaculata* treated with NaSCN and NaI and was unable to demonstrate an increase.

For these reasons we thought it useful to repeat the work of Corti, but on another species, *Xenopus laevis* (Daudin), which appeared to be very sensitive to substances causing alterations of development (for the effect of LiCl see Bäckström, 1954).

**METHODS**

Eggs laid naturally by a couple of *X. laevis* were used; about 93 per cent. of the eggs were fertilized and all the controls developed normally. The eggs of this species appear to be particularly sensitive to NaSCN; a 0.01 M solution produced the familiar malformations (see Ranzi, Tamini, & Storari Offer, 1946) and yet allowed the embryos to survive.

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A 1 M NaSCN solution was made up and diluted 1 to 100 with spring-water; *Xenopus* embryos at stage 8/9 (late blastula) of Nieuwkoop (1956) were placed in the solution at 15° C. and allowed to remain there for 2 hours, i.e. during the first part of gastrulation. Then they were carefully washed with spring-water and allowed to develop in spring-water.

The controls were reared in identical finger bowls in spring-water.

When the control and experimental embryos at the age of 48 hours reached the tail-bud stage, 50 controls and 50 experimental embryos were fixed. During development we noticed a slight retardation in the controls compared with the NaSCN-treated embryos.

The embryos were fixed in Bouin's fluid, paraffin embedded, and sectioned at 10 μ; sections were stained with Ehrlich haematoxylin and eosin. The counting of nuclei was performed with a micrometer disk mounted in a ×7 ocular and a × 62 objective. All the nuclei which appeared in one section were counted; the error in counting twice those nuclei which traversed two sections was then corrected.

RESULTS

We first measured the volume of notochord in the controls and the NaSCN-treated embryos. The volumes expressed in μ³ x 10⁶ (mean and standard error) were 11.5 ± 0.59 and 10.0 ± 0.52 respectively. The difference is not significant.

![Text-fig. 1. Diagram of the crude counts of nuclei in the notochord of 50 controls and of 50 embryos treated with sodium thiocyanate (NaSCN).](image)

In comparing the numbers of nuclei we have avoided any error due to an accelerated development induced by NaSCN for the controls used were, in fact, a little more advanced than the treated embryos, and many of them showed the beginning of vacuolization in notochordal cells.
The results of the nuclear counts are given in Text-fig. 1. The mean number of nuclei of the 50 individuals in each group is: control $1056.6 \pm 18.5$; NaSCN-treated $1137.3 \pm 21.5$. These values must be corrected, taking into consideration the dimension of nuclei (see Abercrombie, 1946). The diameters of the nuclei are $14.1 \pm 0.72 \mu$ for the controls and $13.2 \pm 0.67 \mu$ for the treated embryos. Since the thickness of the sections was $10 \mu$, the corrected number of nuclei is therefore as follows: controls $438.4 \pm 7.67$; treated embryos $490.2 \pm 9.25$. There is here a highly significant difference ($0.01 > P > 0.001$), the increase amounting to 11.8 per cent.

We can therefore determine the ratio $\frac{\text{volume of notochord}}{\text{number of nuclei}}$, i.e. the mean volume of a cell (cells were assumed to be uninucleate). The volume, expressed in $\mu^3$ was: controls $26,232 \pm 1220$, treated embryos $20,400 \pm 567$. The higher standard error of the controls is due to the fact that some embryos had larger notochordal cells due to greater vacuolization.

Since research carried out by Cigada (1957) showed that NaSCN stimulated the division of *Amoeba*, the question arose as to whether the increase of nuclei in the notochord was a specific phenomenon, confined to this organ, or a general
phenomenon detectable in all the rudiments of the embryo. Unpublished work by Ranzi & Tamini had already shown that the nuclei of the ectoderm of NaSCN-treated embryos do not increase in number. In the present investigation we examined the number of nuclei in the somites, from whose presumptive material Ranzi & Tamini (1940) had shown that notochordal cells segregate. The results are shown in Text-fig. 2. The mean number of nuclei of the 10 individuals in each group is: control 13,750 ± 565; NaSCN-treated 10,987 ± 586.

The mean diameter of the nuclei of the controls was 7.9 ± 0.37 μ, that of the treated embryos 7.5 ± 0.36 μ, and the thickness of the sections 10 μ. The corrected number of nuclei is therefore: controls 7793.4 ± 315.8 nuclei; NaSCN-treated embryos 6273.3 ± 334.7 nuclei. This represents a highly significant difference (0.01 > P > 0.001). The decrease of the nuclear number in the somites of the NaSCN-treated embryos is 24.1 per cent.

The conclusion to be drawn is that cells of the somite rudiment are transformed into notochord.

SUMMARY

The number of nuclei of the presumptive notochord and somite cells of embryos of *X. laevis* at early tail-bud stage was determined in normal and NaSCN-treated embryos.

The nuclei of the presumptive notochord are more numerous in the NaSCN-treated embryos by 11.8 per cent., a significant difference.

The nuclei of the somites are less numerous in the NaSCN-treated by 24.1 per cent., a significant difference.

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REFERENCES


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