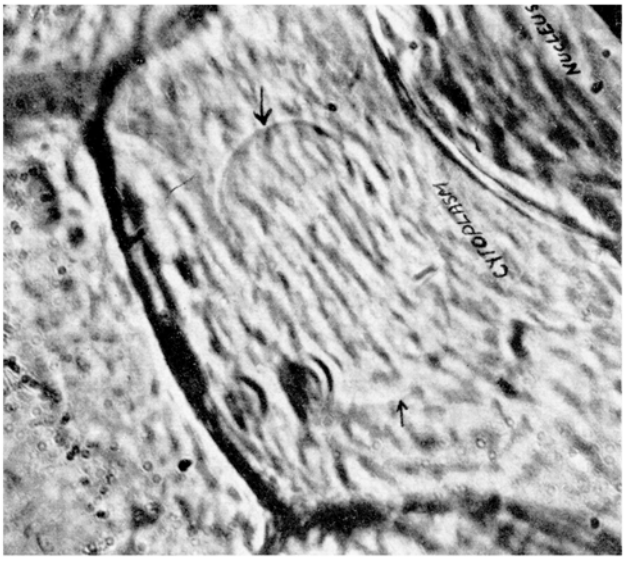

Fig. 1. A portion of oocyte of *N. chrysoleucas*, photomicrographed under phase-contrast microscope, showing 'yolk nucleus'.

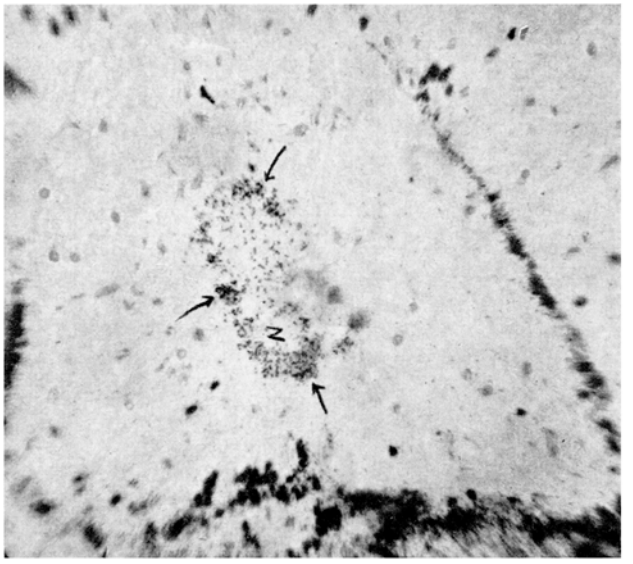

Fig. 2. An early oocyte photomicrographed to show the incorporation of tritium labelled cytidine in the nucleoli.

Autoradiographic Studies of Yolk Nucleus in Fish Oocytes

Since the early days of cytology there had been extensive studies on the extrusion of nucleolar material into the cytoplasm, which is described as a well known phenomenon of 'yolk nucleus' in fish oocytes. CUNNINGHAM[1] and WALLACE[2] were the earliest workers to describe 'yolk nucleus' in the fish oocytes which according to them arises in relation with the germinal vesicle or nucleus and later moves towards the periphery of the egg. They speculated the probability of some sort of relationship of this body with the formation of oil in the egg cell; thus named it as 'yolk nucleus'. WHEELER[3], NARAIN[4], and SUBRAMANIAM[5] have also reported the nuclear origin of the so-called 'yolk nucleus'. Recent studies of SATHYANESAN[6] and STOLK[7], based on classical histological technique such as picroformol-acetic/haematoxylin, describe this structure as an intranuclear body, larger than a normal nucleolus and with varying staining intensity, which pierces through the nuclear membrane to migrate into the cytoplasm where it merges into the surrounding cytoplasmic inclusions.

My present histo- and cytochemical studies by the use of autoradiographic technique help to clarify the origin of 'yolk nucleus' which seems to have been misleading a great many cytologists due to their exclusive use of biological stains, then considered to be specific, but recent advances in cytochemical techniques have revealed that identity of staining reaction by no means implies identical substances.

The appearance of a similar structure, corresponding to 'yolk nucleus', has already been reported by me in fishes *Ophiocephalus punctatus*[8] and *Barbus ticto*[9], and has also been studied in *Labeo dyocheilus* (unpublished). As earlier reported this structure is noticed as a mere circular concentration of mitochondria and Golgi granules; sometimes small vacuoles or tiny vesicles may also be observed. Such a locus can be clearly observed under phase-contrast microscope (as photomicrographed in Fig. 1) in the oocyte of the fish, *Notemigonus chrysoleucas*. My present studies have revealed that this structure has no relationship with nucleolar extrusions.

The various histochemical tests show that in very early oocytes this body 'the so-called yolk nucleus' appears as a juxta-nuclear concentration of mitochondrial granules among which a large number of bigger granules with stronger sudanophil nature are formed. A few sudanophobe vacuoles or vesicles may also be seen appearing in this area. The fine mitochondrial granules show protein contents with certain lipo-protein traces; while the sudanophil bigger granules consist exclusively of phospholipids, as revealed by Baker's acid haematin technique. In the late oocytes this body seems to merge into the surrounding cytoplasmic inclusions.

Further investigations whether the 'yolk nucleus' represents a nucleolus emerged from the nucleus of the oocyte were supplemented by the use of autoradiographic technique in which tritium labelled cytidine was used to follow the extrusion of nucleolar RNA. The oocytes of the fish, *Notemigonus chrysoleucas*, were incubated in the isotope solution (10 μcurie in 2 ml of Ringer's solution) for 12, 24, and 36 h at room temperature. The grains produced on the autoradiographic film showed that there is optimum uptake of labelled cytidine by the RNA of the nucleoli of early oocytes in 24 h (Fig. 2); and that at no stage a nucleolus is seen passing out into the cytoplasm in its complete form. However, it may be probable that some nucleolar material passes out into the cytoplasm in the diffused state, as shown by the fact that nucleoli always plaster against the nuclear membrane in late

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[12] Present address: Division of Cell Biology, Institute for Muscle Diseases, 515 East, 71 Street, New York.

oocytes and seem to lose their certain parts. Also the cytoplasmic RNA is feebly labelled as may be revealed by very few grains in the cytoplasm.

The conclusion of early workers with regard to the nucleolar origin of 'yolk nucleus' fails to be confirmed by my present investigations, and I fully agree with NATH's et al.[10] view advocating that the nucleolar extrusions in fish oocytes are artefacts; and such a conclusion derived by earlier workers from the irregularity of nuclear membrane (sometimes rupturing also) having nuclear pouches is erroneous and may be the effect of fixation and the mechanical disturbance.

Résumé. L'expulsion de la matière nucléolaire dans les œufs du poisson est étudiée ici par autoradiographie. Employant la cytidine-H_3, nous avons vu que le RNA nucléolaire passe dans le cytoplasme sous forme diffuse, et non comme un nucléole complet appelé «noyau-jaune».

H. C. CHOPRA[11,12]

Hargitt Research Fellow, Duke University, Durham (N.C.), November 11, 1960.

Temperature and Mutant Expression

The uniform adaptive phenotypic reactions to environmental change performed by populations of wild-type organisms are thought to be originated by natural selection. Out of a heterogeneous population, with individuals reacting in various directions, only those genotypes showing a useful reaction would be preserved.

There is support for this idea in the fact that wild populations do not react uniformly to such unnatural variations in environment as provided by temperature shock, X-rays and various chemicals (review GOLDSCHMIDT[1]). Moreover, by artificial selection for a special type of reactivity to such abnormal conditions in *Drosophila*, BATEMAN[2] obtained stocks showing predominantly the reaction selected. On this basis one might expect that in an experimental situation, where a mutant gene is introduced in an otherwise wild genotype, a given change in environment would not result in a uniformly directed reaction of the mutant expression. However, in the many experiments done on the reactivity to temperature change of mutant expression in *Drosophila melanogaster*, only uniform reactions were reported. They were in fact described as a property of the mutant gene. We mention the work of ZELENY and his group (a.o. HERSH[3]) on Bar, STANLEY[4], HARNLEY[5] and RIEDEL[6] on vestigial and STERN[7] and HOUSE[8] on cubitus-interruptus. In those experiments, mostly inbred laboratory stocks were used to ensure a uniform response, since the main interest lay in the precise quantitative relations between temperature and mutant expression. These relations were expected to yield information about the nature of gene action (PLUNKETT[9]).

In contrast, for the experiments reported here, the mutant ci^D (cubitus-interruptus dominant) was crossed into three recently caught wild stocks from Colmar (France, courtesy of Prof. H. BURLA, Zürich), Leiden, and Gouda (Netherlands) in such a way that together with ci^D only chromosomes 4 and Y were introduced, amounting to about 0.2% of the total genetic map. As ci^D is homozygous lethal it was in all cases balanced over another 4th chromosome lethal, sparkling-Cataract (spa^{Cat}, abbreviated below as Cat).

The ci^D mutant shows among other features a terminal interruption of the 4th longitudinal wing vein. The expression of ci^D was measured as the percentage ratio of the length of the 4th to that of the 3rd longitudinal vein distal to the anterior crossvein (a/b in Fig. 1). All cultures were set up with 10 cm^3 of a constant food medium (2% agar, 15% sugar, 4% dead dried yeast, 0.1% nipagin) and with a constant population density of 100 larvae. All larvae had been reared at 25° up to 48 $\pm$ $1^1/_2$ h of age. In each culture the left wings of 20 ♀♀ and 20 ♂♂ were measured.

In the first experiment, the three stocks were compared at temperatures 20° and 25°. The results in Table I show clearly that the shift in expression of ci^D is directed differently. The Leiden stock shows a direct relation between temperature and mean expression, the Colmar stock no change and the Gouda stock an inverse relation.

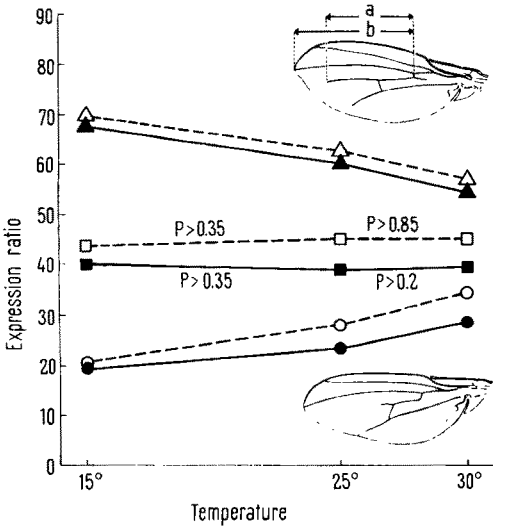

Fig. 1. The mean expression ratio at 3 different temperatures of the Colmar ci^D/Cat base population (□ ■) and the lines selected for high (△ ▲) and low (○ ●) expression ratio. Open figures and broken lines represent females, solid figures and lines the males. The differences between the samples were tested following Wilcoxon and probabilities exceeding 0.001 are given in the Figure. The means of the high line are based on 40, all other means on 60 individuals.

Tab. I. The mean expression ratio of ci^D in 3 different wild stocks at different temperatures. From the Gouda stock for each mean only 40 individuals were measured, the other means are based on 60 individuals. *P* values are calculated after Wilcoxon.

Stock		Mean expression ratio 25°	Mean expression ratio 20°	Difference 25°–20°	*P*
Gouda $\frac{ci^D}{Cat}$	♀	52.3	56.6	−4.3	<0.001
	♂	46.6	50.4	−4.2	<0.001
Colmar $\frac{ci^D}{Cat}$	♀	49.1	48.0	−1.1	>0.4
	♂	40.5	39.8	+0.7	>0.14
Leiden $\frac{ci^D}{Cat}$	♀	43.7	34.7	+9	<0.001
	♂	32.2	29.3	+2.9	<0.001

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