Reduction in DNA and RNA Content during the Development of Haploid *Rana pipiens* Embryos

The development of haploid amphibian embryos is characterized by retarded gastrulation, abnormal neurulation, incomplete differentiation of endodermal derivatives, aberrant body proportions, edema, and reduced viability. The cause(s) for this haploid syndrome is unclear since the factors involved in its development are complex. A single set of chromosomes in the egg results in 3 obvious consequences: (1) heterozygosity is lacking; (2) recessive lethal genes, being without their dominant alleles, can express themselves; (3) the ratio of nuclear to cytoplasmic volume is abnormal. Although there is evidence that each of the above factors is related to haploid abnormality, the cause of the syndrome has not been attributed exclusively to any of them[1].

A remaining possibility is that haploidy per se causes the syndrome; that abnormality results from metabolic inadequacies of haploid nuclei. If this were the case, then haploid embryos might develop deficiencies in their syntheses of gene products. Earlier cytophotometric studies on haploid embryos demonstrated the dependence of nuclear DNA and cellular RNA on ploidy. There is, however, conflicting evidence of the relationship between nucleic acid content and haploid morphological abnormality[2-5].

In this study a biochemical method was used to compare the amounts of DNA and RNA contained in developing haploid and diploid embryos of the leopard frog, *Rana pipiens*. My purpose was to ascertain whether haploid embryos deviate from normal levels of DNA and RNA; and if so whether there is a time correlation between the onset of deviant nucleic acid content and abnormal development.

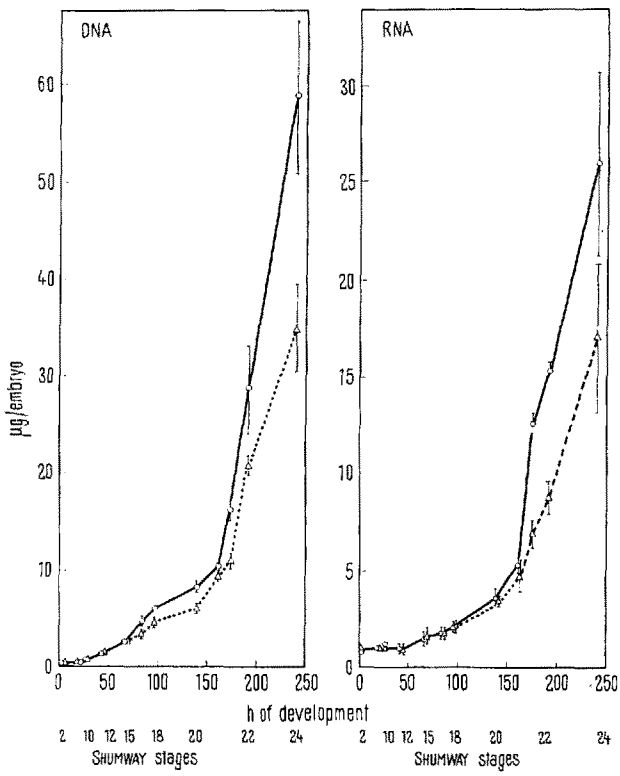
Gynogenetic haploid embryos were produced by 'stripping' eggs into an aliquot of sperm suspension previously irradiated with UV-light. Chromosome analysis of 40 of these embryos demonstrated that haploid production was 100%. An unirradiated aliquot of the same suspension was used to produce a diploid reference control. The embryos were reared in 10% Frog Ringers solution at 18 °C.

Nucleic acids were extracted by the OGUR and ROSEN[6] procedure, as modified by CHEN[7] for single amphibian embryos. Each dejellied embryo was homogenized in hot (60 °C) ethanol for 1 h to extract lipids. Acid soluble materials, including mononucleotides, were removed in cold (2 °C) 0.3*N* perchloric acid (PCA). Lipids were more completely removed by 3 extractions with cold (2 °C) ethanol-ether (3:1 v/v) for a total of 12 h. Afterwards, RNA was extracted with 0.8*N* PCA at 25 °C for 4 h; and DNA was extracted with 1.6*N* PCA at 60 °C for 20 min. The final centrifugation pellet was dissolved in 1.0*N* NaOH and its protein content was measured by the method of LOWRY et al.[8].

The UV absorption spectrum (300–200 nm) of each nucleic acid extract was determined in a Beckman DB spectrophotometer, using a quartz cell (10 mm path length; 60 µl capacity). DNA and RNA concentrations were calculated from the difference between maximum (260 nm) and minimum (230 nm) optical density, from concurrently determined standard curves.

The absorption spectra of the extracts agreed closely with those of standard RNA (Schwartz) and DNA (Nutritional Biochemicals), indicating that the extracts were essentially free from contaminating materials. Preliminary experiments showed the extraction time for each step to be optimal because increasing the duration of extraction gave no greater yields. Pellet proteins per embryo were all similar, ruling out the possibility that differences observed in DNA and RNA content merely reflected differences in mass of the embryos.

Haploid embryos became deficient in their total DNA content by SHUMWAY stage 17 (tail bud)[9], and in RNA content by stage 20 (hatching). Similar haploid and diploid nucleic acid content, prior to these developmental stages, involves a combination of factors: (1) because eggs with haploid nuclei have normal cytoplasms, they contain normal levels of the reserve materials synthesized during oogenesis. Amphibian eggs are described as having cytoplasmic stores of both DNA and RNA[10]. These reserves should mask differences in nucleic acid content of haploid and diploid nuclei during early stages. (2) Between early cleavage and late gastrula stages haploid embryos undergo an additional division. Thus they possess cells $^1/_2$



DNA and RNA content per embryo are plotted against developmental time of diploid controls. Ninety-six haploid (△) and 75 diploid (O) samples were analyzed. A sample contained: 5 blastulae; 3 gastrulae-neurulae; or 1 tail bud-swimming stage embryo.

[1] R. BRIGGS and T. J. KING, in *The Cell I* (Ed. J. BRACHET and A. E. MIRSKY; Academic Press, New York 1959), p. 537.
[2] B. C. MOORE, J. Morph. *101*, 227 (1957).
[3] M. YOSHIDA, Expl Cell Res. *19*, 434 (1960).
[4] J. BRACHET, *Biochemistry of Development* (Pergamon Press, New York 1960), p. 223.
[5] H. ESPER, J. Morph. *111*, 227 (1962).
[6] M. OGUR and G. ROSEN, Archs Biochem. *25*, 262 (1950).
[7] P. S. CHEN, Expl Cell Res. *21*, 523 (1960).
[8] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[9] W. SHUMWAY, Anat. Rec. *78*, 139 (1940).
[10] P. GRANT in *The Biochemistry of Animal Development* (Ed. R. WEBER; Academic Press, New York 1965), p. 483.

diploid size, but twice as numerous. While haploid cells at this stage presumably contain half the diploid content of DNA and RNA, normal total levels per embryo are maintained by its increased number of cells. After undergoing the additional division, haploid cells apparently divide at the same rate as diploids, at least until neurula stages are reached. During this period, the S phase of DNA synthesis occupies similar positions in the cell cycle and lasts as long in haploid cells as it does in diploid cells[11].

The period at which haploid and diploid nucleic acid content becomes dissimilar coincides with one of increasing abnormality for haploid embryos. The increased cell number of earlier haploids is apparently superceded by incomplete differentiation in certain tissues. Mitosis in forebrain and pronephros is similar in both haploids and diploids, but in liver and cartilage the mitotic index is greatly diminished[12]. Both nervous tissue and the notochord of haploid embryos have larger numbers of cells than diploids, but the cell number is reduced in muscle, the circulatory system and the gut[13].

Clearly, the abnormal development of haploid embryos is accompanied by a reduction in the net synthesis of both DNA and RNA. The role of nucleic acid metabolism, however, in the development of the haploid syndrome is uncertain. The first visible sign of haploid abnormality is the retardation of gastrulation. Because differences between haploids and diploids in DNA and RNA content are not detectable for several hours after gastrulation, a direct causal relationship between nucleic acid synthesis and the syndrome may be precluded[14].

Résumé. Une méthode micro-biochimique a été utilisée pour comparer les quantités d'ADN et ARN contenues dans des embryons en développement haploïdes et diploïdes de la grenouille, *Rana pipiens*. Le contenu d'ADN des embryons haploïdes a été réduit au stade de la queue bourgeonnante, et leur contenu d'ARN au stade de l'éclosion. Puisque les irrégularités morphologiques caracteristiques des embryons haploïdes apparaissent beaucoup plus tôt dans le développement, une relation directe et causale entre la réduction observée de la production nette de l'acide nucléique et le syndrome haploïde n'est pas probable.

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Tulane University, New Orleans (Louisiana, USA), 22 May 1967.

[11] C. F. GRAHAM, Expl Cell Res. *43*, 13 (1965).
[12] B. C. MOORE, Chromosoma *4*, 563 (1952).
[13] K. PORTER, Biol. Bull. mar. biol. Lab., Woods Hole *77*, 233 (1939).
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Action de la capsicine sur la multiplication et la virulence de l'*Agrobacterium tumefaciens*

En 1961, nous avons établi qu'en soumettant des cultures bien développées de leptospires à l'action de la capsicine[1] pendant 6 h dans le milieu de KORTHOF dans la proportion de 0.0025% dans 5 ml de culture, il se produit un très fort accroissement du pouvoir de multiplication des leptospires de tous les sérotypes. Cet accroissement atteint dès la 18è h de l'action des quantités de leptospires telles qu'on n'en obtient dans des conditions ordinaires que vers le 10è jour. En outre, les leptospires soumis à l'effet de la capsicine accusent un accroissement de leur pouvoir antigène par rapport aux leptospires normaux, qui est de l'ordre d'environ 60%[2-4]. Au cours d'un autre essai, effectué en 1964 avec de la capsicine sur le *Mycoplasma agalactiae*, nous avons obtenu des modifications morphologiques des colonies du microorganisme et une légère modification de sa virulence[5].

Nous avons continué nos études dans ce sens et entrepris d'autres recherches, cette fois-ci sur l'*Agrobacterium tumefaciens*.

Dans 3 cultures bien développées et virulentes d'*A. tumefaciens* en milieu liquide de pommes de terre, en quantité de 5 ml chacune, nous avons placé respectivement 0.0025, 0.005 et 0.01 g de capsicine, puis laissé les tubes dans l'étuve à 30 °C pendant 6 h. Passé le délai de 6 h, on a repiqué dans un autre tube contenant le même milieu stérile, séparément, 2 gouttes de culture, aussi bien de celle ayant été soumise à l'action de la capsicine que celle de la culture normale et développées simultanément. Après 48 h à l'étuve à 30 °C on a constaté que dans les nouvelles cultures de repiquage provenant de 3 cultures soumises à des quantités différentes de capsicine, la multiplication des bactéries est plus abondante que chez les témoins aussi repiqués. Il est à remarquer en outre, que lors du premier repiquage en milieu stérile des cultures soumises à l'action de la capsicine, environ le 20% des bactéries pendant les 6 h meurent sous l'influence de la capsicine. Cela signifie que la quantité de bactéries des 2 gouttes de culture traitée est plus petite que celle des 2 gouttes de culture normale. Pour obtenir des données encore plus précises, on a ensemencé dans 5 boîtes gélose nutritive à base de pomme de terre une culture soumise à l'action de la capsicine et dans 5 autres une culture normale, en partant de repiquages correspondants d'un milieu stérile, après, bien entendu, une égalisation quantitative des bactéries dans les repiquages mêmes.

L'examen des résultats, effectué après un séjour des boîtes de gélose ensemencées et tenues à 30 °C a permis d'établir que dans la culture soumise à l'action de la capsicine il y avait dès la 24è h une multiplication abon-

[1] Par capsicine on entend l'extrait total du fruit des piments rouges (*Capsicum annuum* L.) qui ont un goût âpre et qui, avec d'autres composants, contiennent aussi l'alcaloïde de même gout — la capsaïcine. La capsaïcine est l'alcaloïde chimiquement pur.
[2] I. KUJUMGIEV, Izv. mikrobiol. Inst., Sof. *13*, 5 (1962).
[3] I. KUJUMGIEV, Zentbl. Bakt. Parasitkae, Abt. I. *185*, 230 (1962).
[4] I. KUJUMGIEV, Izv. mikrobiol. Inst., Sof. *16*, 41 (1964).
[5] I. KUJUMGIEV, C. r. Acad. bulg. Sci. *18*, 79 (1965).