CONDITIONS FOR EXTRACTION OF CYTOPLASMIC DNA FROM EMBRYOS OF A SEA URCHIN[1].

Anatol Eberhard[2] and Daniel Mazia

Department of Zoology, University of California, Berkeley, California.

Received November 2, 1965

The eggs of many species contain large amounts of material identified as cytoplasmic DNA (Hoff-Jorgensen, 1954; Baltus, et al., 1965; Brachet and Ficq, 1965; Durand, 1961; Haggis, 1964). The nature of this DNA is still not known (Gahan and Chayen, 1965; Vacelet, 1963) though at least that portion of it that can be extracted by the phenol method behaves in the ultracentrifuge just like normal DNA (Brachet and Ficq, 1965; Dawid, 1965; Carden, et al., 1965). The solubility properties of cytoplasmic DNA, however, appear to be different from those of nuclear DNA (Agrell, 1964, and personal communication); cytoplasmic DNA may exist as a protein complex (Agrell, 1964; Brachet and Quertier, 1963; Finamore, et al., 1960).

During attempts to measure the amount of DNA in eggs and embryos of the sea urchin Strongylocentrotus purpuratus, difficulties with the classical extraction procedures were encountered: the DNA in the eggs seemed not to be as readily extractable with hot acid as that in older embryos. In order to define this difference in extractability and to obtain a more accurate figure for the DNA content of the eggs and embryos, the kinetics of solubilization of the DNA in acid was determined for unfertilized eggs, developing embryos and sperm. The fluorometric assay of Kissane and Robins (1958) was used to measure the amount of DNA solubilized by the acid treatment.

Unfertilized eggs of S. purpuratus and embryos grown for different lengths

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1) Supported in part by grants no. GM 6025 and GM 10532 from the National Institutes of Health.

2) National Cancer Institute postdoctoral fellow (fellowship no. CA 12,657).

of time at 15° were transformed into acetone powders by washing several times with sea water and treating five times with acetone at 50°. After drying, 0.002 to 0.005 g. samples of these powders or 0.005 ml. samples of undiluted sperm (after counting of the sperm in an aliquot with a Coulter Counter) were extracted three times with 1M HCl at 0°, then suspended in 1 ml. of 1M HCl and heated in a water bath at 60°. From time to time, samples of the extracted material were taken by chilling the tubes in an ice bath, centrifuging them, and withdrawing 0.02 ml. of the supernatants. The hydrolysis tubes were returned to the 60° bath, while the 0.02 ml. aliquots were dried *in vacuo* in scrupulously clean 6 x 50 mm. pyrex tubes. To these tubes was added 0.02 ml. of a 2M solution of freshly recrystallized 3,5-diaminobenzoic acid dihydrochloride (DABA), care being taken to remove any traces of charcoal used in the recrystallization. The tubes were suspended in a water bath at 60° for 45 minutes, removed, 0.25 ml. of 1M HCl added and the fluorescence read with a Turner fluorometer against a blank containing no extract (Hinegardner, personal communication; Kissane and Robins, 1958). The readings obtained were compared to those obtained with known amounts of deoxyadenosine-5'-monophosphate, thus allowing a calculation of the quantity of DNA in the materials brought into solution by hydrolysis (taking into account that only the deoxyribose attached to purines gives rise to fluorescence).

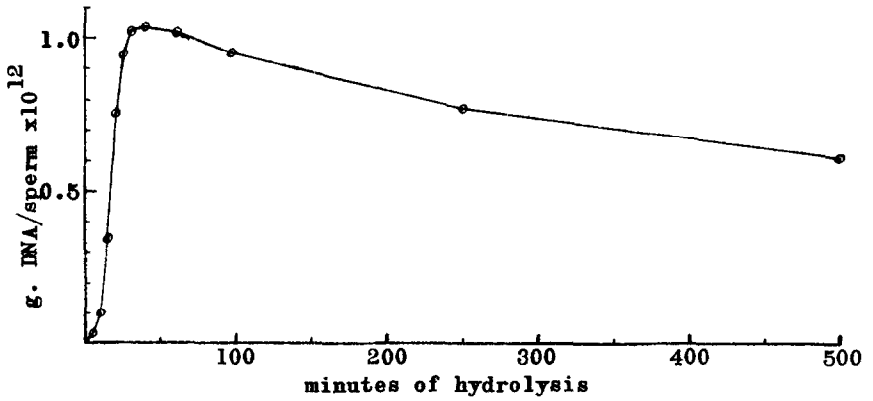

Figure 1. Hydrolysis of sperm; 1M HCl, 60°.

The results obtained with a sample of sperm are shown in figure 1. The initial s-shaped portion of the curve is expected for the hydrolysis of a polymer, long sections of which are insoluble but short portions of which are soluble in acid. The decline after about 40 minutes is due to the decomposition of the deoxyribose portions of the materials in solution (DABA gives fluorescent compounds with deoxyribose derivatives formed by acid depurination of DNA (Kissane and Robins, 1958)). Deoxyribose (or dAMP) after 500 minutes in 1M HCl at 60° gives only one-half the fluorescence with DABA that it gives before hydrolysis. The maximum in the curve at about 40 minutes (1.04×10^{-12} g. of DNA/sperm) can be taken to indicate the total amount of DNA present in the sample. Analyses of dried sperm, without acid hydrolysis, using the DABA method yielded the value 0.9×10^{-12} g. of DNA/sperm (Hinegardner, 1961, gives an average value of 0.84×10^{-12} g.). The reasons for the higher results obtained with HCl hydrolysis are not known.

Figure 2 shows the results of the hydrolysis of 30 hour-old embryos. The DNA in these embryos behaves essentially like that in sperm. Embryos older than about 10 hours all give hydrolysis curves similar in shape to the one shown in figure 2. The hydrolysis behavior of 3 hour-old embryos is shown in

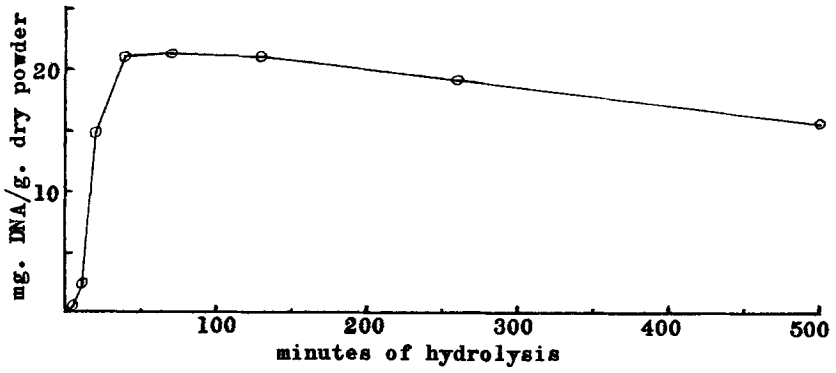

Figure 2. Hydrolysis of 30 hour-old embryos; 1M HCl, 60°.

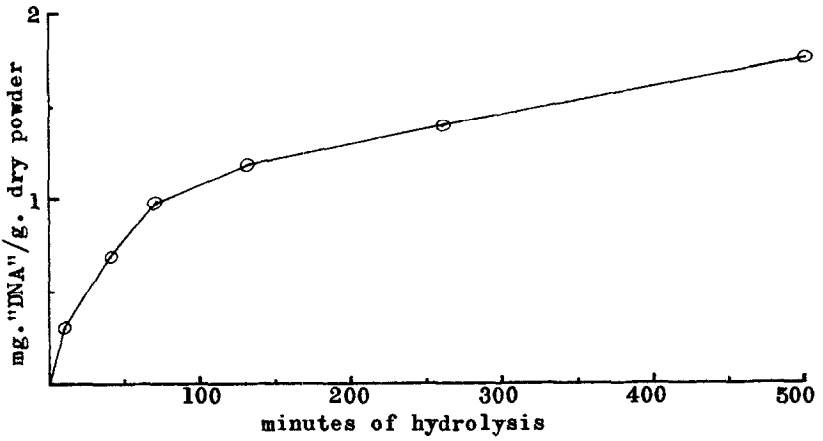

Figure 3. Hydrolysis of 3 hour-old embryos; 1M HCl, 60°.

figure 3. There is no "induction period", the DNA "titer" rising slowly over a long period of time in spite of the decomposition of those deoxyribose compounds already in solution. Hydrolysis curves for unfertilized eggs or for embryos of up to 6 hours of age resemble the curve shown in figure 3. (Embryos between the ages of about 6 and 10 hours give hydrolysis curves of a form intermediate to those shown in figures 2 and 3.) Figure 3 does not allow the assignment of a definite value to the DNA content of the 3 hour-old embryos, though the sample must contain more DABA-reactive material than is indicated by the highest value of the curve. Whether or not this material corresponds exclusively to DNA cannot be decided on the basis of these experiments (see Izawa, et al., 1963).

Using both the Lowry and the Biuret methods for protein analysis, all the embryo powders were found to contain about 60% of protein; using the Biuret method with samples of eggs counted directly with a Coulter Counter and converted to acetone powders, each egg was found to contain 6.1×10^{-8} g. of protein (Mazia and Roslansky, 1956, found 6.2×10^{-8} g. of protein per embryo using the Lowry method). Thus one gram of dried acetone powder contains about

10^7 embryos. Using the highest value in figure 3 (about 0.0018 g. of "DNA" per gram of dried powder), each embryo contains at least 1.8×10^{-10} g. of "DNA". Since each sperm contains about 10^{-12} g. of DNA (figure 1), each embryo contains enough "DNA" for about 90 diploid cells[1]. The total amount of "DNA" in each embryo, measured in this way, remains nearly constant for the first 6 to 8 hours after fertilization until about the 32 to 64-cell stage, after which it increases rapidly.

Young and old embryos were dissolved in 1M NaOH by heating for one hour at 60°, then were hydrolyzed further at 60° in 1M HCl. In all cases, fluorescence readings of aliquots after DABA treatment were maximal before 40 minutes of acid hydrolysis, longer hydrolysis times leading, as usual, to lower values. (These maximal values, however, were always lower by some 10% than the maximal values observed for the same samples without prior NaOH treatment.) The difference in behavior upon acid hydrolysis between the material in unfertilized eggs or young embryos and that of the DNA in older embryos is thus abolished by prior alkaline treatment. The material in the unfertilized eggs may, therefore, be quite similar to the DNA in older embryos, though presumably it is bound differently. Such differences show the importance of determining at least the solubility characteristics of the DNA (or other material) in the sample under consideration before a given analysis may be said to have yielded an absolute value for DNA.

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1) The fluorometric DNA assay used here is presumably specific for the group RCH_2CHO. Thus any compound not soluble in acetone or cold 1M HCl, but soluble in 1M HCl at 60° which contains this group or can give rise to it in acid, would appear in this assay as having arisen from DNA (Burgos, 1955; Haggis, 1964; Izawa, et al., 1963; see also Schrader, 1951). The possibility that the DNA content of a given batch of eggs may depend on the length of stay of the eggs in the ovary before shedding, on the season or on the adequacy of the nutrition of the sea urchins was not explored here (see Bieber, et al., 1959).

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