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CYTOCHEMICAL EVIDENCE FOR VARIED DNA COMPLEXES IN THE NUCLEI OF UNDIFFERENTIATED CELLS

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It has been found that hetero- and euchromatin incorporate thymidine at different times. Lima-de-Faria (6) observed that in the nuclei of grasshopper testes the label was incorporated differently into sex chromosomes and autosomes, whereas in nuclei of young rye leaf the difference in the timing of DNA synthesis occurred within each chromosome. The same was found for dipteran polytene chromosomes (Ficq and Pavan (4)). The incorporation of label was confined to specific puffings at exact stages of larval life. Possibly this metabolic heterogeneity of DNA is a result of a varied linkage of DNA to other constituents of the chromosome material. Interest has been directed especially to basic proteins. Bloch (3) underlines the difference between nuclei during autosynthesis and heterosynthesis. During autosynthesis all DNA should be bound to histone, while during heterosynthesis DNA appears to be linked to a metabolically active fraction of residual protein. Agrell (2) found in the nuclei of rapidly dividing cells a complex comprising RNA, DNA, and basic protein. The stability of this complex weakens with advancing differentiation. The present article deals with an observation of DNA complexes of different stabilities as revealed by Feulgen staining with different hydrolysis times.

MATERIAL AND METHODS

The materials employed were frogs, *Rana temporaria*, in embryonic stages and ciliates, *Tetrahymena pyriformis*, in logarithmic and stationary phases of population growth, and during growth in the blocked division (Zeuthen and Scherbaum (13)), a period which, after a series of thermal shocks, precedes synchronous division. The frog embryos and frog liver were fixed in buffered formol, 4 per cent, embedded in polyester wax with low melting point, +37°C (Steedman (11)), and sectioned in 10 μ sections. Interfering pigment was bleached in buffered 3 per cent hydrogen peroxide. To obtain uniformity the frog liver sections were also treated in this way. The *Tetrahymena* cells were attached to glass slides by a rapid coagulation with alcohol according to the technique of Agrell (1). The fixation was made in alcohol-acetic acid, 3:1. The preparations from one and the same homogeneous material were hydrolyzed for different times with 1 N HCl at +58°C. The nuclei were stained with Feulgen. The leuco-fuchsin was prepared following Rafalko (10) and the subsequent steps were performed according to Leuchtenberger (5). The intensity of the stain was evaluated microspectrophotometrically. The apparatus was similar to that used by Pollister (9). The spectrophotometric readings were made with Mendelsohn's two-wavelength method (7). Only interphase nuclei were considered. The absorption spectrum for the Feulgen stain in the nuclei was found to be independent of the hydrolysis time. Unhydrolyzed nuclei gave no measurable staining. There was never any measurable stain appearing in the cytoplasm of the investigated cells.

The results are given in Figs. 1 and 2. Each point on the curves represents measurements on an average of 25 separate nuclei. The standard deviation of the mean has never exceeded 10 per cent. Each curve shown represents a separate experiment in which all preparations were hydrolyzed and stained together in the same acid and dye respectively. Moreover, the sections from frog liver and middle gastrula were stained in the same dye. Accordingly, in the curves the demonstration of a succession of maxima and minima is of significance. The placements of this succession on the abscissa should not be strictly compared from one curve to another.

RESULTS

To state the optimal conditions for the Feulgen stain, the intensity of the stain should be plotted against hydrolysis time. As far as we have found, all such curves hitherto reported from animal tissue concern adult cells. Uniform, single-peaked curves were always obtained. As an example of such a curve, our own measurements on mouse liver nuclei are reproduced in Fig. 1. A related curve was obtained for the nuclei of adult frog liver (Fig. 1). However, the corresponding curves for the nuclei of the early frog embryo show a distinct heterogeneity. At least two different and statistically significant peaks are demonstrated, (Fig. 1). The experiments do not allow any safe conclusions about conceivable differences in the DNA heterogeneity between the two embryonic stages observed. An indication of a third peak at short hydrolysis time can be seen in the late

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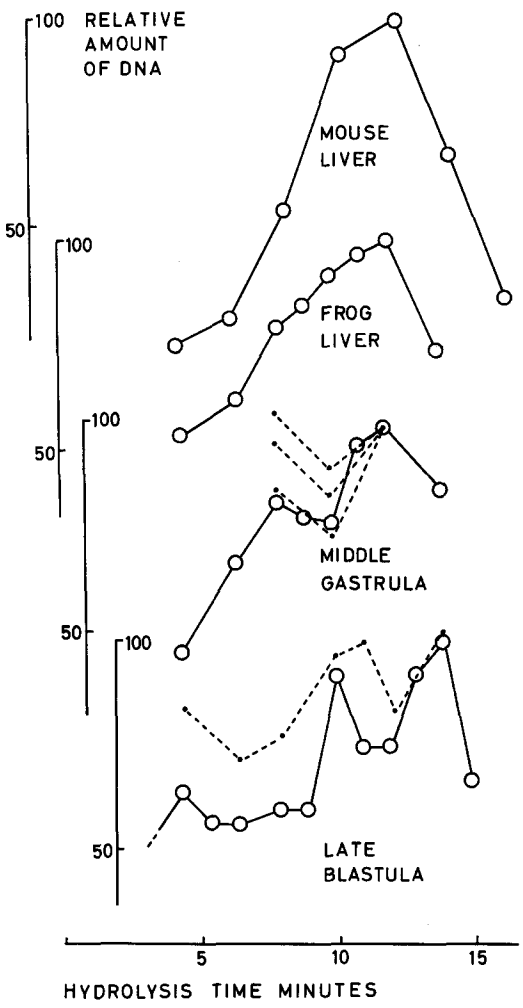

FIGURE 1

Changes in Feulgen stainability, relative amount of DNA, in relation to hydrolysis time, of nuclei from different developmental stages of the frog. For the middle gastrula, the minimum at 10 minutes' hydrolysis time has been tested in three additional series of embryos. For the late blastula, measurements from one supplementary series are included. These complementary curves are represented by the broken lines. At the top of the diagram is our curve for mouse liver nuclei, fixed in alcohol-acetic acid.

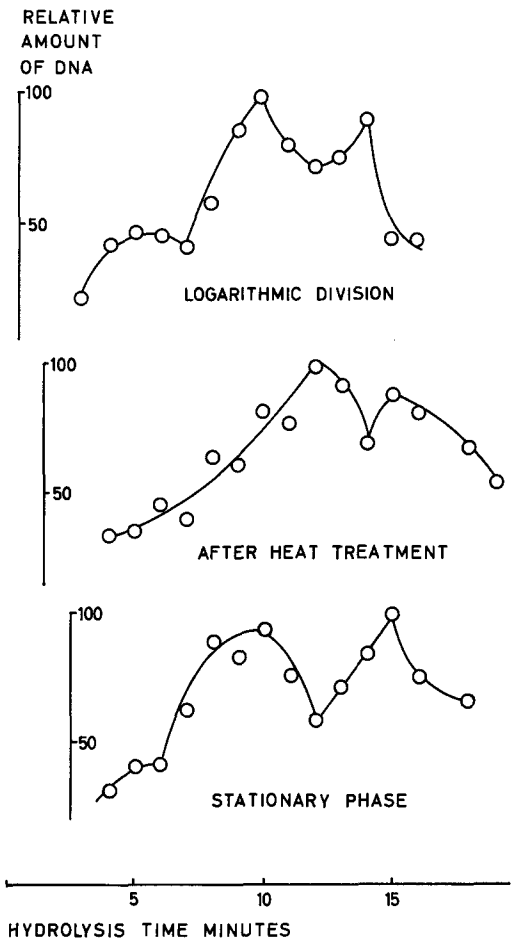

FIGURE 2

Changes in Feulgen stainability, with varied hydrolysis time, of nuclei from different phases of *Tetrahymena*. The middle curve represents cells treated with heat shocks to induce capacity for synchronous division. The cells are fixed at the same moment as heat treatment was over.

blastula. In any case, the heterogeneity has almost disappeared in the nuclei of the adult tissue, the frog liver. The microspectrophotometric measurements recalculated from the peak values above 12 minutes' hydrolysis to represent the total amount of DNA per nucleus are well located within the diploid-tetraploid range for both adult and embryonic stages. The results obtained from the ciliate nuclei are closely consistent (Fig. 2). In this material also, two distinct peaks in the curve were found, as well as a tendency to a third peak on short hydrolysis time. During suppressed cellular division, after seven thermal shocks, the peaks are leveled out to a certain extent.

The different peaks in the hydrolysis curves suggest different types of DNA complexes, which are characterized by their varying lability towards acid hydrolysis. We believe that the demonstrated variations in stability depend upon divergent linkages of DNA to other macromolecules in

the nuclei by which DNA is differently protected. Probably the heights of the peaks do not reflect the true relative amounts of DNA in the different complexes. When using the Feulgen procedure one must weigh the development of the stain intensity against an undue decomposition of DNA through hydrolysis (*cf.* also Woods (12)). Thus more DNA may be lost from some complexes than from others.

The more labile DNA complexes characterize rapidly proliferating nuclei and seem to disappear with advancing embryogenesis and increasing differentiation. It may be that undifferentiated nuclei contain, besides a firmly protein-linked DNA, a more labile RNA-bound DNA (2), which is acid labile simply because it is less protected by protein. Perhaps the observed disappearance of labile DNA during differentiation can be placed in conjunction with successive elimination of DNA from somatic cells, contrary to germ cells, observed during embryogenesis in some animal groups as a visible loss of true chromosomal structures (*cf.* Painter (8)).

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REFERENCES

1. AGRELL, I., Whole mounts of small embryos attached directly to glass slides, *Stain Technol.*, 1958, **33**, 265.
2. AGRELL, I., Cytochemical demonstration of a varied linkage of nuclear RNA during growth and division, *Pathol. et Biol., Semaine Hop.*, 1961, **9**, 775.
3. BLOCH, D. P., Changes in the deoxyribonucleoprotein complex during the cell cycle, *in* Frontiers in Cytology, (S. Palay, editor), New Haven, Yale University Press, 1958, 113.
4. FICQ, A., and PAVAN, C., Autoradiography of polytene chromosomes of *Rynchosciara angelae* at different stages of larval development, *Nature*, 1958, **180**, 983.
5. LEUCHTENBERGER, C., Quantitative determination of DNA in cells by Feulgen microspectrophotometry, *Gen. Cytochem. Meth.*, 1958, **1**, 219.
6. LIMA-DE-FARIA, A., Differential uptake of tritiated thymidine into hetero- and euchromatin in *Melanoplus* and *Secale*, *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 457.
7. MENDELSOHN, M. L., The two-wavelength method for microspectrophotometry. I and II, *J. Biophysic. and Biochem. Cytol.* 1958, **4**, 407 and 415.
8. PAINTER, T. S., The elimination of DNA from soma cells, *Proc. Nat. Acad. Sc.*, 1959, **45**, 897.
9. POLLISTER, A. W., Photomultiplier apparatus for microspectrophotometry of cells, *Lab. Inv.*, 1952, **1**, 231.
10. RAFALKO, J. S., A modified Feulgen technic for small and diffuse chromatine elements, *Stain Technol.*, 1946, **21**, 91.
11. STEEDMAN, H. F., Polyester wax, *Nature*, 1957, **179**, 1345.
12. WOODS, P. S., A chromatographic study of hydrolysis in the Feulgen nucleal reaction, *J. Biophysic. and Biochem. Cytol.*, 1957, **3**, 71.
13. ZEUTHEN, E., and SCHERBAUM, O., Synchronous divisions in mass cultures of the ciliate protozoon *Tetrahymena pyriformis*, as induced by temperature changes, *Colston Papers*, 1954, **7**, 141.