

# THE RELATIONS BETWEEN DNA, RNA, AND PROTEIN IN NORMAL EMBRYONIC CELL NUCLEI AND SPONTANEOUS TUMOUR CELL NUCLEI

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## ABSTRACT

Interferometric and photometric measurements have been made successively on individual cell nuclei derived from normal embryonic tissues and spontaneous tumour tissues of the mouse grown *in vivo*. From the measurements, the relations between nucleic acid and dry mass content have been studied in the two types of nuclei and the results shown to be consistent with differences in cell metabolism previously reported to exist *in vitro*. In the nuclei of normal embryonic cells, the syntheses of DNA, nuclear RNA, and protein appear to be closely associated, whereas in the tumour cell nuclei an appreciable fraction of the chromatin RNA and protein synthesis is dissociated from the replication of DNA.

## INTRODUCTION

Investigations of the time synthesis curves of cells grown in culture (Seed, 1961, 1962, 1963) have shown that, in rapidly dividing cells freshly prepared from normal tissues, the processes of DNA and the bulk of nuclear RNA and protein syntheses for a new cell begin at approximately the same time in interphase and thereafter proceed approximately together. However, in rapidly dividing cell strains derived from or capable of producing tumours, a large part of nuclear RNA and protein synthesis proceeds continuously, in contrast with DNA synthesis which begins only after a waiting time has elapsed as in normal cells. Because of the genetic role currently attributed to DNA, I have suggested that this time disconnection of onset of nuclear protein (with RNA) synthesis reflects a fundamental loss of control in tumour strain cells (Seed, 1961, 1962, 1963).

The method adopted in the above experiments was to make photometric nucleic acid determinations and interferometric dry mass determinations

on individual cell nuclei, the various ages of which in interphase had been found by prior time-lapse photography. Thus by plotting the values obtained for individual cells against the corresponding cell ages (*i.e.*, the time since the last mitosis), average synthesis curves could be constructed. The DNA and RNA cycles were also confirmed by autoradiographic observations of the uptake of nucleosides (Seed, 1962, 1963). Because of the importance of the inferences drawn from the results, clearly it was desirable to extend the measurements to normal cells and spontaneous tumour cells grown *in vivo* and the aim of the present paper is to describe some of these measurements. At the start, it was deemed impracticable to film a section of a tissue grown *in vivo* in the same way as was done for cells grown *in vitro*, nevertheless it was thought possible to make useful deductions from the correlation plots of dry mass, total nucleic acid and DNA measured on the same individual cell nuclei without having a knowledge

of the cell ages. This approach had been demonstrated (Seed, 1963) for the *in vitro* measurements to lead to conclusions equivalent to those of the timing experiments.

## MATERIALS AND METHODS

### *Biological Material*

In the present experiments, the normal cells were prepared from mouse embryo limb bud, and the tumour cells from the growing edge of a spontaneous tumour arising in the flank of a C+ strain mouse: this strain was obtained originally from the Chester Beatty Research Institute by Dr. J. T. Hemingway and Mr. E. A. King. Smears were prepared from the tissue by touching a freshly cut surface, as dry as possible, onto a clean quartz coverslip: the cells were then allowed to air-dry, after which they were immersed in methanol for 15 minutes. When prepared in this way, the smears showed areas containing numbers of individually separated cell nuclei having apparently little overlapping cytoplasm. However, the cells were not so flat as those grown in culture. After fixation, the low molecular weight compounds were extracted from the cells by a 30-minute treatment in 1 per cent perchloric acid at 4°C.

### *Experimental Procedure*

The fixed cells adhering to a quartz coverslip were mounted in glycerol and were photographed in the ultraviolet at 2536 Å, using Cooke quartz optics. Prints were then made from the photographic plates to facilitate identification of the individual cells during the remainder of the measurements, and from optical density measurements on the plates the integrated absorption of each cell nucleus was obtained. This absorption is proportional to the amount of nucleic acid (DNA plus RNA) in the nucleus. Although the ratio of RNA to DNA in the chromatin of the present material is unknown, in other rapidly dividing cells *in vitro* (monkey kidney, HeLa strain) it was estimated to be in the region of two-fifths (Seed, 1963, see also Walker and Yates, 1952, Bonner *et al.*, 1961).

Next, the cells were remounted in distilled water and the optical retardation of each of the same cell nuclei was measured on the Cooke-Dyson interferometer microscope, from which the dry masses could be determined. These are effectively the protein contents (Barer, 1951 *a, b*, Davies and Wilkins, 1951). As before (Seed, 1962, 1963), the nucleoli were not included in the photometric or optical retardation measurements. No corrections were applied to the ultraviolet measurements for protein absorption, nor to the dry mass measure-

ments for nucleic acids, since these contributions were insufficient to affect the conclusions drawn.

Finally, the cells were stained using the Feulgen reaction and the amount of stain in each cell nucleus determined with the aid of a microspectrophotometer (Mendelsohn, 1957) to give the relative amounts of DNA.

The complete procedure was carried out for each type of cell, derived from mouse embryo and mouse spontaneous tumour tissues respectively.

## RESULTS

The Feulgen stain values and ultraviolet absorptions for individual cell nuclei are plotted separately against their corresponding nuclear dry masses in Fig. 1, for normal embryo mouse cells, and in Fig. 3, for spontaneous tumour cells. In addition, the distributions of values obtained for Feulgen stain, UV absorption and dry mass are plotted in Figs. 2 and 4 for the two types of cells.

Considering first the normal embryonic cells, in Fig. 2 *a* the Feulgen stain measurements show a large group corresponding to the post-telophase value and a smaller one corresponding to the preprophase value, with a few intermediate values. This is because, in most types of mammalian cells preparing for a further division, DNA synthesis occurs only during the latter part of interphase (Swift, 1950; Walker and Yates, 1952); thus there is a waiting time before onset of synthesis and another shorter one after completion, and it is more probable to find DNA values corresponding to one of these periods. In Fig. 2 there will also be a number of differentiating cells not in a synthetic cycle leading to a further division; these also will presumably form part of the post-telophase peak. The value distributions in Figs. 2 *b* and 2 *c* for nuclear dry mass (protein) and UV absorption (total nuclear nucleic acid) are similar to that for DNA for mouse embryo cells.

Examination of the plots of dry mass against DNA and against total nuclear nucleic acid (Fig. 1) shows that in each case the values are closely grouped. From these plots it is clear that there is a close relation between the amount of DNA in a cell nucleus and the dry mass (protein) content, and also a close relation between the nuclear UV absorption (total nucleic acid) and dry mass content. A least squares analysis of the points in Fig. 1 gives the high correlation of 0.918 between DNA and nuclear dry mass, and a similar correlation of 0.919 between total nuclear nucleic acid and dry mass. Using Fisher's *z* transformation

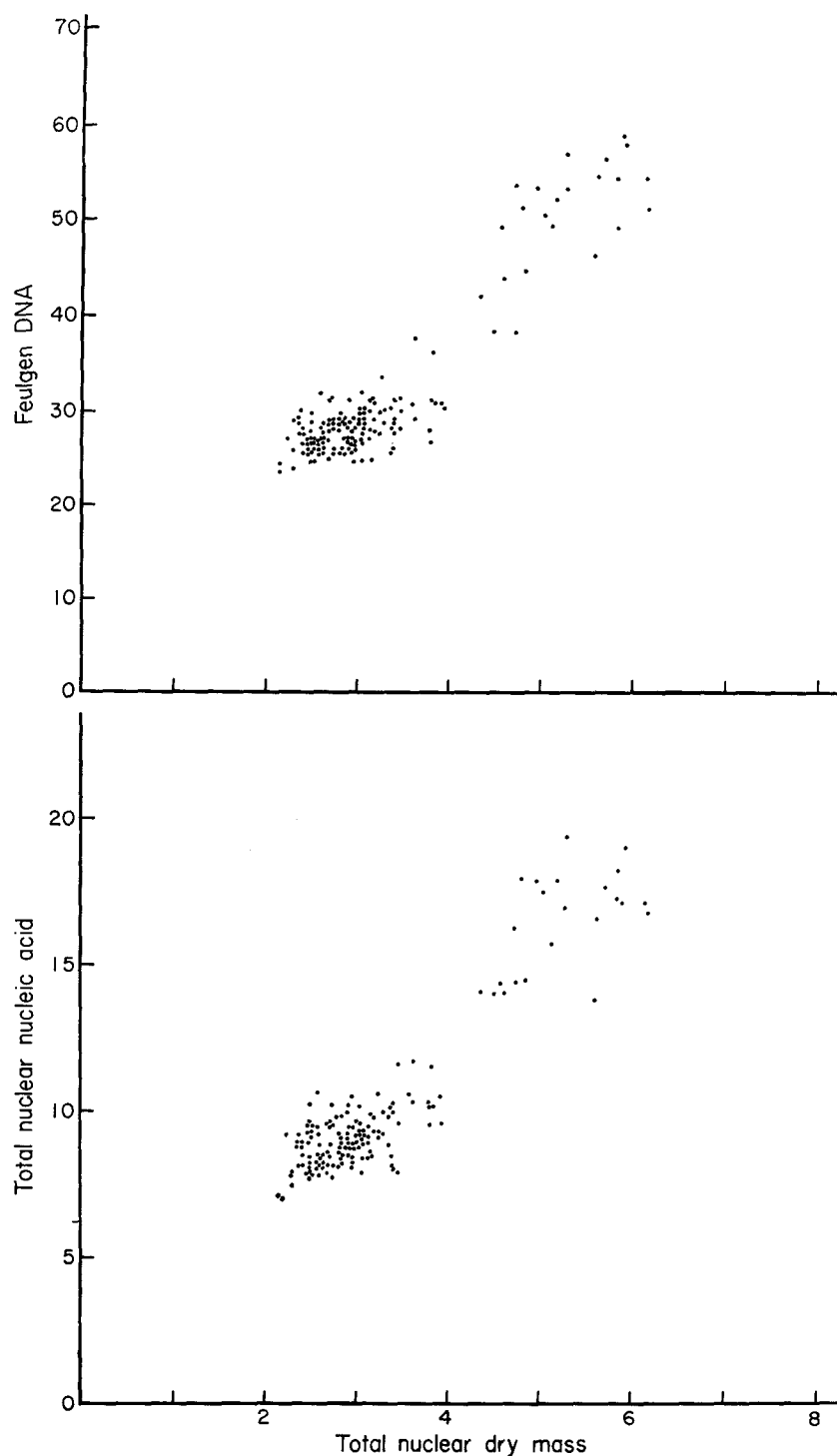


FIGURE 1 Feulgen stain (DNA) and ultraviolet absorption at 2536 Å (total nuclear nucleic acid) measurements for individual mouse embryo cell nuclei, plotted against the corresponding nuclear dry masses. All quantities are in arbitrary units.

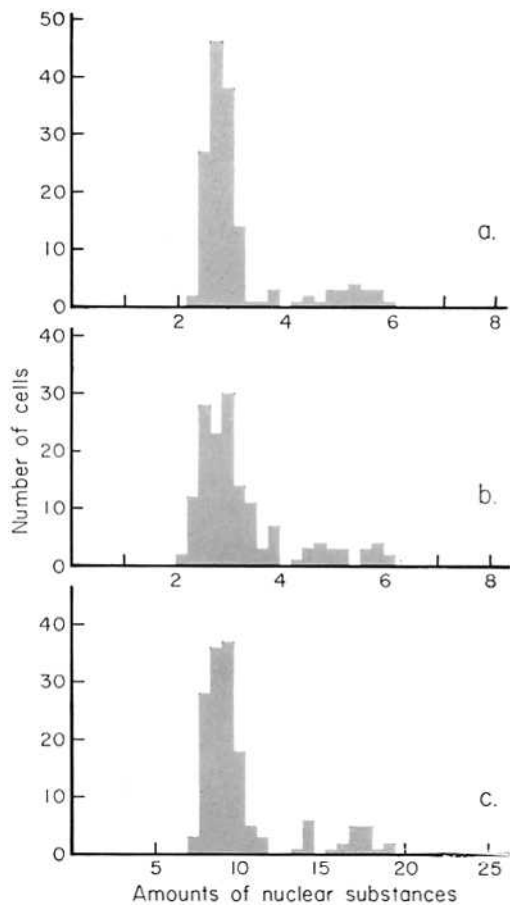


FIGURE 2 Distributions of values of (a) Feulgen stain (DNA), (b) nuclear dry mass, and (c) nuclear ultra-violet absorption (total nuclear nucleic acid) for mouse embryo cells. (From Fig. 1: all quantities are in arbitrary units).

(Fisher, 1936) to test the significance of the differences between the coefficients,  $t = 0.078$  which does not reach the value to be attained at the  $P = 0.05$  level ( $t = 1.97$ ) nor even at the  $P = 0.5$  level ( $t = 0.67$ ).

It is reasonable to conclude, therefore, from the similarity of the high correlations between DNA and dry mass and between total nuclear nucleic acid and dry mass, that the syntheses of all three components, DNA, RNA and protein, occur closely together in interphase, in the chromatin of nuclei of normal mouse embryonic cells *in vivo*. This conclusion is identical with that reached for DNA and nuclear protein synthesis in mouse embryo cells *in vitro* (Seed, 1961, 1962),

and for DNA, RNA and nuclear protein synthesis in normal monkey kidney cells *in vitro* (Seed, 1961, 1962, 1963), where time-lapse photography was used to show that the three time-synthesis curves were similar in pattern. Moreover, for the timed normal monkey kidney cells it was shown that similar high correlations existed between DNA and nuclear dry mass and between total nuclear nucleic acid and nuclear dry mass: there was no significant difference between the two correlations (Seed, 1963). However, there were relatively more intermediate and high values in the correlation plots for monkey kidney cells *in vitro*, presumably in the main because the period of synthesis and premitotic delay occupied a relatively longer fraction of the short interphase times ( $\sim 17$  hours) prevailing in the cultures *in vitro* and also because of the presence *in vivo* of cells not proceeding to another division. Analysis of variance of the monkey kidney cell plots also showed that there was no significant non-linearity in either of the correlations (Seed, 1963): because of the relatively small number of intermediate values in the *in vivo* experiment it was not possible to perform non-linearity tests here.

Turning next to the results for the spontaneous tumour cells (Fig. 3), and to the value distributions (Fig. 4), it is seen that the distribution of Feulgen stain values again has two groups, corresponding to the two waiting periods, post-telophase and prophase, in the cell cycle for DNA synthesis. However, in Fig. 4 *b* the dry mass distribution is continuous in pattern. Moreover, the correlation plots in Fig. 3 show that, whereas the dry mass (protein) and total nucleic acid in a cell nucleus are approximately proportional to one another and increase approximately together, the relation between DNA and dry mass is more complex: the difference between the two plots is presumably due to the synthesis of non-nucleolar RNA associated with the synthesis of protein during the cell cycle. A least squares analysis of the plots in Fig. 3 confirms a high correlation coefficient of 0.945 between total nuclear nucleic acid and nuclear dry mass but gives a lower correlation of 0.867 between DNA and nuclear dry mass. Applying Fisher's  $z$  transformation (Fisher, 1936), a significant difference is found between the two correlation coefficients, giving  $t = 3.78$  which exceeds the value to be attained at the  $P = 0.001$  level ( $t = 3.30$ ). Again, analysis of variance of the results in Fig. 3 shows a significant non-linear-

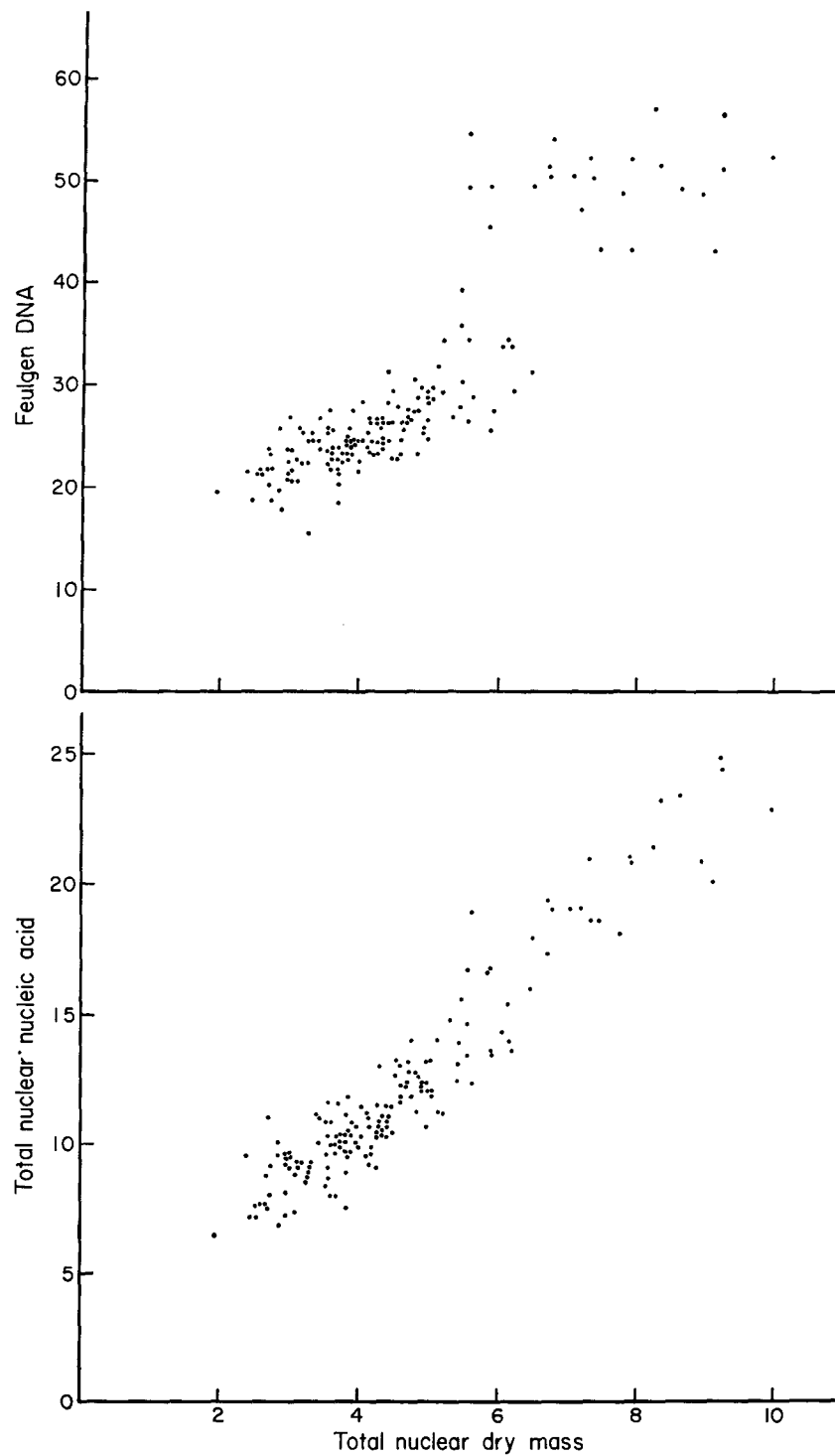


FIGURE 3 Feulgen stain (DNA) and ultraviolet absorption at 2536 Å (total nuclear nucleic acid) measurements for individual nuclei of mouse spontaneous tumour cells, plotted against the corresponding nuclear dry masses. All quantities are in arbitrary units.

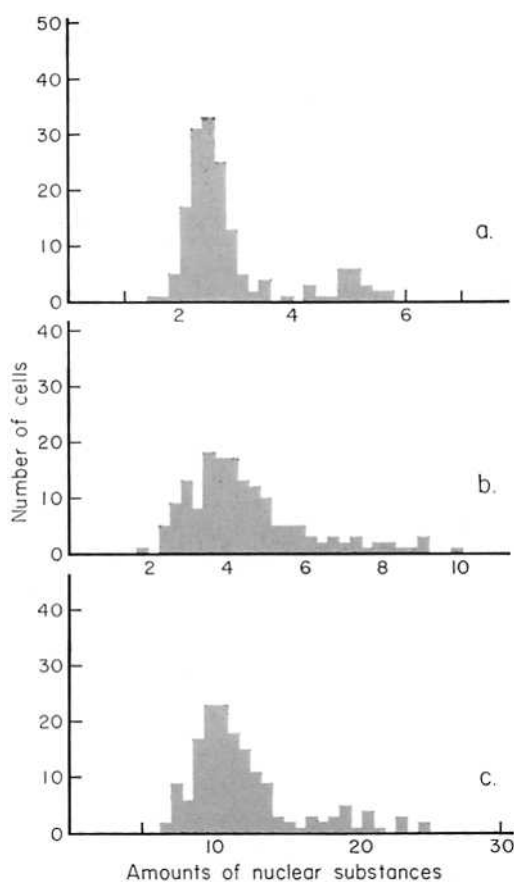


FIGURE 4 Distribution of values of (a) Feulgen stain (DNA), (b) nuclear dry mass, and (c) nuclear ultra-violet absorption (total nuclear nucleic acid) for mouse spontaneous tumour cells. (From Fig. 3: all quantities are in arbitrary units).

ity in the regression function of DNA on nuclear dry mass, giving  $F = 4.68$ , which exceeds the value to be attained at the  $P = 0.01$  level ( $F = 3.44$ ). On the other hand, analysis of variance shows no significant non-linearity in the regression of total nuclear nucleic acid on dry mass, giving  $F = 2.28$ , which does not reach the value to be attained at the  $P = 0.05$  level ( $F = 2.43$ ).

It would appear, therefore, that during the interphase of the spontaneous tumour cells, the nuclear protein and total nuclear nucleic acid increase approximately together, whereas the nuclear protein and DNA contents increase independently to an appreciable extent. These results on the correlation plots are in good agreement with those found for HeLa tumour strain

nuclei in culture, where the correlation between nuclear dry mass and DNA content was found to be significantly lower than the correlation between dry mass and total nucleic acid content: a significant non-linearity also existed in the nuclear dry mass—DNA correlation, whereas no significant non-linearity was found in the nuclear dry mass—UV absorption correlation. By using time-lapse photography, these results for HeLa tumour strain cells were shown to be associated with differences in timing between DNA synthesis on the one hand, which began only after a waiting time had elapsed after telophase, and nuclear RNA and protein synthesis on the other hand, which proceeded continuously in the chromatin of the HeLa strain nuclei (Seed, 1963). As with the normal cells *in vivo*, there are in Fig. 3 relatively fewer cells with high values than in the HeLa cell correlation: this is presumably because in the HeLa cell *in vitro* the period of DNA synthesis with the premitotic delay occupies a much bigger fraction of the shorter interphase time ( $\sim 21$  hours) and the presynthetic delay is correspondingly shorter.

In the present type of experiment, it is worth noting that by the restriction of comparisons of correlations to within the one cell population, the effects of certain variations or errors of measurement, for example of cell size, will be minimized because the same cell areas are involved in all three sets of measurements: in addition, the correlations are compared over the same ranges of dry mass values. Thus the conclusions drawn here are based on the similarity of the high correlations between dry mass and DNA and between dry mass and total nucleic acid within the one population of normal embryonic cells, and the dissimilarity of these correlations within an equivalent population of tumour cells.

As I have previously pointed out (Seed, 1962), the cytophotometric measurements of McLeish (1959, 1960) on normal plant nuclei are consistent with my work on normal animal cells. McLeish found similar bimodal distributions for DNA and for arginine (Sakaguchi stain), and from his results he concluded that the syntheses of DNA and bound arginine occurred closely together in interphase in the cell nuclei derived from root meristem. On the other hand, Feulgen and dry mass measurements on ascites tumour cell nuclei (Richards and Davies, 1958) gave a bimodal distribution for DNA but a continuous distribution for dry mass: from their findings the

authors derived dissimilar synthesis curves for DNA and nuclear protein which are equivalent to those I have reported for tumour strain cells *in vitro* (Seed, 1961, 1962, 1963). Both of the experiments quoted above represent *in vivo* synthesis, and both lend supporting evidence to that presented here.

#### BIBLIOGRAPHY

- BARER, R., 1952 *a*, *Nature*, **169**, 108.  
BARER, R., 1952 *b*, *Nature*, **169**, 366.  
BONNER, J., HUANG, R. C., and MAHESHWARI, N., 1961, *Proc. Nat. Acad. Sc.*, **47**, 1548.  
DAVIES, H. G., and WILKINS, M. H. F., 1952, *Nature*, **169**, 541.  
FISHER, R. A., 1936, *Statistical Methods for Research Workers*, London, Oliver & Boyd, Ltd.  
MCLEISH, J., 1959, *Chromosoma*, **10**, 686.  
MCLEISH, J., 1960, in *The Cell Nucleus*, (J. S. Mitchell, editor), London, Butterworth and Co. Ltd., 91.  
MENDELSON, M. L., 1957, Ph.D. Thesis, University of Cambridge, Cambridge, England.  
RICHARD, B. M., and DAVIES, H. G., 1958, in *General Cytochemical Methods*, (J. F. Danielli, editor), New York, Academic Press, Inc., **1**, 130.  
SEED, J., 1961, *Nature*, **192**, 944.  
SEED, J., 1962, *Proc. Roy. Soc. London, Series B*, **156**, 41.  
SEED, J., 1963, *Nature*, **198**, 147.  
SWIFT, H. H., 1950, *Physiol. Zool.*, **23**, 169.  
WALKER, P. M. B., and YATES, H. B., 1952, *Proc. Roy. Soc. London, Series B*, **140**, 274.

I am grateful to the Council of the Royal Society for the loan of a recording microdensitometer. I thank Mr. B. Turner for technical assistance.

Part of the work was done during the tenure of the Royal Society Foulerton Gift Research Fellowship.

*Received for publication, March 29, 1963.*