6. Computerized karyotype analysis.

A meaningful analysis of the variation in arm lengths and arm ratios of chromosomes requires a large number of measurements and repetitious calculations. Preceding such an analysis a survey was initiated to investigate the possibilities of computerized analysis.

Computerized analysis of human chromosomes is being attempted in several laboratories. The Chloe film scanner, F.I.D.A.C., and the Cydac system are three devices being used. The first two systems provide as computer output the image of the chromosome spread, analyzed as to arm ratio and total length of each chromosome. Negatives of the cells to be analyzed must be fed into the Chloe scanner and F.I.D.A.C. whereas the Cydac system takes the image directly from the prepared slide. Cydac appears to be the most efficient system for an analysis of the proposed type since it would both collect and analyze the data.

Manual measurements of chromosome lengths and arm ratios can be done with good precision. A computerized project would also permit the determination of mean arm lengths and ratios with a small standard error. Whether any greater precision can be obtained using the computer, has not been established. Thus, the merit of computer use appears to lie in its relative speed and precision and removal of the limitation of small sample size.

Computerized chromosome analysis could be useful in any area where somatic chromosomes are to be examined. For instance, development of an aneuploid series could be accelerated. As well, trisomic and nullisomic analysis could be computerized. Since the computer can be programmed to identify, record and recall, normal and abnormal chromosome complements can be determined. Computerization, then, could increase quantity without a sacrifice on quality.

Computerized analysis of corn root tip preparations have been attempted using the Chloe film scanner. Many technical problems unique to the corn material, have yet to be overcome. Whereas the human leucocyte cultures are in a monolayer due to the air drying process used in slide preparation, corn root tip smears are thicker and therefore result in a more dense background. As well, the corn chromosome arms are not spread as widely as the human material. The net result of these technical differences is the inability to locate accurately the centromere and define the limits of the arms. Attempts are being made to correct the technical problems and hopefully a satisfactory solution can be found.

W. G. Filion

7. Nuclear cycle in maize root tips.

An investigation of the nuclear cycle, and its components, in corn was undertaken in preparation for evaluation in specific chromosomes of the pattern of incorporation of $H^3$ - thymidine during DNA synthesis.

Seeds of "Seneca 60" hybrid ($\underline{su}_1/\underline{su}_1$) were germinated and grown at 28°C on filter paper kept moist with distilled water. Germinated kernels with roots at least 1 cm in length were placed for 0.5 hours in a solution of $H^3$ - thymidine (6.3 c/m M diluted to 1.0 uc/m M distilled water) after which they were incubated in distilled water (without chaser). Root tips were fixed subsequently at hourly intervals. Liquid emulsion autoradiographs (Kodak KTB-2) were prepared from Feulgen squashes of this material, and slides were scored for the frequency of labelled division figures. Over 6000 division figures were examined.

Per cent prophase and metaphase figures were plotted against time after labelling (Fig. 1). The adjusted prophase line (short dashes) indicates the line for the end of prophase.

Following Wimber's (1960) interpretation, the duration of $G_1$, S, $G_2$ and mitosis was calculated from the data. Dissatisfaction with the lack of precision of these estimates obtained by extrapolation from the curve led to a probit regression analysis, yielding more appropriate calculations of the mean and standard deviation of the durations of the various phases of the nuclear cycle.

The results are tabulated below, and are compared with other information on the nuclear cycle of corn (Clowes, 1965).

| | Duration (hours) of nuclear cycle of maize | | |
|---|---|---|---|
| Phase | Mean | Standard Deviation | Clowes (1965) |
| $G_1$ | -0.75 | 0.66 | 3 |
| S | 6.02 | 0.07 | 11 |
| $G_2$ | 3.08 | 0.09 | 3 |
| M - Prophase | 0.97 | | |
| Metaphase | 0.37 | 0.66 | |
| Anaphase | 0.13 | | |
| Telophase | 0.37 | | |
| sub total (M) | 1.84 | | 2.5 |
| Total | 10.19 | 0.67 | 19.5 |

The difference between Clowes' report and the present study in the total nuclear cycle time can be attributed to differences in the S and $G_1$. Clowes' seed was germinated and incubated at 20°C, whereas the present study was conducted at 28°C. We suggest that the difference in the duration of S is due to the accelerated rates of biochemical processes at the higher temperature. The negative value calculated for $G_1$ indicates that $G_1$ cannot be accommodated within the total mitotic cycle.

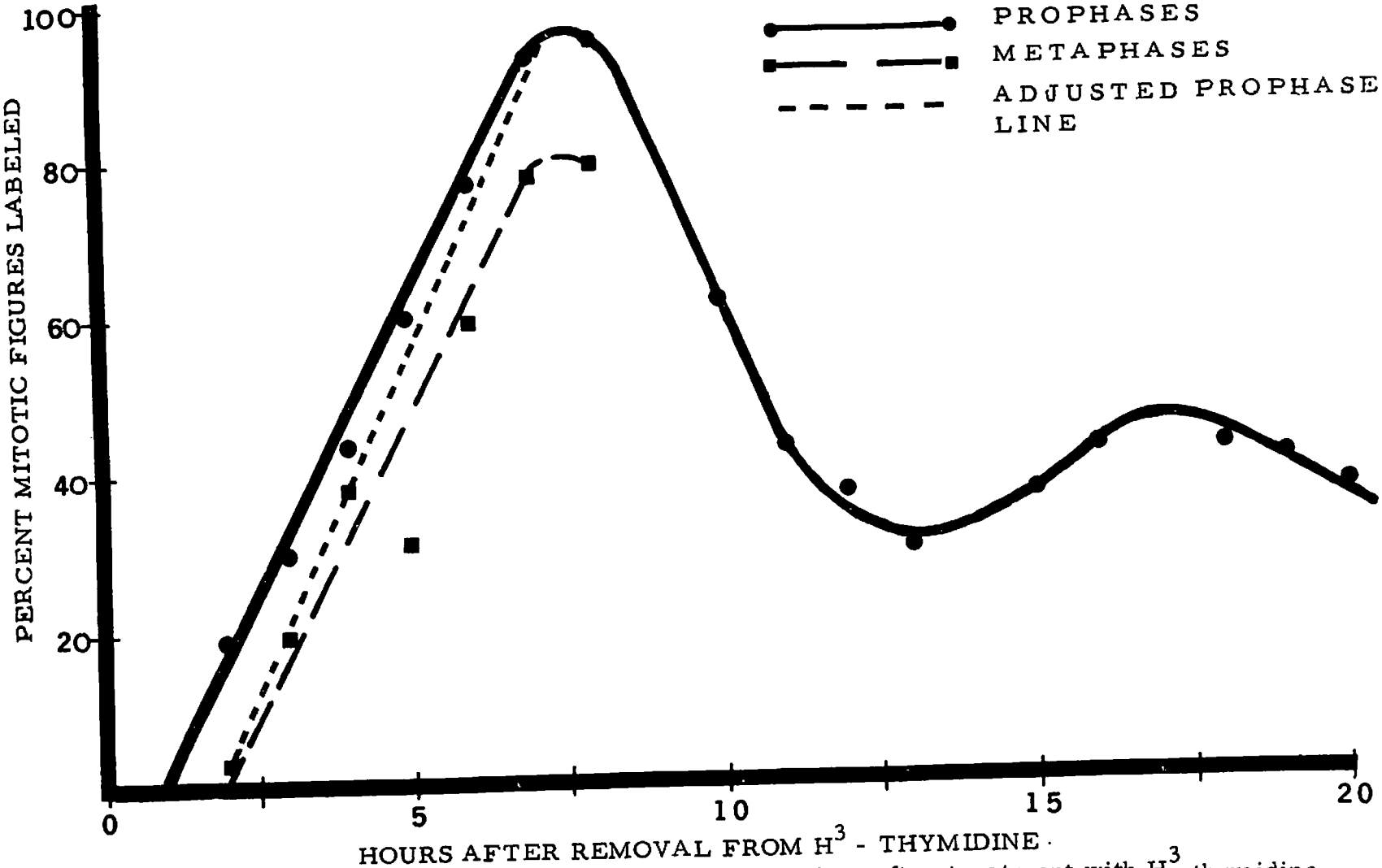


Figure 1. Percentage of divisions labeled vs. time after treatment with $H^3$ thymidine

Clowes (1967) has shown that in rapidly dividing cap initials of corn, $G_1$ is telescoped such that DNA synthesis can start as early as telophase. In our case the short cell cycle indicates that most cells of the root tip were rapidly dividing, leading to an overlap of the S period with the end of telophase, and thus, effectively eliminating $G_1$.

## Literature Cited

Clowes, F. A. L. 1965. The duration of the $G_1$ phase of the mitotic cycle and its relation to radiosensitivity. New Phytol. 64:355-359.

_____. 1967. Synthesis of DNA during mitosis. J. Expt. Bot. 18:740-745.

Finney, D. J. 1962. Probit Analysis. Cambridge University Press, London.

Wimber, D. E. 1960. Duration of the nuclear cycle in Tradescantia paludosa root tips as measured with $H^3$ - thymidine. Am. J. Bot. 47:823-833.

G. R. Douglas

UNIVERSITY OF WISCONSIN
Madison, Wisconsin
Department of Genetics

## 1. Effects of ionizing irradiation on paramutation at the R locus in maize.

### A. Effects of X-irradiation on pigmenting potential of standard $R^r$ and paramutants from standard $R^r$

Standard $R^rR^r$ seeds and pollen from $R^rR^r$ plants were irradiated with 10,000r and 1,200r, respectively, following which testcrosses were made on $r^gr^g$ plants. The data obtained by evaluating the one dose $R^rr^gr^g$ aleurone of the testcross ears indicated that these treatments produced no effect on the pigmenting potential of $R^r$. On the other hand, the pigmenting potential of strongly repressed $R^{r'''}$ alleles (passed through heterozygotes with the $R^{st}$ allele for three generations) was partially restored, and to different levels, after X-irradiation of seeds (10,000r) and pollen (1,400r). Complete restoration, however, occurred only rarely. The frequencies of detectable changes were 21 per cent for treated seeds and 12 per cent for treated pollen. Other paramutant alleles at different levels of repression were tested by the same methods; all gave a response similar to that of the $R^{r'''}$ alleles. Further tests showed that the X-ray-induced increases in pigmentation were heritable, and that all the paramutant alleles whose pigmenting ability had been increased by X-irradiation were still paramutable. The high frequency of X-ray-induced changes support the suggestion (Brink 1964) that there is a component at or near the R locus responsible for repression, and that this component, rather than the R structural gene itself, is involved in the response to irradiation. That the increases in paramutant pigmenting ability were to different levels also supports the postulate that more than one unit of the genetic component is involved in repression.