that becomes more evident towards the 4th day after irradiation and reaches normal values on the 6th day.

In the activity of the acid phosphatase we noted a more marked inhibition in the irradiated cultures.

The enzymatic activity of the culture and the number of cells present at the times of determination have been correlated, as illustrated only for the dose of 300 r in Figures 4 and 5.

From these Figures it appears that the activity of the alkaline phosphatase calculated per single cell is actually higher in the irradiated cultures, because fewer cells are present in them, as compared with the control. The increase in this activity is maximal on the 2nd day and decreases to normal by the 6th day. In contrast, there are only minor variations in the level of acid phosphatase.

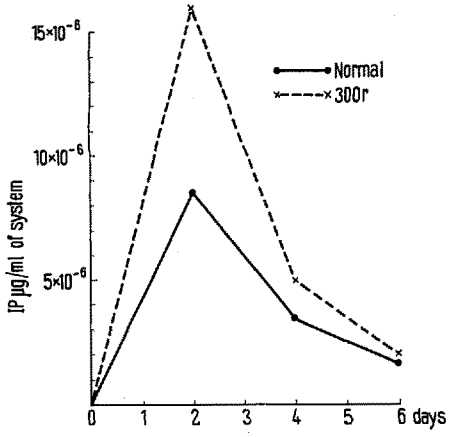

Fig. 4. Effect on alkaline phosphatase calculated per single cell.

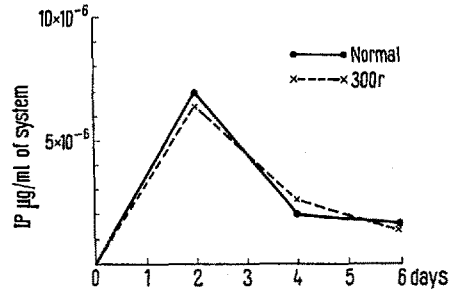

Fig. 5. Effect on acid phosphatase calculated per single cell.

Conclusions. We have studied the behaviour of primary monkey kidney cultures after irradiation with 300, 1000, and 3000 r. At the same time, on these cultures we have made determinations aimed at showing cell growth rate and alterations in the acid and alkaline phosphatase activity to determine whether it is possible to detect enzymatic changes preceding the growth alterations and the appearance of cells with micronuclei.

Considering the modifications in the enzymatic activity calculated per single cell, it was concluded that after irradiation with 300 and 1000 r alkaline phosphatase activity increases whilst acid phosphatase does not differ in behaviour from normal cells. The increased activity of the alkaline phosphatase reaches a maximum on the 2nd day after irradiation when the growth rate begins to decrease.

Considering the diffuse distribution of alkaline phosphatase in the cells the increased activity of this enzyme could be the expression of an early generalized stimulation or lesion that later disappears. The radioresistance of the acid phosphatase may be unusual because the activity of several other enzymes localized in the lysosomes is increased after irradiation[6].

We have noted that, in irradiated monkey kidney cell cultures, very particular cells appear which are characterized by the presence of one or more micronuclei arranged around the principal nucleus. These micronuclei frequently contain a small nucleolus.

The study of the formation of these cells is at present in progress.

Riassunto. Colture primarie di rene di scimmia (*M. rhesus*) presentano, dopo l'irradiazione, un aumento nell'attività della fosfatasi alcalina, mentre l'attività della fosfatasi acida non risulta modificata.

P. VERANI, D. BALDUCCI, M. A. CHIBBARO, M. CHIOZZOTTO, S. PERUGINI, and G. PENSO.

Istituto Superiore di Sanità, Roma (Italy), September 19, 1963.

S. OKADA, E. R. GORDON, R. KING, and L. H. HEMPELMANN, Arch. Biochem. Biophys. *70*, 469 (1957).

Chromosomal Control of Nucleolar Synthesis

The discovery of enzymes like polynucleotide phosphorylase[1] and RNA-polymerase[2], and their experimental use in synthesising polyribonucleotides, have indicated that the biosynthesis of ribonucleic acid (RNA) is mediated by deoxyribonucleic acid (DNA). This can be concluded from the observation that incorporation of ribonucleotides is significantly increased in the presence of primer DNA, which also influences the base composition of the synthetic 'RNA'. While studies of this type have been undertaken on cell-free systems, autoradiographic analysis[3,4] in different tissues has also shown that incorporation of labelled RNA precursors is first initiated in the nucleus, close to the chromatin regions, and only at a later stage the radioactive RNA makes its appearance in the cytoplasm, presumably after its migration from the initial sites of synthesis in the nucleus. These findings are

[1] M. GRUNBERG-MANANGO, P. J. ORTIZ, and S. OCHOA, Biochem. biophys. Acta *20*, 269 (1956).
[2] J. HURWITZ, J. J. FURTH, M. ANDERS, P. J. ORTIZ, and J. T. AUGUST, Cold Spr. Harb. Symp. Quant. Biol. *26*, 91 (1961).
[3] R. MCMASTER-KAYE, J. Histochem. Cytochem. *10*, 154 (1962).
[4] C. P. LEBLOND and M. AMANO, J. Histochem. Cytochem. *10*, 162 (1962).

consistent with the cytological observation that the nucleolus, a nuclear component known to be rich in RNA content, is organized in close association with chromosomal segments. The normal process of nucleolar organization, however, is not such that the role of the chromosomal DNA in this respect becomes particularly evident. This is primarily due to the fact that the formation of the nucleolus, at a specifically differentiated region in the nuclear complement, undermines the chromosomal control of the process, the differentiated region being considered by some to be a collecting centre rather than a synthetically active site (see VINCENT[5]). It may, therefore, be possible to demonstrate chromosomal control of nucleolar synthesis in a more striking manner if the regular organizing region is experimentally inactivated. This has been done in *Lolium* by means of high temperature treatment of plants as described fully elsewhere[6]. Following such inactivation of the organizer, it is observed that only those chromosomes in the nucleus which show normal stainability and regular prophase condensation are able to synthesise nucleolar material, others which are abnormal in these respects fail to indicate any nucleolar activity. These observations on the varying number of 'nucleolar' and 'non-nucleolar' chromosomes in different cells, the former showing normal prophase development and the latter failing to do so (Figures 1 and 2), clearly indicate a close correlation of nucleolar synthesis with those chromosomal characteristics which are known to be a function of its DNA component. It could be concluded, on the basis of their relatively poor stainability, including that with Feulgen stain, and retarded prophase development, that, as a result of the high temperature treatment, some of the chromosome pairs are affected in their normal DNA synthesis and these are the ones which fail to show any nucleolar activity. The present observations supplement the earlier analysis of MCCLINTOCK[7] on maize, which indicated that all the chromosomes in a nucleus can take up nucleolar synthesis when the organizer is inactive. The observations on *Lolium* show that non-specific chromosomes can take up this activity only if they are normal with regard to their DNA-conditioned organization, indicating thereby the nature of the chromosomal control of nucleolar synthesis.

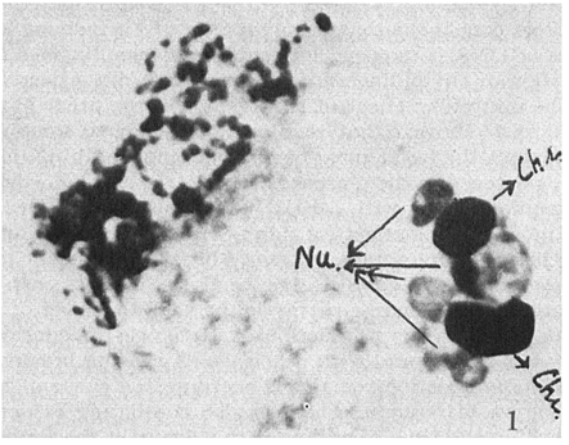

Fig. 1. PMC showing the formation of nucleolar bodies (Nu) by two of the chromosome pairs (Chr) following the inactivation of the organizer. These two pairs are the only ones showing normal prophase development; the remaining chromosomes are retarded in their condensation. Carnoy-acetocarmine squash.

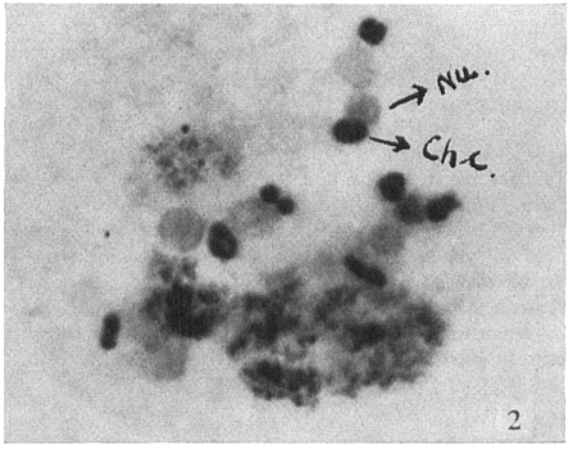

Fig. 2. A 'binucleate' PMC in which as many as 8 bivalents form nucleoli (see Figure 1). The 'non-nucleolar' chromosomes in this cell also fail to show normal prophase development. Carnoy-Feulgen squash with counterstaining in acetocarmine.

Zusammenfassung. Aus cytologischen Inaktivierungsexperimenten wird der Beweis abgeleitet, dass die Nucleolus-Synthese auf jenen Chromosomeneigenschaften beruhe, die mit ihrer pro-phasischen Verdichtung und Färbbarkeit eng verknüpft ist. Es scheint dies darauf hinzudeuten, dass DNA derjenige Bestandteil des Chromosoms ist, welcher die Bildung von Nucleolen steuert.

H. K. JAIN

Division of Botany, Indian Agricultural Research Institute, New Delhi (India), July 5, 1963.

[5] W. S. VINCENT, Intern. Rev. Cytol. *4*, 269 (1955).
[6] H. K. JAIN, Heredity *11*, 23 (1957).
[7] B. MCCLINTOCK, Z. Zellforsch. *21*, 294 (1934).