The Identification of some Specific Meanings in Infant Vocalization

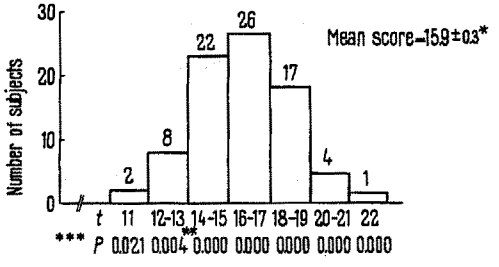
In her experiment, SHERMAN[1] collected some empirical data that led her to the conclusion that the situational content of preverbal infant vocalizations cannot be recognized by graduate students in psychology, medical students and nurses. The cries used by SHERMAN were actual vocalizations obtained in the course of the experiment, in situations of hunger, dropping the infant towards the table, restraint of the face of the infant towards the table, and sticking with a needle. The infants varied in age from three to seven days. They could not be seen by the subjects who were asked to try to identify the situations in which the cries were obtained.

By our preliminary studies, we found some evidence that the negative results of SHERMAN – extensively referred to in various textbooks – could possibly be disconfirmed by using recorded vocalizations typical to the situations of birth, pain, hunger, and pleasure, and by giving these response categories in advance to the subjects (multiple choice technique).

After our first experimentations on the subject, we constructed a magnetic tape consisting of 24 selected vocal responses that seemed, by an auditive analysis, to be typical for the four situations: six birth vocalizations (obtained during the first 5 min of the life of the child), six pain vocalizations (recorded when vaccinations were administered, from two weeks to eight months of age), six hunger cries (recordings were made about 4 h after previous mealtime, from babies one week to eight months of age), and six pleasure cries (obtained after meal from babies 4–8 months old). All material used was obtained from normal children and normal deliveries. In every type of vocalization, attempts were made to eliminate other emotional states than the main one. The mean length of the vocalizations was 12.3 sec, the shortest one being 5.0 sec and the longest one 17.7 sec. The vocalizations were on the test tape in random order. For more details about our vocalization material, see WASZ-HÖCKERT et al.[2–4].

Our first trial sample tested by the material described above consisted of 80 trained nurses, aged 23–37 years. No one had children of her own, and some had been working with children after having completed their training. The ordinary split-half reliability coefficient (corrected for the entire test) was computed to be 0.67.

The Figure shows the distribution of the nurses according to how many correct choices they made. The p-values are binomial probabilities for at least the corresponding t-value to be obtained by mere chance. The average nurse in our sample made 16 right choices out of 24, the corresponding chance probability being as small as 0.00002. Even the poorest score could be obtained only in two cases out of a hundred by chance. Thus, the identification of the vocalizations was not complete. On the average, however, 67% were identified.



Distribution of 80 nurses according to their total scores. t = total score, or number of correct choices.

* 0.3 = Standard error of the mean. ** interpolated.

*** $P = \sum_{t}^{24} \binom{24}{t} 0.25^{t}\, 0.75^{24-t}$.

Résumé. Un test de type du choix multiple montre que les infirmières peuvent reconnaître d'après une bande magnétique la signification des émissions vocales préverbales répondant à des situations données (naissance, douleur, faim, plaisir) chez les enfants de moins de 8 mois. Chez les 80 infirmières testées l'aptitude moyenne à l'identification fut de 67%. Nos études portant sur d'autres groupes humains seront publiées plus tard.

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T. J. PARTANEN, V. VUORENKOSKI,
K. MICHELSSON, and E. VALANNE

Children's Hospital, University of Helsinki (Finland), August 29, 1963.

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[4] O. WASZ-HÖCKERT, V. VUORENKOSKI, E. VALANNE und K. MICHELSSON, Rev. Mex. Pediatr., in press.

The Effects of Radiation on Enzymes in Tissue Cultures

One of the tasks of the experimental biologist is to correlate the effect of radiation *in vivo* and *in vitro*. Experiments *in vivo* are complicated by the fact that in the animal there are several cell types, each having a different radio-sensitivity; tissue cultures therefore have the great advantage of being constituted of a cell population that can be considered homogeneous.

Cells that have received sublethal doses of X-rays have survived and continued to multiply. We have investigated some morphological and biochemical aspects of sublethally irradiated cells and non-radiated cells to see whether differences are detectable between the two systems.

Materials and Methods. Rhesus monkey kidney cells obtained by trypsinization have been used throughout the experiments[1].

Irradiation. The method of crystal violet uptake by the nuclei was used to check cell viability.

The cells, at a concentration of 500,000/ml in petri dishes, were irradiated with 300, 1000 and 3000 r. The

C. RAPPAPORT, Bull. Wld. Hlth. Org. *14*, 147 (1956).

covered Petri dish was exposed for measured intervals of time to a Picker 260 KVP[2] X-ray tube operating at 230 KVP and 14 mA, filtered with 1 mm of aluminium and 0.5 mm of Cu. An exposure dose rate of 57.8 r/min was employed. After irradiation, the cells were diluted 1:5 in growth medium, distributed in culture bottles and maintained in an incubator at 37°C.

Growth determination. The ability to form colonies and the rate of growth of the cultures were followed by counting the average number of cells per colony in a microscopic field at different intervals of time. The growth rate of irradiated cells has been expressed as a percentage of the controls.

Enzymatic determinations. Synthetic medium without inorganic phosphorus has been used. This medium is constituted of sucrose, glucose, amino acids and one organic compound – sodium-β-glycerophosphate – that is the substrate for the phosphatases. The inorganic phosphorus liberated by the enzymes was determined by the FISKE-SUBBAROW method[3].

Control cultures were incubated in medium without substrate. The value of the inorganic phosphorus in these cultures was considered to be the baseline.

The difference between the amount of inorganic phosphorus in cultures incubated with medium containing substrate and in those without substrate represented the activity of the phosphatases. This activity has been expressed as γ of phosphorus per ml liberated in 24 h.

Results. Figure 1 illustrates the growth variation as the number of cells per colony. The cultures that received 300 and 1000 r show increased or normal growth on the first day and progressively slower growth on successive days. There were no differences in the number of colonies between the control cultures and the cultures irradiated with 300 r, thus the ability to form colonies was not affected at this dosage level. At 1000 r the number of colonies was reduced by 15%.

In the first four days a slowing of the growth rate therefore results from the fact that cells grow slowly after irradiation and not from increase in cell mortality. Therefore the growth rate of cells per microscopic field as function of time is parallel to the number of cells per colony (Figure 2). The irradiated cultures continued to grow up to the formation of confluent cell sheets.

Normal monkey kidney cells cultured *in vitro* can be classified in 8 groups, already described[4], on the basis of the shape and number of nuclei. We observed in the irradiated cultures the appearance of a very peculiar type of cell containing micronuclei (Figure 3). The percentage of these cells depends on the irradiation dose and increases progressively during the time of observation. These observations were made on fixed and stained cultures.

Enzymatic determinations were carried out directly on cells maintained in the special synthetic medium already mentioned. In this way it was possible to study the activity of intracellular enzymes under controlled experimental conditions, avoiding the variations introduced by the use of sera, proteins, nucleic acids and enzymes. This method has already been used[5] to detect early intracellular changes during viral infections that precede morphological alterations.

The following enzymes have been studied in irradiated cultures: alkaline phosphatase, which is increased during the early stage of poliomyelitic virus infection, and acid phosphatase, which is slightly decreased.

We have calculated the level of alkaline phosphatase activity in control cultures and cultures irradiated with 300 or 1000 r. There is a slight inhibition at the beginning

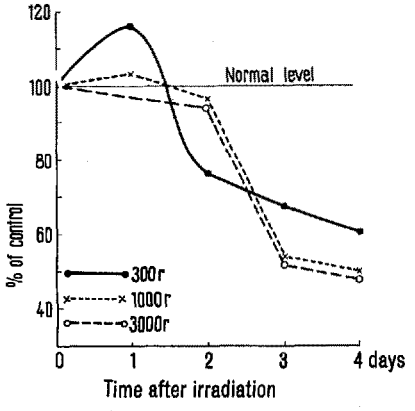

Fig. 1. Monkey kidney cell growth rate. Cells per colony.

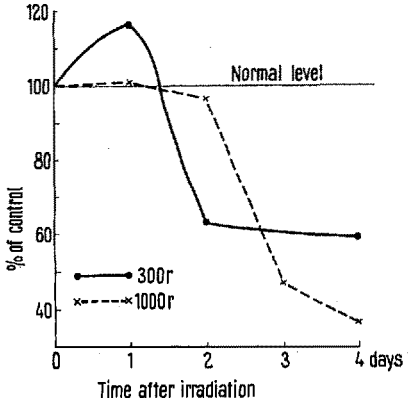

Fig. 2. Monkey kidney cell growth rate. Cells per microscopic field.

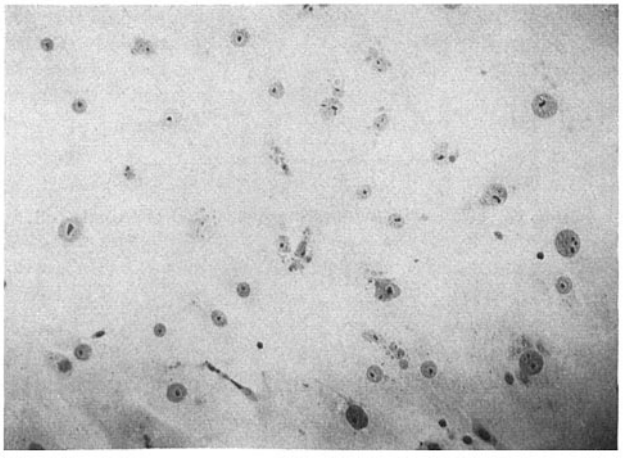

Fig. 3. Culture irradiated with 1000 r. Cells containing micronuclei.

[2] Kilovolt peak.

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[5] E. KOVACS, G. WAGNER, and V. STURTZ, Z. Naturforsch. *15b*, 506 (1960).

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.

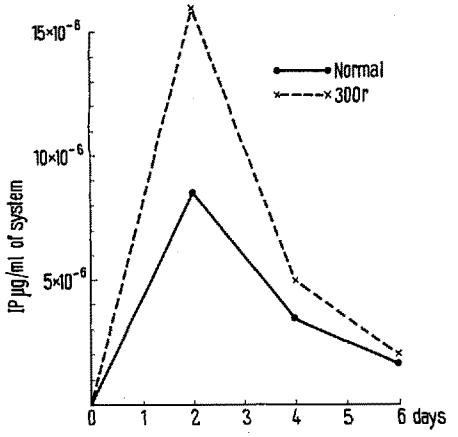

Fig. 4. Effect on alkaline phosphatase calculated per single cell.

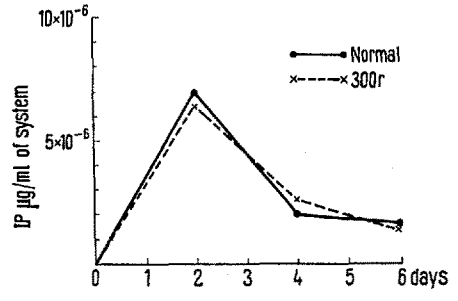

Fig. 5. Effect on acid phosphatase calculated per single cell.

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.

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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

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