Tissue Culture Studies on *Vicia Faba*

IV. Effect of growth factors on mitotic activity¹

S. Venketeswaran and Eliot B. Spiess

Department of Biology, University of Pittsburgh,
Pittsburgh 13, Pennsylvania, U. S. A.

Received July 31, 1963

In a previous paper (Venketeswaran 1963 b) an account was presented of the variations in nuclear behavior and chromosome morphology of tissue culture cells of *Vicia faba* L. when incubated in a culture medium containing an exogenous supply of nucleic acids, their salts or their precursors. The present paper reports observations made on the relative effect of these nucleic acid additives on the mitotic activity of the cultured cells. The effect of nucleic acid on growth activity of plant cells cultured *in vitro* have been reported by other workers (Nickell *et al.* 1950, Nickell 1954 a, b, Sharma and Sen 1954, Hildebrandt *et al.* 1957, Nitsch and Nitsch 1957, Vasil 1959).

Materials and methods

The culture procedures and the culture medium employed were similar to those described in previous papers (Venketeswaran 1962, 1963 a, b). A modified Bonner-Devirian medium (Venketeswaran 1962) supplemented with 40 gm sucrose, 1×10^{-6} M 2, 4-dichlorophenoxy-acetic acid (2, 4-D) and 15% coconut milk (C. M.) per liter served as the control medium. The following substances (purchased from Nutritional Biochemicals Corporation Cleveland, Ohio) were added to this control medium at different levels of concentration: (a) Deoxyribonucleic acid (DNA)-sperm; (b) Ribose nucleic acid (RNA); (c) Sodium desoxyribonucleate (DNAate)-fish; (d) Sodium ribonucleate (RNAate)-thymus; (e) Thymidine; (f) Cytidine; and (g) Uridine. The medium was autoclaved in 125 ml Erlenmeyer flasks containing 50 ml aliquots. Particular attention was given to the influence of different concentration of these additives on the tolerance, stimulation or inhibition of growth of the small cell aggregates when they were incubated in these respective media. By running controls at the same time in the basal control media, increase in the frequency of mitoses in cell aggregates grown under different media was calculated.

To determine whether any specific changes occurred when the nucleic

¹ Part of a dissertation in fulfillment of the requirements for the degree of Doctor of Philosophy in Botany at the University of Pittsburgh, Pittsburgh, Pa., U. S. A.

acids or their salts were autoclaved, a chromatographic analysis of these compounds was carried out before and after autoclaving. A one-dimensional chromatogram was run on Whatman No. 1 filter paper with a solvent mixture consisting of n-butanol saturated with a 10 per cent aqueous urea solution. The detection of spots was made by spraying the dried chromatogram with molybdic acid reagent (5.0 ml of perchloric acid-sp. g 1.61, 10 ml of 1 N HCl, 25 ml of 4% $(\text{NH}_4)_2\text{MoO}_4$ and 60 ml of water) and observing the formation of "molybdenum-blue" upon exposure to UV illumination (Block, Durrum and Zweig 1958).

A dialysis experiment was carried out to determine how much of DNAate and RNAate (0.1% and 1.0%) were dialysed against the solvent, viz., the modified Bonner-Devirian basal control medium. The vitamin mixture, 15% C.M. and 1×10^{-6} M 2, 4-D which are generally added to make up the control medium, were omitted. The measurements were made at 260 m μ on a Beckman D. U. spectrophotometer.

Tissue pieces were inoculated aseptically into the appropriate media and the inoculated flasks were transferred to a gyrorotatory shaker (175 r.p.m) in a culture room maintained at $25 \pm 1^\circ\text{C}$ with 12 hours illumination. Material was removed aseptically at periodical intervals and fixed in a 3:1 alcohol acetic acid mixture. Standard methods of squash preparations were made using either the Feulgen technique or the acetic orcein method (Darlington and LaCour 1960). Preparations were made permanent by the quick-freeze method (Conger and Fairchild 1953).

Scoring of nuclear stages was standardized in the following manner: prophase—first distinct chromosomal threads apparent; metaphase—the chromosomes visible as distinct double-stranded bodies arranged on the metaphase plate or flattened out, with sister chromatids together; anaphase—the chromatids separate, starting to separate or in the process of moving towards the two poles; and telophase—the chromosomal strands indistinct with nuclei either fully or partly formed. As there were no significant shifts in the relative proportion of these four main stages of nuclear division, they were all pooled together and presented as percentage of nuclei (cells) in division, i. e. % mitosis.

It has been pointed out that the calculations based on mitotic frequency of cells described above might be misleading because a decrease in mitotic frequency could be either due to a relative shortening of any one of the stages or to stimulation or inhibition of any one stage (see Setterfield and Duncan 1955). In an attempt to overcome this error, the percentage of cells having completed their division was recorded by adding up the nuclei observed in anaphase and telophase stages and representing the mitotic index as percentage of cells with anaphase and telophase nuclei. Mitra *et al.* (1960) have remarked that the statistical probability of finding stages of nuclear division in any one cell aggregate is very low. To estimate the

probability of nuclear division stages in cells, recording from nine different flasks were made for each treatment and an analysis of variance was calculated to test significance of differences between treatments and controls.

General observations on the mitotic activity in cell aggregates indicated that a more active mitotic rate occurred during the night rather than during the day. To confirm this observation, materials from activity growing cultures were fixed at time intervals of two hours over a 24 hour period. Frequency counts of mitotic stages revealed a higher percentage of mitoses during night hours. Hence most of the experiments were carried out during the night hours.

Observations and results

The relative frequency of nuclear divisions in *V. faba* calli when grown in the basal control medium containing a low concentration of nucleic acids or their sodium salts is indicated in Table 1.

Table 1. Frequency of mitoses observed in *V. faba* calli after 24 hours of incubation in different culture media

Culture medium	Total no. mitoses	Per cent of nuclei in mitoses
Basal medium (control)	18/650	2.8
+DNA 0.001 g/l	18/636	2.8
+RNA 0.001 g/l	21/656	3.2
+DNAate 0.001 g/l	29/624	4.6
+RNAate 0.001 g/l	32/613	5.2

No appreciable effect in term of a stimulation of growth was observed when cell aggregates were incubated in a medium containing nucleic acids. In comparison, cells growing in a medium containing sodium salts of nucleic acid revealed that the sodium salts had a slightly increased effect on the mitotic activity of the cells. On the basis of

this initial observation and from experiments with the salts of nucleic acids by other workers (Huskins 1948, Patau and Patil 1951, Sharma and Sen 1954) a detailed study was carried out on the effect of the sodium nucleates (viz., DNAate and RNAate) at three concentrations, i.e. 0.1%, 0.5% and 1.0% (1.0, 5.0 and 10.0 g/l).

Hildebrandt *et al.* (1957) have distinguished the effects of DNA and RNA on the growth of marigold and tobacco tissues in culture when these components were added to the media after sterilization by autoclaving or by filtering. In all experiments reported here the sodium nucleates were added to the medium before autoclaving because the results of the chromatographic analysis of the two nucleates at two different concentrations, viz., 0.1% and 1.0% revealed that there was no detectable difference in the components of these salts before and after autoclaving. Consequently autoclaving itself was not producing any identifiable chemical change in the material.

[illegible]

maximum concentration in cells within the first two or three hours.

It is evident from the tables, that in the first 2–12 hours after the cells had been incubated, there was no appreciable increase in the number of mitoses or in the percentage of anaphases and telophases between the control and the nucleate-treated cell aggregates. But after the cells had been incubated for 24 hours, in medium containing DNAate or RNAate, at the low concentration of 0.1%, the frequency of mitosis and the frequency of anaphase and telophase stages pooled together showed a rate much higher than the frequency in the control. As the incubation time went beyond 24 hours, the frequency in mitoses showed a relative increase over the control for a short period beyond which there appeared to be a drop in the mitotic frequency in both cases of DNAate and RNAate. This decreased frequency indicated a possible decreased effect of the sodium salt on the rate or mitoses.

Table 4. Relative frequency of % mitoses and % anaphases and telophases observed in *V. faba* calli growing in basal control medium containing three different concentrations of sodium DNAate

	Control			+0.1% DNAate			+0.5% DNAate			+1.0% DNAate		
2 hrs.	606	2.30	0.32	463	3.24	1.51	517	3.09	0.77	451	1.99	0.44
4 hrs.	666	2.85	0.45	465	3.01	0.64	506	2.17	0.98	—	—	—
12 hrs.	600	1.83	0.66	670	2.23	0.15	—	—	—	—	—	—
24 hrs.	601	4.04	0.54	686	11.66	2.04	—	—	—	—	—	—
40 hrs.	558	1.97	0.53	631	5.38	1.11	—	—	—	—	—	—
3 days	650	2.85	0.46	605	7.60	1.81	685	4.54	0.72	—	—	—
15 days	565	3.85	0.58	598	6.89	1.60	520	4.04	0.77	555	2.70	0.37
45 days	—	—	—	—	—	—	497	1.81	0.22	666	3.00	0.45

At the high concentrations of 0.5% and 1.0%, the cell aggregates revealed an initial increase in mitotic frequency within the first 2–4 hours followed by a linear drop until there was a total inhibition of mitosis at the end of 12 hours. Feulgen preparations of these cultures did not give good squash preparations and the cells seemed to be clumped together. Nuclei were lightly stained and showed the poisoning effects generally brought out by toxic chemicals on nuclei. At the end of three days' incubation, the cell aggregates which had shown an inhibition of mitosis in cells in the medium containing 0.5% and 1.0% DNAate appeared to have recovered. Visual observations indicated the development of small globular masses of cells on the darkly colored surface of the initial inoculum. Cytological counts made on squashes of these regenerated cell aggregates confirmed the occurrence of active mitosis.

Presumably, a selectivity of certain cells from the cell aggregates which were able to adapt to the environmental condition was taking place at this higher percentage of DNAate in the medium. This was observed only in

the case of DNAate and not in the case of RNAate. To confirm whether selection was actually taking place within cell aggregates, some of these cell aggregates which recovered were reinoculated into their respective media with the high concentrations, viz., 0.5% and 1.0%. After 2-3 days the inoculated aggregates showed evidence of renewed growth in the form of budlike outgrowths. Counts of division could not be made because on the surface of the cells which had been active in division, there was the debris of the old dead cells. These observations indicated that cell selection was taking place in terms of an environment, viz., chemical composition of the medium.

Table 5. Mean percent of mitoses and percent of anaphases + telophases from nine separate flasks of each category

	Control	0.1% RNAate	0.1% DNAate
	Mean percent mitoses ± S. E. of mean	Mean percent mitoses ± S. E. of mean	Mean percent mitoses ± S. E. of mean
2 hrs.	1.79±0.12	2.22±0.19	2.23±0.17
4 hrs.	1.71±0.12	1.93±0.18	1.93±0.13
12 hrs.	2.56±0.09	2.61±0.20	2.66±0.22
24 hrs.	2.64±0.14	3.72±0.17	3.51±0.26
	Mean percent anaphases and telophases ± S. E. of mean	Mean percent anaphases and telophases ± S. E. of mean	Mean percent anaphases and telophases ± S. E. of mean
2 hrs.	0.358±0.05	0.490±0.07	0.434±0.09
4 hrs.	0.320±0.05	0.526±0.08	0.541±0.09
12 hrs.	0.722±0.07	0.523±0.11	0.636±0.14
24 hrs.	0.545±0.05	0.660±0.11	0.758±0.13

Table 6. Analysis of variance for percent mitosis on 27 flasks, nine in each category for 24 hr. growth

Source of variance	d. f.	Σ sq's.	Mean sq's. (variance)	F
Total	26	13.9715	—	
Between Nucleates and control	1	6.4204	6.4204	21.654*
Between RNAate and DNAate	1	0.4355	0.4355	1.469
Between treatments (subtotal)	2	6.8559	3.4279	
Error (within treatments)	24	7.1156	0.2965	

* F at 1% level $\left(\frac{1 \text{ d. f.}}{24 \text{ d. f.}}\right) = 7.82$; at 5% level = 4.26

The first set of experiments reveals that 0.1% of nucleic acid salts produce a relative increase in the frequency of mitoses in the inoculated cell aggregates. To obtain data on limits of variation, further observations were made on three different sets, each with 9 flasks, i.e. 9 of basal medium control, 9 of 0.1% RNAate and 9 of 0.1% DNAate. The 'means' of these

sets are given in Table 5.

Analysis of variance made on the 24 hour mitotic percent data, using an orthogonal contrast method for obtaining sums of squares between RNA-ate vs. DNAate and between the two nucleates vs. control is shown in Table 6.

The difference between the nucleates is not significant but each is highly different from the control ($F=21.654$, $p<0.01$). These nucleates then significantly increase mitotic activity by about 50% of the control within 24 hours.

Mitotic frequency in polyploid cells: It has been pointed out that the addition of an external supply of nucleic acids or their precursors to a culture medium specifically induces division among polyploid cells (Venkateswaran 1963 b). Table 7 illustrates the frequency of cells in polyploid metaphases relative to the total number of metaphases, viz., diploid or near-diploid and polyploid metaphases when cells were grown under the present experimental conditions.

Table 7. Frequency of polyploid metaphases relative the total number of metaphases in *V. faba* calli after 21 days growth in different experimental media

	Diploid or near-diploid	No. of polyploid metaphases	% polyploid
Basal medium (control)	15	1	6.2
+ DNA	41	19	31.6
+ DNAate	27	18	40.0
+ RNA	39	12	23.5
+ RNAate	24	8	25.0

Concentration of additive ranged from 0.1 g/l and less.

Mean number of nuclei counted per treatment $\approx$ 1800.

It is seen from Table 7 that a medium containing DNA or its sodium salt had a greater effect in induction of division among polyploid cells than a medium containing RNA or its salt. Besides the existence of a mixture of diploid or near-diploid cells with polyploid cells in a cell aggregate, distinct areas could be noted in which the entire cell population appeared to be all polyploid (determined by nuclear size) in cell aggregates growing in DNA or DNAate medium (Fig. 1). Torrey (1959) reported observations on pea root callus tissue of essentially diploid nuclei and tetraploid nuclei when grown in a medium containing yeast extract in contrast to a completely synthetic medium.

When cell aggregates were incubated in culture medium containing nucleic acid precursors (thymidine, cytidine or uridine) at concentrations of 0.1, 0.01 and 0.001 g/l, initial observations at the end of 24 hours on frequency change of nuclear division did not reveal any appreciable change. When the cultures were incubated for 20 days or more, squash preparations

of cell aggregates revealed a number of polyploid nuclei in division (Table 8).

Table 8. Frequency of polyploid metaphases relative to total number of metaphase cells in *Vicia faba* calli after 21 days growth

	No. of diploid or near-diploid	No. of polyploid	% of polyploid
Basal medium (control)	64	6	8.5
+Cytidine 0.001 g/l	44	7	13.7
0.01 g/l	22	4	15.3
0.1 g/l	44	7	13.7
+Uridine 0.001 g/l	36	25	40.9
0.01 g/l	87	20	18.7
0.1 g/l	80	15	15.8

Mean number of nuclei counted per treatment $\approx$ 1800.

Fig. 1. Appearance of squashes of a diploid or a near-diploid region (A) and an essentially polyploid region (B) relative to nuclear size from a single cell aggregate of *V. faba* growing in the basal control medium with 0.01 g/l DNA. $\times$ ca. 200.

No significant changes were seen with thymidine in medium; but both cytidine and uridine appeared to have specifically stimulated division of polyploid nuclei, the uridine effect being very highly pronounced at low

concentrations. Presumably, the exogenously added precursors were incorporated by the cells, and polyploid cells were stimulated to divide in the presence of new precursors. Further experiments are in progress to determine the specific effect of these precursors.

Discussion

The effect on mitotic activity of nucleic acid salts and their precursors incorporated separately in the culture medium of *V. faba* cell aggregates growing in liquid culture have revealed some points of considerable interest.

The addition of both DNAate and RNAate at low concentrations stimulated cell aggregates to divide rapidly as revealed by a higher frequency or mitosis than that in the control medium. Nitsch and Nitsch (1957) observed in their experiments stimulation growth of Jerusalem artichoke tissue above that of the controls with addition of autoclaved commercial preparations of nucleic acids in the culture medium. Vasil (1959) showed that the addition of sodium salts of DNA and RNA separately to the culture medium supported the development of excised anthers up to the formation of tetrads or even 1-celled microspores although he indicated that RNA salt proved more useful than the DNA salt.

But, Hildebrandt *et al.* (1957) pointed out that growth *in vitro* of plant tissues of crown gall and hybrid origin was strikingly stimulated by both filtered and autoclaved RNA at certain concentrations whereas DNA had no effect at low concentrations and strikingly inhibitory at higher concentrations. Similarly, Nickell (1954 a, b) showed that the addition of alkaline hydrolysate of RNA stimulated growth up to 0.8 mg/ml whereas DNA hydrolysates showed slightly inhibitory effect at all levels tested. The significance of such differential response with DNA and RNA still remains open. Since the results of the dialysis experiments indicate that a major portion of the sodium nucleates added to the basal culture medium did not degrade into short chained nucleotides (Table 2), it is possible that the stimulatory effect of the sodium nucleates in mitotic activity observed in the present set of experiments may be partly due to the effect of the exogenously added sodium nucleates and partly due to an effect induced by the small percent of breakdown products of the nucleates during autoclaving, etc. It is well known that breakdown products of commercial nucleic acids increase mitotic frequency of plant tissues grown *in vitro* (Miller *et al.* 1955 a, b., Das *et al.* 1956).

Previous workers studying the effect of sodium nucleates on growing intact roots have used concentration ranges of 1-8% or even higher for varying periods of time (Huskins 1948, Kodani 1948, Wilson and Cheng 1949, Patau and Patill 1951, Sharma and Sen 1954). Present observations indicate that a growing callus tissue is less resistant to high concentration of the sodium nucleates than the intact roots. Higher concentrations than 0.1% produced a gradual cessation of growth of the callus and in some

cases even evidence of poisoning effects, although some of these cell aggregates recover after 4-5 days. At concentrations of 0.1% DNAate or RNAate in the culture medium, the cell aggregates revealed a higher frequency of mitosis than in the control medium at the end of the first 24 hours of incubation. After 24 hours, there was a linear decrease in the percent of mitoses and no sharp rise in growth rate. This possibly indicates that the sodium nucleate enters the cell within the first few hours.

Increasing the concentration of sodium nucleate in the surrounding medium over a threshold concentration did not influence the subsequent growth rate. Patau and Patil (1951) made similar observation and indicated that sodium nucleate or a physiologically active derivative reaches a maximum concentration in the cell during the first two or three hours without much change thereafter.

Stimulation of division in polyploid cells in plant callus tissues grown *in vitro* in the presence of various growth factors like coconut milk, yeast extract, nucleic acids and their derivatives, etc. have been reported by various workers (Straus 1954, Torrey 1959, Mitra *et al.* 1960, Mitra and Steward 1961, Venketeswaran 1963 b). In the present study, this stimulation of division in polyploid cells was particularly significant in cell aggregates growing in media containing DNA, DNAate, cytidine or uridine. The occurrence of polyploid cells through endopolyploidy is well known in different tissues of *V. faba* (Coleman 1950, Therman 1951, D'Amato 1952 a, b). Presumably under normal conditions of culture, there are a large number of polyploid cells occurring in the cell aggregates and these polyploid cells are induced to divide by such additive like DNA, DNAate, cytidine or uridine. It is possible that such additives act as "specific mitotic stimulants" or as "kinin factors" triggering off the polyploid cells to divide. Kinetin, which is a degradation product of DNA is well known for specifically triggering division in polyploid cells (Torrey 1961).

The present study has revealed the heterogeneity of cell types present in cultured cells of *V. faba* and the selective effect of different growth factors on the mitotic pattern of these cell types, like stimulation of division in polyploid nuclei, recovery of mitotic activity following high concentrations of nucleic acid salts, etc. Chemical substances which are incorporated in the culture medium and which get transported to the vicinity of the cell can be considered as a simulation of cellular environment. The environment of the cells within a callus tissue includes not only those stimuli which are external to the callus but also those which arise within the callus but external to the cell under consideration. As a result of such stimuli, specific cell types are stimulated to undergo division and express themselves as part of the cell population. Since the fate of a cell generally depends in part on its innate genetic potentialities and in part upon the environment to which it is exposed, a study of such interaction of genetic constitution

(including cytoplasmic determiners) and environment becomes possible by studies of this type. Techniques employed here are intended to be used in attempting to find evidence of such interactions.

Summary

The effects of commercially available nucleic acids, their salts or their precursors added to a culture medium on the mitotic activity of cultured cells of *Vicia faba* under liquid shaking conditions have been reported.

The addition of intact DNA or RNA to the control medium did not reveal any considerable effect on the growth rate of the cell aggregates whereas the addition of sodium salts of DNA or RNA at low concentration of 0.1% showed a very significant stimulatory effect on the percent of mitoses in the cell aggregates than in the control.

Analysis of variance made on the 24 hour mitotic percent data using an orthogonal contrast method indicated that the difference between the two nucleate treatments was not significant; but each was highly significantly different from the control.

At high concentrations of 0.5% or 1.0% DNAate in the medium, although the cells usually showed a complete cessation of mitoses in the beginning, after 4-5 days, the cell aggregates recovered revealing actively growing bud-like outgrowths from the original inoculum. Further incubation in these high concentrations did not appear to inhibit growth.

Cell aggregates growing in medium containing the nucleates or nucleic acid precursors (cytidine or uridine) also revealed an appreciably high incidence of polyploid cells in division. This was particularly pronounced in cell aggregates growing in medium containing DNA, DNAate, cytidine or uridine.

The present study thus reveals the heterogeneity of cell types present in *V. faba* tissue cultures and the selective effect of growth factors on the mitotic pattern of different cell types. It is suggested that such evidences like stimulation of division in polyploid cells and recovery of mitotic activity following high concentrations of nucleic acid salts may be the result of certain stimuli brought out by the chemical additive incorporated in the culture medium. Specific cell types are thus stimulated to undergo division and express themselves as part of the cell population. The possibilities of using these techniques in studying interactions between the genetic potential of cells and the environment (chemical) to which they are exposed is suggested.

Acknowledgments

We wish to express our appreciation to Dr. Paul G. Mahlberg and Dr. Ian M. Sussex for their encouragement and interest in the course of this

investigation. To Dr. Joan E. Gottlieb, one of us (S. V.) extends acknowledgement for providing facilities to work in her laboratory. Our thanks are due to Dr. Fred J. Gottlieb for suggestions in manuscript and Miss Mary Jane Jost and Mrs. Janet A. Natowitz for secretarial assistance.

References

- Block, R. J., Durrum, E. L. and Zweig, G. 1958. A manual of paper chromatography and paper electrophoresis. Academic Press Inc., New York.
- Coleman, L. C. 1950. Nuclear conditions in normal stem tissue of *Vicia faba*. Can. J. Res. 28: 328-391.
- Conger, A. D. and Fairchild, L. M. 1953. A quick-freeze method for making smear slides permanent. Stain Techn. 28: 281-283.
- D'Amato, F. 1952a. New evidence of endopolyploidy in differentiated plant tissues. Caryologia 4: 121-144.
- 1952b. Polyploidy in the differentiation and function of tissues and cells in plants. Caryologia 4: 311-358.
- Darlington, C. D. and La Cour, L. F. 1960. The handling of chromosomes, 3rd ed. Allen and Unwin Ltd., London.
- Das, N. K., Patau, K. and Skoog, F. 1956. Initiation of mitosis and cell division by kinetin and indoleacetic acid in excised tobacco pith tissue. Physiol. Plant. 9: 640-651.
- Hildebrandt, A. C., Riker, A. J. and Muir, E. 1957. Growth *in vitro* of marigold and tobacco tissue with nucleic acids and related compounds. Plant. Physiol. 32: 231-236.
- Huskins, C. L. 1948. Segregation and reduction in somatic tissues. I. Initial observations on *Allium cepa*. Jour. Hered. 39: 310-325.
- Kodani, M. 1948. Sodium ribose nucleate and mitosis. Introduction of morphological changes in the chromosomes and of abnormalities in mitotic divisions in the root meristem. Jour. Hered. 39: 327-335.
- Miller, C., Skoog, F., von Saltza, M. H. and Strong, F. M. 1955a. Kinetin, a cell division factor from deoxyribonucleic acid. Jour. Amer. Chem. Soc. 77: 1392.
- , —, Oomura, F. S., von Saltza, M. H. and Strong, F. M. 1955b. Structure and synthesis of kinetin. Jour. Amer. Chem. Soc. 77: 2662.
- Mitra, J. and Steward, F. C. 1961. Growth induction in cultures of *Haplopappus gracilis*. II. The behavior of the nucleus. Amer. J. Bot. 47: 358-368.
- , Marion O. Mapes and Steward, F. C. 1960. Growth and organized development of cultured cells. IV. Behavior of the nucleus. Amer. J. Bot. 47: 357-367.
- Nickell, L. G. 1954a. Nutrition of pathological tissues caused by plant viruses. Premier Colloque International sur la Physiologie des Cultures de tissus Vegetaux. 107-121.
- 1954b. Nutritional aspects of virus tumor growth. Abnormal and Pathological Plant Growth. Brookhaven Symposia in Biology 6: 174-186.
- , Greenfield, P. and Burkholder, P. R. 1950. Atypical growth of plants. III. Growth responses of virus tumors of *Rumex* to certain nucleic acid components and related compounds. Bot. Gaz. 112: 42-52.
- Nitsch, J. P. and Nitsch, C. 1957. Auxin-dependent growth of excised *Helianthus tuberosus* tissues. Amer. J. Bot. 44: 555-563.
- Patau, K. and Patil, R. P. 1951. Mitotic effects of sodium nucleate in root tips of *Rhoeo discolor* Hance. Chromosoma 4: 470-502.
- Setterfield, G. and Duncan, R. E. 1955. Cytological studies on the antimetabolite action of 2, 6-diaminopurine in *Vicia faba* roots. J. Biophys. Biochem. Cytol. 1: 399-419.
- Sharma, A. K. and Sen, S. 1954. Induction of division through nucleic acid treatment. Caryologia 6: 151-159.

- Straus, J. 1954. Maize endosperm tissue grown *in vitro*. II. Morphology and cytology. Amer. J. Bot. 41: 833-839.
- Therman, Eva 1951. The effect of indole-3-acetic acid on resting plant nuclei. Ann. Acad. Sci. Fennica. Ser. A. IV. 16: 1-40.
- Torrey, J. G. 1959. Experimental modification of development in root. In Cell, organism and milieu. Rudnick, Dorothea (Ed.). Ronald Press Co., New York. pp. 189-222.
- 1961. Kinetin as trigger for mitosis in mature endomitotic plant cells. Exper. Cell Res. 23: 281-299.
- Vasil, I. K. 1959. Cultivation of excised anthers *in vitro*—Effect of nucleic acids. Jour. Exptl. Bot. 10: 399-408.
- Venketeswaran, S. 1962. Tissue culture studies on *Vicia faba*. I. Establishment of culture. Phytomorph. 12: 300-306.
- 1963a. Tissue culture studies on *Vicia faba*. II. Cytology. Caryologia 16: 91-100.
- and Spiess, E. B. 1963b. Tissue culture studies on *Vicia faba*. III. Effect of growth factors on chromosome morphology. Cytologia 28: 201-212.
- Wilson, C. G. and Cheng, K. C. 1949. Segregation and reduction in somatic tissues. II. The separation of homologous chromosomes in *Trillium* species. Jour. Hered. 40: 2-6.
-