colysis is inversely dependent upon the concentration of ATP (*5*). Since none of the tissues have undergone sudden changes necessitating quick energy, thus lowering ATP and stimulating glycolysis, glycogen levels do not show any significant differences (*6*).

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Factors Influencing the Effect of β-Propiolactone on Chromosomes of Vicia faba

Abstract. The effect of β-propiolactone on chromosomes is independent of *p*H, oxygen tension, and some metabolic inhibitors. It is influenced by temperature, concentration, and pretreatments with dinitrophenol. Interphase sensitivity and localization of breaks are discussed in terms of the action of β-propiolactone and the relationship of β-propiolactone to other radiomimetic compounds.

Several studies (for example, Kihlman's, *1*) have indicated that with few exceptions each of a now long list of radiomimetic compounds differs from the others in its mode of action (that is, in interphase sensitivity and preferential breakage) or in its dependence on oxygen tension, temperature, and metabolic activity. In general, most radiomimetic chemicals can be grouped into two classes: (i) weakly reactive compounds such as maleic hydrazide and 8-ethoxycaffeine whose observed effects are much enhanced and depressed by factors that alter the metabolic state of the cell at the time of exposure; (ii) strongly reactive compounds whose observed effects seem to be somewhat independent of experimentally altered exposure conditions, acting as if by the law of mass action. It seems, then, that the less reactive chemicals are dependent on, and the more reactive chemicals independent of, oxidative metabolism systems for their observed effect. The work reported in this paper (*2*) indicates that β-propiolactone (BPL) is a substance belonging to the more reactive group, although differences in action are discernible.

β-Propiolactone is the simplest of a number of related compounds. It is used industrially as a plasticizer. Our interest in an understanding of its action, apart from determining its place in our study of the comparative effects of mutagens, stems partly from its promise as a chemotherapeutic agent in oncology. Its mutagenic properties have been demonstrated in yeast, bacteria, and *Neurospora*, while Smith and Lotfy (*3*) have demonstrated its effectiveness as a radiomimetic agent, testing it on the chromosomes of *Vicia* and *Allium*.

Our work is unlike that of Smith and Lotfy in two basic respects: metaphase rather than anaphase chromosomes were scored and environmental conditions were altered to test their influence on the effect of β-propiolactone in an attempt to elucidate the mechanism by which it acts in producing chromosomal aberrations. The lateral root-tip chromosomes of *Vicia* were used. Smith and Lotfy employed primary roots in their study, but the erratic and often high frequency of spontaneous aberrations in the primary roots makes the use of laterals, roots, in which spontaneous aberrations are generally absent or are present in a frequency of less than 1 percent, advantageous in this type of work. There is also the added advantage that chromosomes from roots of the same bean, presumably with the same genetic constitution, can be treated and tested.

The techniques of culture, treatment and recovery, and slide preparation have been previously described (*4*). Fresh preparations of BPL were always used in treatments because BPL has a tendency to break down in solution. A variety of BPL concentrations were tested, but $7 \times 10^{-3} M$ seemed to be the most effective since it resulted in a high frequency of aberrations and a low frequency of cell death. Only experiments in which this concentration was used are reported. The roots were treated at 17°C for 30 minutes and were allowed to recover for 48 hours at 25°C before fixation.

β-Propiolactone seems to act on chromosomes most effectively when the chromosomes are in early interphase. Few aberrations are observed after 24 hours' recovery, a peak frequency is reached after 48 hours, a somewhat lower frequency occurs after 72 hours, and an appreciable decline sets in by 96 hours. The prolonged delay of the peak frequency is due largely to the depressing effect of BPL on the mitotic rate.

The first burst of mitosis following the suppression of cell division coincides with the peak frequency, but whether BPL causes cells in prophase to regress to earlier stages is not known, although

Table 1. Effect of temperature on the activity of BPL.

Temperature (°C)	No. of cells	Isochromatids (%)	Exchanges (%)
5	100	22	3
17	100	43	10
25	100	43	29
37	Lethal		

no polyploid cells result from the suppression. It can be assumed, therefore, that the principal delay occurs at a stage prior to DNA synthesis and chromosome replication. When a low frequency of aberrations was encountered, it was paralleled by a low mitotic rate and a high frequency of dead cells. Lower concentrations of BPL interfered less with the mitotic rate but did not appreciably affect the time of appearance of aberrations. Our findings in this regard are in accord with those of Smith and Lotfy. We do not agree, however, on the time that definitive chromosome aberrations occur. One can see abnormal cells so far as the presence of sticky bridges and lagging chromosomes is concerned. This is especially true with regard to the satellites of the long chromosomes which often lag at anaphase to the extent that they appear to be fragments. Some of the latter abnormalities are seen at 24 hours, but they are very few in number, mitosis being severely inhibited at this time. The results reported here are similar to those reported by Smith and Srb (*5*) so far as time of appearance of aberrations is concerned.

The aberrations induced were entirely of the chromatid rather than the chromosome type. As is usual, isochromatid aberrations were predominant; interchanges were about one-third as frequent as isochromatids, and single chromatid deletions—never a large class of experimentally induced aberrations in *Vicia*—were conspicuously absent. The presence of fragment types, as contrasted to the anaphase bridges reported by Smith and Lotfy, cannot be confirmed by these experiments. We disagree therefore with Smith and Lotfy's conclusion that sister and nonsister fusion of broken ends is

Table 2. Effect of dinitrophenol (DNP) ($1 \times 10^{-4} M$ at 17°C for 2 hours) on BPL activity.

Treatment	No. of cells	Isochromatids (%)	Exchanges (%)
BPL	200	41.5	24.5
DNP + BPL	100	96	22
BPL + DNP	100	98	51

low; in fact, the absence of single deletions and the apparent completeness of isochromatid and exchange aberrations leads to the conclusion that just the opposite situation prevails—that is, the frequency of fusion of broken ends is very high. It might be well, however, to point out that the fusion or exchange scored in metaphase does not always result in an anaphase bridge. Wolff (*6*) has discussed this phenomenon in some detail, while Conger (*7*) has presented evidence that some of the discrepancy between these scoring systems is due to "free fall" and broken bridges.

Like other chemicals capable of inducing aberrations, BPL is preferential in its action. The short chromosomes were much more frequently broken than the long pair. The S/L ratio (determined by dividing the number of long chromosomes affected into the number of short chromosomes affected) is a measure of this preference. X-rays, which break short and long chromosomes at random, induce an S/L ratio of 2.5. Treatments with BPL result in an S/L ratio higher than 6.0 (only 464 chromosome breaks were analyzed). Actually, the action of BPL is even more restricted than the S/L ratio indicates. β-Propiolactone selectively results in breakage in those segments known to be heterochromatic and to be located in the long arm of the short chromosomes. The few breaks produced in the long chromosomes were located in the heterochromatic regions on either side of the centromere. β-Propiolactone is similar, then, to mustard and diepoxide so far as site of breakage is concerned and is dissimilar to 8-ethoxycaffeine and maleic hydrazide, which attack the nucleolar organizer region and the centric heterochromatin of the long chromosome, respectively. The breakage frequency induced by BPL treatments is not modified by changes in *p*H or oxygen tension or by such metabolic inhibitors as NaN_3 or NaF. It is modified, however, by temperature change. The higher the temperature the higher the breakage frequency within the range tested. These data are presented in Table 1. The influence of temperature on the BPL effect is similar to its influence on the action of mustard, diepoxide, and maleic hydrazide but dissimilar to its influence on KCN, the final effect of which is independent of temperature and *p*H changes but dependent on oxygen tension.

It has previously been demonstrated (*1*) that dinitrophenol has a marked inhibitory influence on the effect of maleic hydrazide and 8-ethoxycaffeine as a pretreatment but not as a posttreatment and on diepoxide as a posttreatment but not as a pretreatment. It has no observable effect on KCN action. The influence of dinitrophenol on BPL is strikingly different in that both pre- and posttreatments with it result in an increased frequency of aberration (Table 2). Since dinitrophenol is believed to uncouple oxidation from phosphorylation, it is tempting to suggest that BPL, despite its reactive nature, is more reactive in the absence of an intact energy source. Whether this source is similar to that described for intact nuclei (*8*) or is of a cytoplasmic nature remains to be determined. It is conceivable that the influence of dinitrophenol on BPL is on rejoining and not on breakage, although the exchange rate is not affected as much as the isochromatid rate.

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An Explanation of the Liesegang Phenomenon

Abstract. Periodic band precipitation in porous media is explained by means of the Hirsch effect (specific semipermeability of the precipitate occurring only as long as both the precipitate-forming ions are present). The possibility of getting Liesegang bands in immunological precipitates is underlined, and a suggestion is made how to avoid them.

Many authors have attempted to explain the periodic precipitation phenomenon in porous media or gels, found by Liesegang (*1*), in different ways, which, although often more or less plausible, are never wholly satisfactory, since they are either incomplete or do not convincingly demonstrate that the proposed mechanisms *must* give rise to band formation. Hedges (*2*) and Veil (*3*) give complete reviews of different theories, mentioning Ostwald's supersaturation theory (*4*) [refuted by Hatschek's experiments (*5*)], Dhar and Chatterji's coagulation theory (*6*), Bradford's adsorption theory (*7*), Fricke's diffusion theory (*8*) (see also *9*), and McLaughlin and Fischer's membrane theory (*10*). A more recent review is given by Stern (*11*). Different aspects of the diffusion theory are further given by Yanagihara (*12*) (influence of d-c and a-c electric fields), Wagner (*13*) (a mathematical analysis), and Matalon and Packter (*14*) (protecting influence of the gel).

The Hirsch effect (*15*), described 60 years after Liesegang's first paper on this phenomenon, now permits a satisfactory and general explanation. Hirsch (*15*) observed that, in certain cases, two solutions of electrolytes which can precipitate with one another, when diffusing toward one another through a membrane (thin slice of a gel), endow the membrane with quite remarkable permeability properties. The membrane becomes in such cases perfectly impermeable to the ions that formed the precipitate but remains permeable to other ions and to the solvent (see also McLaughlin and Fischer, *10*).

The remarkable impermeability to the ions that form the precipitate layer is illustrated by the following experiment [see *15* (*a*), Table I]: A cellophane membrane separating solutions of (i) $0.1N$ $Ba(OH)_2$ and (ii) $0.1N$ H_2SO_4 gave rise to a membrane potential of 670 mv. This value corresponds well with the value E calculated from

$$E = 2.3026 \frac{RT}{F} (pH_i - pH_{ii})$$

This is in perfect accord with the assumption that the membrane is completely impermeable to the ions Ba^{++} and So_4^{--} but permeable to H^+ and OH^-. The same membrane, after the formation of the $BaSO_4$ barrier inside it, but with Na^+ substituted for Ba^{++}, or with Cl^- substituted for SO_4^{--}, showed a membrane potential of approximately 65 mv, equal to the membrane potential found with untreated cellophane under the same conditions; the semipermeability now had vanished.

At first sight this observation appears to exclude any formation of multiple precipitate bands, because the formation of the first band, which is impermeable to the forming ions, would stop the ions from crossing it to form a second band farther on. But Hirsch (*15*) also observed that the precipitate layer inside the membrane remains impermeable to the forming ions only as long as small quantities of the forming ions are present in solution on either side of the membrane. As soon as one of these ions is lacking on one side of the membrane, the precipitate layer in the membrane can be crossed, after a longer or shorter lapse of time, by the other ion.

Without attempting for the moment to discuss the explanation of this Hirsch-effect, expressed by the condition "specific semipermeability of the precipitate occurs only as long as both the precipitate-forming ions are present," the effect