

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 pH 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 pH 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 dif-

ferent 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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References and Notes

1. B. A. Kihlman, *J. Biophys. Biochem. Cytol.* 2, 543 (1956).
2. This work was supported in part by the Atomic Energy Commission, the National Science Foundation, and the National Institutes of Health.
3. H. H. Smith and T. A. Lotfy, *Am. J. Botany* 42, 750 (1955).
4. T. Merz, *J. Biophys. Biochem. Cytol.* 5, 135 (1959).
5. H. H. Smith and A. M. Srb, *Science* 114, 490 (1951).
6. S. Wolff and H. E. Luippold, *Nature* 179, 208 (1957).
7. A. Conger, *Abstr. 10th Intern. Congr. Genetics* 2, 57 (1958).
8. V. G. Allfrey, A. E. Mirsky, S. Osawa, *Nature* 176, 1042 (1955).

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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 Hattschek'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 dif-

fusion 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 $\text{Ba}(\text{OH})_2$ 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

$$E = 2.3026 \frac{RT}{F} (p\text{H}_i - p\text{H}_{ii})$$

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

by itself suffices to explain the Liesegang phenomenon. As a matter of fact, Liesegang (16), Hedges (2), Bradford (7), and Holmes (17) observed that between two precipitate bands the solution in the gel is completely or almost completely depleted of one of the forming ions (generally the ion initially present in the lowest concentration is the one that is lacking). Stanfield (18) observed that a strong concentration difference between the two reagents predisposes to the formation of macroscopic multiple bands [see also Bradford (7) and Holmes (17)]. Wilson and Pringle (19) and Salvinien and Kaminsky (20) noticed the same tendency with immunological precipitates.

Our explanation of the Liesegang phenomenon is most nearly approached by McLaughlin and Fischer's hypothesis (10), which states that while the precipitate bands may be somewhat permeable to many substances, they certainly are impermeable to the forming substances. According to these authors, the barriers then lose their impermeability after some time, by a mechanism for which they do not offer a satisfactory explanation. The only thing lacking in their theory, to make it conclusive, is the condition from the Hirsch effect: that these precipitates constitute a membrane that is impermeable to the forming substances only as long as the forming substances are both present on either side of the membrane. Thus, once a band is formed by precipitation of two reagents that meet in a gel (or another porous medium such as filter paper) [see Van Oss, Fontaine, Dhennin, and Fontaine (9) and Milone, Cetini, and Ricca (21)], that band remains impermeable until one of the reagents is exhausted by precipitation or by absorption on the precipitate, at least in the immediate vicinity of the membrane [see Bradford's adsorption experiments (7)]. Only from that moment can the other reagent cross the barrier, until it again encounters farther on a sufficient amount of the first reagent, with which it will form a second band, and so on.

Our theory may also throw some light on the formation of immunological precipitate bands in porous media, where it is of great importance to be able to avoid confusion of Liesegang bands with bands due to a multiple immunological system [Wilson and Pringle (19); Salvinien and Kaminsky (20); Van Oss, Fontaine, Dhennin, and Fontaine (9)]. The mobility of the bands [Oudin (22)] does not necessarily exclude the occurrence of a Liesegang phenomenon, particularly not in the cases where the precipitate is soluble in an excess of one of the reagents, like colloidal and immunological precipitates (Bechhold, 23), and even

sometimes precipitates of electrolytes [Pringsheim (24), and Cetini and Ricca (25)]. The best way to avoid the formation of multiple macroscopical Liesegang bands is to operate with equivalent concentrations of reagents.

Many other phenomena of periodic structure may be explained in a similar way: the very creation of the first structural elements opposes a temporary barrier to the transport of a forming substance. The barrier then loses its function as such for reasons inherent to its growth, thus leaving the way free to the construction of the next structural element. The periodicity is determined by the magnitude of negative feedback arising out of the interaction between the formation and the degeneration of the barrier.

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References and Notes

1. R. E. Liesegang, *Naturw. Wochschr.* 11, 353 (1896).
2. E. S. Hedges, *Liesegang Rings and Other Periodic Structures* (Chapman and Hall, London, 1932).
3. S. Veil, *Les périodicités de structure* (Hermann, Paris, 1934).
4. W. Ostwald, *Z. physik. Chem. (Leipzig)* 23, 365 (1897).
5. E. Hatschek, *Kolloid-Z.* 10, 124 (1912).
6. R. N. Dhar and A. C. Chatterji, *ibid.* 31, 15 (1922); 37, 2, 89 (1925).
7. S. C. Bradford, in *Colloid Chemistry*, J. Alexander, Ed. (Chemical Catalog Co., New York, 1926), vol. 1, p. 790.
8. R. Fricke, *Z. Physik. Chem. (Leipzig)* 107, 41 (1923).
9. C. J. van Oss, M. Fontaine, L. Dhennin, M. Fontaine, *Compt. rend.* 245, 407 (1957). By rheophoresis, Liesegang phenomena can be created with hydrodynamic flow as the transport method, which proves that diffusion is not necessary.
10. G. D. McLaughlin and M. H. Fischer, *Kolloid-Z.* 30, 13 (1922).
11. K. H. Stern, *Chem. Revs.* 54, 79 (1954).
12. A. Yanagihara, *Nippon Kagaku Zasshi* 76, 161, 165 (1955).
13. C. Wagner, *J. Colloid Sci.* 5, 85 (1950).
14. R. Matalon and A. Packter, *ibid.* 10, 46 (1955).
15. P. Hirsch-Ayalon, (a) *Rec. trav. chim.* 75, 1065 (1956); (b) *J. Polymer Sci.* 23, 697 (1957).
16. R. E. Liesegang, *Z. angew. Chem.* 23, 2124 (1910).
17. H. N. Holmes, in *Colloid Chemistry*, J. Alexander, Ed. (Chemical Catalog Co., New York, 1926), vol. 1, p. 796.
18. J. Stanfield, *Am. J. Sci.* 43, 1 (1917). Stanfield observed that with equimolar concentrations only one band can be obtained, which, however, consists of a great many microscopically thin bands.
19. M. W. Wilson and B. H. Pringle, *J. Immunol.* 73, 232 (1954).
20. J. Salvinien and M. Kaminsky, *Compt. rend.* 240, 257 (1955).
21. M. Milone, G. Cetini, F. Ricca, *J. chim. phys.* 55, 320 (1958).
22. J. Oudin, *Ann. inst. Pasteur* 75, 30 (1948).
23. H. Bechhold, *Z. physik. Chem. (Leipzig)* 52, 185 (1905).
24. N. Pringsheim, *ibid.* 17, 473 (1895).
25. G. Cetini and F. Ricca, *J. chim. phys.* 55, 323 (1958).

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Prolongation of Response of Node of Ranvier by Metal Ions

Abstract. The response of the node of Ranvier can be prolonged up to 5 to 6 seconds by addition to the medium of minute amounts of certain metal ions. This prolongation involves a change from a triangular to a rectangular configuration. The properties of the prolonged nerve-fiber responses are very similar to those of heart muscle.

Electrical responses of the node of Ranvier whose falling phase is markedly prolonged can be obtained by exposure of the node to such alkaloids as brucine, emetine, sinomenine, and heroine (1), to certain derivatives of morphine (3), and to strychnine (4), as well as by exposure to hypertonic solutions (2). Prolonged responses were also obtained in the giant axon of the squid with the intracellular injection of tetraethylammonium chloride (5, 6). Following repetitive stimulation, many excitable cells show a type of "memory" in the form of a prolonged response. This phenomenon has been observed in the node of Ranvier of the toad (7, 8), giant axon of sepia (9), aplysia nerve cell (9), the ommatidium of the horseshoe crab (10), the supradullary ganglion cell of the puffer fish (11) and in *Noctiluca*, a protozoan (12). Somewhat less marked prolongations of nerve fiber responses have been obtained with low temperatures (13), high pressures (14, 15) and by replacing H₂O in the fluid medium with D₂O (16).

The prolonged responses (especially the markedly prolonged responses) show many properties in common with those of the normal response of heart muscle. These similarities involve the configuration, the instability of the duration, the time course of the impedance changes, the refractory period, the resistance to lowered sodium or increased potassium, and the effects upon the duration and configuration of temperature changes, of pressure changes, of polarization, of frequency of stimulation, and of a large number of chemicals (5, 7, 17). In the experiments reported here (18), a prolongation of the response of the node of Ranvier was obtained by the external application of metal ions. Brief reference to these experiments was made in a previous publication (6).

Both the node of Ranvier and the giant axon preparation were used in these experiments. Single myelinated nerve fibers were isolated from the nerve innervating the semitendinosus or sartorius muscle of the toad (*Bufo marinus*). This technique has been described previously (1). Action currents of the node of Ranvier were recorded by the "bridge-insulator" technique (1). Action potentials