represents relative resistance to DDT, computed as the ratio of the LD_{50} of the more resistant strain over that of the less resistant strain. Thus, it expresses how many times the RS strain is more resistant than the NS strain (dotted section; up to generation 7) or than the SS strain (dashed section; generations 8*ff*.) As of generation 2, we find the RS strain more resistant. These differences persist and are statistically significant (P mostly < 0.01, never > 0.05). Violent fluctuations in relative resistance prior to generation 19 are probably due to crude methods of resistance assay. In spite of the fluctuations, the increase in relative resistance on continued selection is evident.

A curious behavior pattern was soon noted in the selected strains. The resistant strain tended to pupate at the margin of the medium, whereas the susceptible strain pupated largely in the medium away from the margin. This phenomenon occurred in nontoxic medium and has been quantified as percentage of peripheral pupation—that is, percentage of pupae at margin of medium or up the wall of the vial. In Fig. 2, the broken line expresses differences in percentage of peripheral pupation between the RS and the NS strains up to generation 7 (dotted section) and between the RS and SS strains as of generation 8 (dashed section). These differences are significant (P at least < 0.05, mostly < 0.01) beyond generation 5. Since the various strains were tested on the same batch of medium at the same time and handled alike, pupation site differences between them are not likely to be due to environmental factors, such as humidity. The concomitant increase on selection in differences for the two characters indicates the presence of genetic correlation between DDT resistance and pupation site.

To test this hypothesis, two new strains were selected for pupation-site differences, starting with newly pooled stock. Vials with a high percentage of peripheral pupation were chosen as parents for the peripherally pupating (PP) strain, and vials with a low percentage of peripheral pupation engendered the centrally pupating (CP) strain. Continued selection resulted in increasing and statistically highly significant differences in percentage of peripheral pupation between the two strains, as illustrated by the solid line of Fig. 2. These pupation site strains are now in the 19th generation of selection and their differences in percentage of peripheral pupation are considerably larger than those between the RS and SS strains. Assays of DDT resistance of the CP and PP strains (solid line, Fig. 1) reveal rapidly diverging, statistically significant levels of tolerance. At generation 18, the PP strain was 3.8 times as resistant as the CP strain. The hypothesis of genetic correlation between DDT resistance and peripherality in pupation site appears justified. Selection for one brings on the other.

The foregoing experiments in themselves are insufficient to discriminate between the three aforementioned hypotheses on character correlation. The maintenance of the correlation during reciprocal selection suggests linkage or pleiotropism rather than unconscious selection of a physiologically essential character. Experiments under way now, attempting to separate DDT resistance from peripheral pupation, plus planned genetic analysis, should evaluate the possibility of linkage. A number of experiments on the behavior and physiology of *Drosophila* larvae in toxic and nontoxic medium suggest that resistance as well as peripheral pupation are both measurable end-products of a physiological process, the threshold of which is raised or lowered during selection for either character. This would make the present correlation a case of spurious pleiotropism *sensu* Grüneberg (*6*).

It is evident that there is no perfect correlation between the two characters, since response of the correlated character lags behind that of the selected character. This suggests fixation of modifying genes for the selected trait, which tend to enhance the latter but not the correlated character.

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Received February 4, 1954.

Diffusion Lines in Silver Chromate Gelatin

H. Friedeberg

British Columbia Research Council
University of British Columbia, Vancouver

In 1943, the formation of radial lines occurring when silver nitrate diffuses into a layer of chromate gelatin exposed to mercury vapor was described (*1*). These lines are so close together that they form a diffraction grating, and the appearance of bright diffraction colors makes the phenomenon conspicuous to the naked eye. By passing a beam of light through the plate, a diffraction spectrum can be produced on a screen. Microscopic examination reveals the lines, which seem to be located in or near the surface of the gel and whose distance from one another varies somewhat in different experiments. The maximum density so far observed is 600 lines/mm.

The fundamentally new feature of these lines as compared with Liesegang Rings and, indeed, all rhythmical structures is their radial orientation. Whereas rhythmical structures always extend in a direction normal to that of the generating movement (diffusion, progressing crystallization, and so forth), the present line pattern is running in the direction of the diffusion. The formation of Liesegang Rings is adequately explained by the theories of Ostwald (*2, 3*) and Chatterji and Dhar (*4*); an explanation of the origin of the radial lines, for which I propose to use the term *radii*, is still lacking. A further study of the phenomenon therefore seemed worth while.

In the first experiments, the gelatin layer was exposed to zinc sheets previously moistened with a solu-

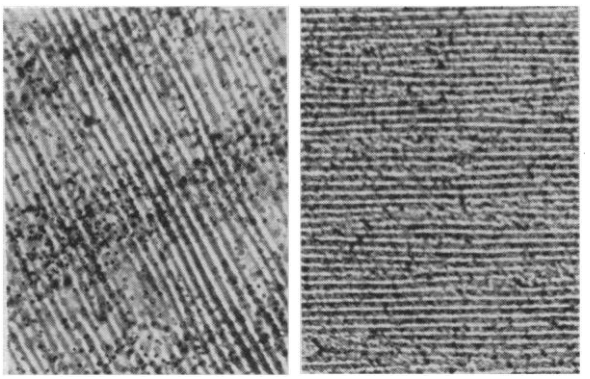

Fig. 1. (left). Radii and secondary Liesegang Rings formed by diffusion of $AgNO_3$ into gelatin containing 0.01 percent K_2CrO_4 under Hg vapor (×260). Fig. 2 (right). Radii formed in gelatin with 0.01 percent K_2CrO_4 in an electric field between Ag electrodes under Hg vapor (×340).

tion of mercuric chloride; later, metal sheet (zinc or copper) covered with mercury metal was used. The fact that the radii could also be produced by inverting the plate bearing the gelatin layer over a dish containing mercury only proves conclusively that mercury vapor is responsible for their appearance.

The lines are much more stable when the chromate concentration in the gelatin is reduced to 0.01 percent compared with 0.1 percent used previously. Figure 1 shows a preparation photographed 3 to 4 wk after it had been made. The dark zones extending across the radii are parts of three of the so-called secondary, or micro, Liesegang Rings that result from the chloride content of commercial grade gelatin.

In order to determine the minimum amount of mercury necessary to produce the radii, plates were covered with pieces of zinc sheet of about 100-cm^2 area, each of which had been moistened with 0.5 ml of mercuric chloride solution of different concentration in each case. The results appear in Table 1. They show that the minimum amount of mercury that will bring about the appearance of radii is 2 to 3 μg per square centimeter. Obviously, the 50 μg of mercury available in this experiment was spent when diffusion of the silver nitrate had reached about two-thirds of the total

TABLE 1. Minimum amount of mercury to produce radii.

No.	Conc. of $HgCl_2$ sol.	Amount of Hg(g)	Effect
1	1 ppm Hg	0.5×10^{-6}	None
2	10 " "	5.0×10^{-6}	None
3	100 " "	50×10^{-6}	Radii extending over two-thirds of diffusion area
4	0.1% Hg	500×10^{-6}	Radii of full length
5	1 % "	0.005	Radii of full length as 4
6	5 % "	0.025	Radii of full length but weaker than 4 and 5

radius—that is, within about 10 hr since no radii had formed in the outermost zone. Sheet No. 4 without further treatment produced radii in a second experiment that was started 24 hr after the first.

Regarding the conditions for the formation of radii as far as the gel is concerned, the occurrence of diffusion in it is a necessity. No radii are formed when gelatin previously mixed with silver nitrate and potassium chromate is exposed to mercury vapor. Diffusion of silver nitrate into purified gelatin not containing chromate is equally ineffective. However, if commercial grade gelatin is used, weak radii of lower density are formed showing that the impurities in this gelatin can replace chromate to a certain extent. Weak radii are also produced when potassium chromate is allowed to diffuse into gelatin that contains silver nitrate. Whether the radii are actually formed by mercury or are only made visible by it through deposition of metallic silver is still an open question. If an answer could be found, this would allow us to decide whether the phenomenon is inherently linked up with the diffusion process or whether it results from interaction of the gel surface with the gas phase.

As previously stated (*1*) formation of radii takes place only at the periphery of the expanding diffusion circle—that is, during or immediately after the reaction of silver with chromate. Silver chromate forms quite stable colloidal solutions in gelatin at concentrations up to well above those used in these experiments. It seemed possible, therefore, that formation of radii results from migration of particles of colloidal silver chromate, especially since such migration appears to take place during the formation of Liesegang Rings when the space between the last two layers changes color from orange to yellow.

In order to test the validity of this explanation, colloidal silver chromate in a layer of gelatin was placed between platinum electrodes to which a potential of 12 v was applied, and the whole system was covered with a piece of copper sheet bearing a layer of mercury. Under these conditions, part of the colloidal silver chromate migrates toward the anode, while the rest undergoes electrolysis. No radii are formed, showing that movement of colloidal particles is not responsible for their appearance.

When the same experiment is carried out with silver, instead of platinum, electrodes, radii of high density giving the usual bright diffraction spectrum are formed running normal to the anode and extending from the central part of the plate almost to the edge of the gel with a short interruption at the anode. No radii are formed in the cathode half of the plate. Figure 2 shows a section of the radii produced in this way. The same result is obtained when only potassium chromate is added to the gel. In this case the necessary silver ions are supplied by the anode.

It may be of interest to mention that secondary Liesegang Rings are also formed in these experiments.

On the basis of this investigation, the following conclusions can be drawn: (i) radii are formed when silver ions migrating in gelatin react with chromate

ions in presence of mercury vapor; (ii) impurities in commercial grade gelatin can replace chromate, although the radii are weaker in this case; (iii) migration of colloidal particles is not responsible for the formation of radii; (iv) a minimum of 2 to 3 μg of mercury per square centimeter of active gel surface is necessary. Further work on the subject is in progress.

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Received November 6, 1953.

A New Technique for Quantitative Paper Chromatography

Itsuhiko Mori

Kobe College of Pharmacy, Kobe, Japan

Paper chromatography has been adapted to quantitative analysis. At first only a rough approximation by means of matching colored spots, it has developed into a precise technique of photoelectric scanning (*1, 2*). But even these methods using a densitometer have errors at times as large as 5 to 17 percent (*2, 3*), because the exact technique of measuring the area of a spot that has been spread over on the filter paper is rather difficult. The author has devised a technique involving optical scanning that makes the measurement easier and the quantitative determination of the paper chromatogram more accurate.

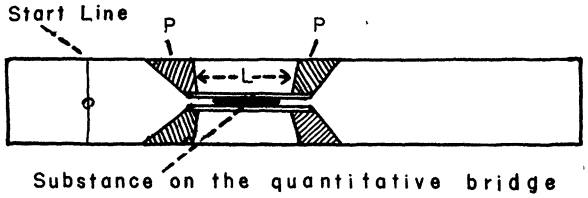

Fig. 1. Diagram showing technique employed.

Figure 1 shows this technique. Make a narrow passage (quantitative bridge) where the substance should be developed. The width of the passage should be 2 to 5 mm and the length about 30 to 100 mm, these dimensions being made commensurate with the quantity of substance. Cut off the side of the passage (bridge) with a sharp razor. Heated solid paraffin should be absorbed at P, so that the developer may ascend only through the bridge.

When a substance is developed into this bridge, the length of the colored zone is determined by the degree of the concentration of the substance, and this length can easily be determined by eye-measurement after some experience. Figure 2 shows this relationship between the concentration C of the substance and the length L of the colored zone. This relationship is

$$\log C = 1.26 \log L - 0.78, \qquad C = 0.167\, L^{1.26}.$$

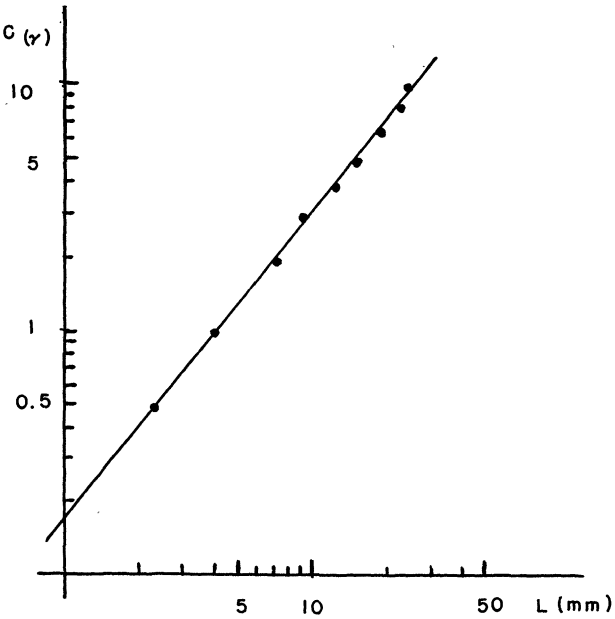

Fig. 2. Concentration of substance versus length of colored zone. Developer: butanol; athanol; 0.5N $NH_{aq.}$-6: 2: 3. Substance, auramine, 0.5 to 10 gamma. The bridge, of width 2 mm, was situated about 20 mm from the start line. Temperature, 28°C.

In measuring a mixture of two or more substances, one should first find separately the locus where each substance would develop itself and then make a bridge on the suitable locus.

Figure 3 shows the relationship between the length L of the colored zone and the width W of the quantitative bridge when we used 10 gamma auramine. The length is calculated by the following equation with error less than 1.8 percent:

$$\log L = 1.83 - 0.48 \log D, \qquad L = 67.5 \times D^{-0.48}.$$

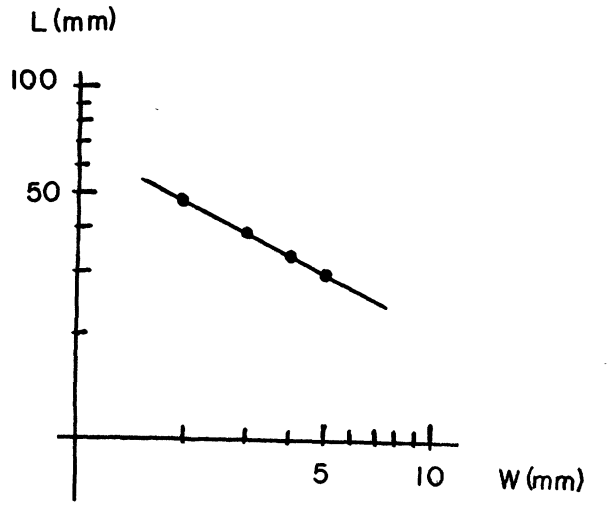

Fig. 3. Length of colored zone versus width of bridge for 10 gamma auramine.