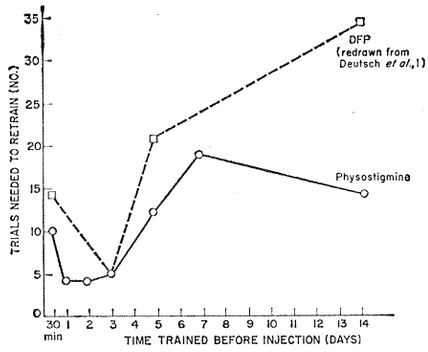

Fig. 1. Mean number of trials needed for retraining of animals injected with physostigmine compared with those required by animals injected with DFP.

the injection were used as a basis for forming these seven experimental groups: the 30-minute ($N=9$), 1-day ($N=11$), 2-day ($N=8$), 3-day ($N=8$), 5-day ($N=12$), 7-day ($N=8$), and the 14-day ($N=10$). One additional group, the 14-day control group ($N=6$), was run but received no injection. Rats were assigned at random to each of the above groups following initial training. After they received the injections, all animals were placed in the home cage for 30 minutes and were then retrained in the Y-maze with the same procedure and criteria as before.

For the initial training, an average of 23 trials (standard deviation = 5.86), not including the ten criterion trials, was needed to learn the task. The fact that after 14 days, control animals required a mean of four trials to relearn the task indicated that there was very little natural forgetting over the longest time interval used. The mean number of trials needed for the retraining of animals injected 30 minutes after training was ten; for those injected after 1 day, four. This difference was significant ($P<.01$ by Mann-Whitney U-test). That the degree of forgetting is decreased with increased time between training and treatment has often been reported (*5*).

Rats injected 2 and 3 days after training also required a mean of four and five trials, respectively, for retraining. The possibility that animals were unable to demonstrate a memory of the task 30 minutes after injection of physostigmine was ruled out by the scores for the 1-, 2-, and 3-day groups. After 5 days, retraining required a mean of 12 trials, significantly greater than that required after 1, 2, and 3 days ($P<.001$). After 7 days, injected animals needed an average of 18 trials to relearn; after 14 days, 14 trials. After 5 days the amount of retraining needed was at least equal to that needed after 30 minutes, and an even greater amnesic affect was demonstrated 7 days after training. The numbers of trials needed for the retraining of the groups injected at 5, 7, or 14 days after training were not significantly different. The number of trials required for the 14-day injected group was significantly different from that needed by the 14-day control group ($P<.01$).

A comparison of the results obtained in this experiment with those Deutsch *et al.* (*1*) obtained by using intracerebral injection of DFP can be seen in Fig. 1. Similar training and testing procedures were used in the two experiments. The intervals between training and injection used by Deutsch were repeated. The interval between injection and retraining was different. The use of DFP injected intracerebrally made necessary anesthetization of the animal with nembutal before the stereotaxic operation; hence, 1 day was allowed for the animal to recover before it was retested. The use of physostigmine administered intraperitoneally required no anesthesia; therefore animals were tested 30 minutes after injection. This interval remained constant for all groups in this experiment and in the corresponding groups in Deutsch's experiment.

In both studies, significantly fewer retraining trials were needed 3 days after training than were needed after the shorter or longer intervals (Deutsch *et al.* did not have a 1- or 2-day group). The conclusion that the amnesia reported by Deutsch *et al.* (*1*) was due to an altering of the cholinergic balance and had nothing to do with the lesions produced nor the place of application is supported by my data. The same U-shaped function was produced by the different anticholinesterase. Therefore, this temporal pattern is important and warrants further investigation.

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3 April 1967

Continuous Gas Chromatography

Abstract. *Continuous gas chromatography has been achieved with a radial-flow chromatographic channel free of packing and formed between two closely spaced (50 to 75 microns) disc surfaces (optically flat and solvent-coated) rotating at one-half or one revolution per second. This technique provides high capacity and immediate response (in a fraction of a second). Mixtures of hydrocarbon gases have been separated at flow rates of 6 to 30 cubic centimeters per minute with 100 to 150 cubic centimeters per minute of nitrogen carrier gas in a chromatographic channel only 39 millimeters long.*

Continuous gas chromatography would have obvious advantages in all chromatographic monitoring and preparative applications. Schemes for continuous operation were suggested as early as 1949 by Martin (*1*), and literature on patents described devices which claim to perform chromatography continuously (*2–4*). Giddings (*5*) published a theoretical analysis of a continuous chromatographic system; Barker and Huntington (*6*) presented operating data on a toroidal bed instrument resembling that of Luft (*3*).

All previous continuous gas chromatographs used moving packed beds, with disadvantages arising from the inherent nonuniform flow resistance of a packed bed. Nonuniform flow resistance prevents the establishment of flat solute profiles (chromatographic bands) and severely limits the resolving power of a bed. The loss of resolution resulting from the nonuniformity of flow resistance was treated theoretically and experimentally by Golay (*7*) and others (*8*) who concluded that packed-bed resolution decreases roughly as the square of the bed radius, although column baffles may reduce the resolution loss accompanying increased column diameter (*7*).

Also relevant to our work are investigations (*7, 9, 10*) which have shown that column HETP (height equivalent to a theoretical plate, a resolution index) is of the same magnitude as the column diameter in a capillary chromatograph. The smaller the HETP, the greater is the separation that can be accomplished in a unit length of column.

These considerations led us to use two parallel optically flat disc surfaces, separated by 50 to 75 microns and

coated with a thin layer of solvent, as a high-resolution continuous chromatographic channel. The close disc spacing was expected to produce an HETP comparable in magnitude to the disc separation, so that very short gas-flow paths would contain a sufficient number of equilibrium stages to produce gas separation. Furthermore, elimination of packing obviously eliminates problems of packing nonuniformity. Flow-resistance uniformity is further insured by using optically flat discs held apart by precision spacers. (A thin cylindrical annulus presents similar advantages but greater fabrication problems.)

In the continuous disc chromatograph (Fig. 1), the chromatographic channel is formed by the opposing parallel disc surfaces which are 98 mm in diameter and coated with silicone grease by spraying with a 5 percent solution of grease in benzene. The discs are held apart by three small spacers whose thickness determines the depth of the gas channel. Both discs are fastened to a turntable which rotates at either ½ or 1 rev/sec in the direction shown by the arrow. Carrier gas enters the chromatograph through the central orifice by means of a stationary bonnet and rotary seal; it then flows radially outward through the space between the discs over 360°C. The mixture to be separated enters continuously through a line which terminates in a tapered nozzle, directly opposite the inlet periphery of the radial chromatographic channel.

If we assume that the mixture consists of components A and B whose retention times in a conventional chromatograph are t_A and t_B, then these components will travel radially through the opposing disc channel and emerge continuously at fixed characteristic angles, Θ_A and Θ_B, which are functions of the conventional column retention time. Detectors, or collectors placed at Θ_A and Θ_B, continuously record or collect the separated components. The gas retention time may be less than 1 second. The continuous disc chromatograph transforms a difference in retention time into a difference in angular discharge position.

The variation of composition with discharge angle for mixtures of methane and propane and for mixtures of methane and butane is shown in Figs. 2 and 3. The fact that separation is achieved in an extremely short chromatographic channel length and at very high feed-flow rates bears out the effectiveness of the concept of the close-spaced flat-plate chromatographic channel. Improved separation can probably be obtained by lowering mixture feed rate, decreasing plate separation, using a more effective solvent phase, and increasing channel length. A new longer channel apparatus is being built.

A preliminary theoretical analysis of continuous disc chromatography has yielded Eqs. 1 and 2 which permit estimation of the peripheral arc length over which a solute gas is discharged (H_{c_i}); and the angle, measured from the feed point, of maximum solute discharge (Θ_i).

$$H_{c_i} = \left[\frac{B}{u_i} \sec \phi_i + C \cdot u_i + r_B\right] \cot \phi_i \qquad (1)$$

where H_{ci} is the length of a solute i vapor discharge zone; B is the molecular diffusion constant; u_i is the average solute zone radial velocity; C is the mass transfer resistance term; ϕ_i is the angle at which the solute i discharge zone intercepts the disc periphery ($\tan^{-1}$ zone radial velocity/peripheral disc velocity); and r_B is the channel loading factor, reflecting the volume of mixture gas fed per centimeter of channel inlet periphery.

$$\Theta_i = \frac{360\, \pi w (k d_G + d_L)(r_2^2 - r_1^2)}{F_c k} \qquad (2)$$

where w is the disc rotation speed, revolutions per second; k is the distribution coefficient; d_G is the disc separation (cm); d_L is the solvent phase thickness (cm); r_2 and r_1 are the inner and outer radii, respectively, of the annular disc (cm); and F_c is carrier gas flow rate (cm^3 per second).

The capillary chromatography equation of Golay (*7*, *10*) and others (*9*) is modified in Eq. 1 by including the resolution *decrementing* effect of (i) lateral molecular diffusion (sec ϕ); (ii) channel load (r_B); and (iii) the angle at which the discharging solute intercepts the outer channel periphery (cot ϕ).

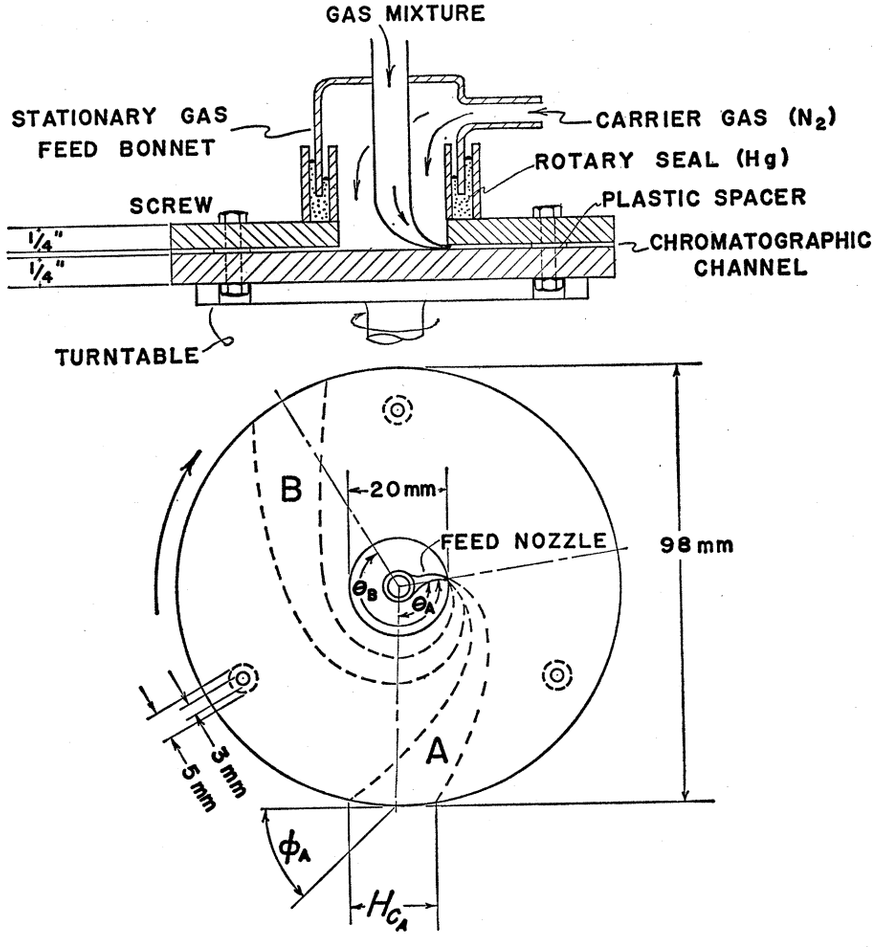

Fig. 1. Continuous disc chromatograph. (Top) Vertical section. Chromatographic channel is formed between solvent-coated surfaces of flat glass discs separated by three small plastic spacers. (Bottom) Discs showing central supply orifice and paths of hypothetical mixture components A and B. H_{CA} is the length of discharge zone for component A. The discs rotate in the direction of the outer arrow.

Together with Eq. 2, it provides a criterion for complete separation. Two components (A and B) may be separated completely if the difference between their discharge angles ($\Theta_A - \Theta_B$) multiplied by π times disc diameter is greater than their average discharge zone length ($H_{CA} + H_{CB}/2$).

Continuous disc chromatography has inherently lower resolution than a capillary column of diameter equal to the plate-separation distance. However, plate-separation distances with this chromatography can be made less than the smallest practical capillary diameters so that the loss in resolution may be partially offset. In addition, capacity is magnitudes higher; response time is shorter, usually a fraction of the rotational period.

By making analysis a function of an angular displacement rather than a time displacement, continuous disc chromatography potentially simplifies all preparative, automatic analysis and monitoring applications of chromatography. Speed of analysis becomes less dependent on the response time of gas detectors, and different kinds of detectors can be used for each effluent species. We believe the concept can lead to a broad spectrum of new instruments and processes for separating and analyzing mixtures.

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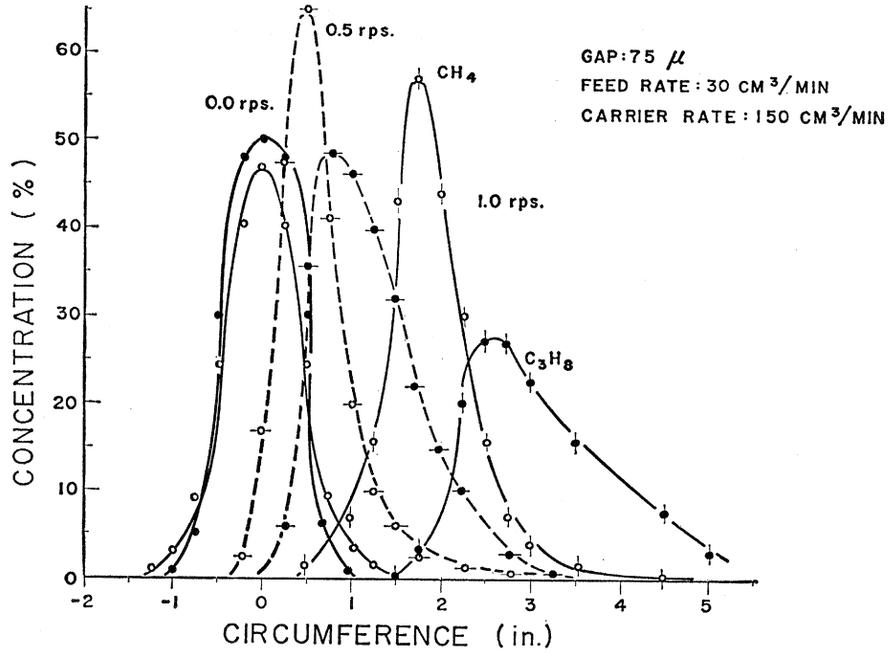

Fig. 2. Effluent concentration as a function of peripheral position for three disc-rotation speeds. Mixture of methane and propane (50 : 50) is used. High loading condition (30 cm³/min).

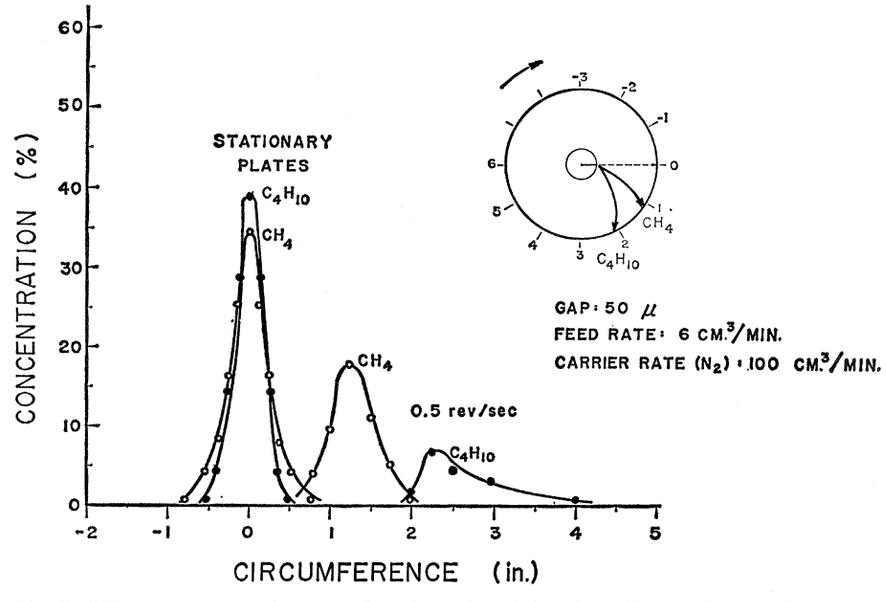

Fig. 3. Effluent concentration as a function of peripheral position. Mixture of methane and butane (37 : 63); 6 cm³/min.

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Tetrodotoxin Derivatives: Chemical Structure and Blockage of Nerve Membrane Conductance

Abstract. *The nerve-impulse-blocking actions of derivatives of tetrodotoxin have been tested on lobster and squid axons. The block produced by deoxytetrodotoxin was similar to that produced by tetrodotoxin and was probably caused by tetrodotoxin contamination. Tetrodaminotoxin and anhydrotetrodotoxin also produced a similar block but at such high concentrations that tetrodotoxin contamination cannot be ruled out. The hydroxyl group of C_4 and the hemilactal oxygen links play an important role for the nerve-blocking action.*

Tetrodotoxin (TTX), the active ingredient of the puffer fish poison, has become a useful tool in the study of electrophysiology since its blocking mechanism of nerve conduction was unveiled (*1*). The TTX blockage, which is not accompanied by any change in