active and numbs the mouth. The sensitivity of buccal tissue to such secretions should offer these frogs partial protection against certain kinds of predators. Conseqently, within a species that tends toward bright coloration and which is likely the prey of diurnal birds, selection might result in a combination of aposematic coloring and a high level of toxicity. Therefore, the chaotic interpopulational variation of color and toxicity in *D. pumilio* seems at first paradoxical. Some of the hues seem cryptic and others flamboyant, and the various populations appear to be evolving different ways of life. Conceivably, any protective aspects of the toxic compounds might be secondary, with the true physiological function entirely unrelated to toxicity.

With all its intraspecific differences—as in appearance, habits, and chemical composition of skin—*Dendrobates pumilio* is the most variable species of vertebrate known to us. A tentative hypothesis would contain the several interacting factors that must be involved, namely, isolation and small population size, inherent variability, chance, and selection. A map of Atlantic-side Costa Rica and Panama reveals that opportunities for isolation are greater along some of the flooded coastal reaches of the latter country than elsewhere. This partly explains the chaotic variation of *D. pumilio* in northwestern Panama; the variation in Costa Rica (*3*), on the other hand, seems largely clinal in nature. Presumably the predecessor of existing populations had a high mutation rate and harbored tremendous genetic variability, making the frog potentially capable of adjusting to diverse conditions in a forest environment. Either the chance fragmentation of this stock, or the chance founding of new colonies in a geographic mosaic, resulted in small populations each of which possessed a unique and greatly limited mixture of alleles on which selection could operate. There was consequent adaptation of different populations not only to different micro- and macrohabitats, but seemingly even to different ways of avoiding predation.

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3. Norman J. Scott, Univ. of Southern California, kindly supplied information on color variation of *D. pumilio* in Costa Rica, on the basis of populational samples from five localities spaced nearly the length of the coastal plain. Individual frogs all have reddish bodies, but there is an interpopulational trend from red to reddish orange and, in a southward direction, a correlated increase in the amount of black spotting on the dorsum. The frogs in northeastern Costa Rica are sparsely dotted with black and have bright blue (rather than black) hind limbs.
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23. Preparation of this paper was supported in part by NIH grant GM 12020.

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Retrograde Amnesia Produced by Intraperitoneal Injection of Physostigmine

Abstract. *Intraperitoneal injection of physostigmine in rats produced a retrograde amnesia of a trained task of escaping shock. This amnesic effect was a U-shaped function of the length of the interval between initial training and injection. In all cases, retraining occurred 30 minutes after injection. A substantial effect was produced by physostigmine if its application was made 30 minutes after training; there was no effect if application and tests were made 1, 2, or 3 days after the original training. When the substance was injected and the rats were retrained 5, 7, or 14 days after the original training, a substantial effect again appeared. These results are similar to those reported in experiments in which another anticholinesterase, diisopropyl fluorophosphate, was applied intracerebrally. The data demonstrate a similar pattern of change of the amnesia with time, and they substantiate the view that neither the place of application nor the brain lesions caused the reported amnesia.*

Recently Deutsch, Hamburg, and Dahl (*1*) and Deutsch and Leibowitz (*2*) produced an amnesia in rats by injecting an anticholinesterase drug, diisopropyl fluorophosphate (DFP) into their cerebrums. Physostigmine, another anticholinesterase, intraperitoneally injected into rats (*3*) and intravenously injected into dogs (*4*) inhibited trained intracranial chemical and electrical self-stimulation behavior. I have attempted to confirm Deutsch's finding that the amnesia is due to an upset of cholinergic balance and to confirm the pattern he reported.

Similar procedures were used in both experiments. Male rats (Holtzman strain, 300 to 350 g) were trained to escape shock in a Y-maze by running to the illuminated arm. The safe side was varied randomly from trial to trial. Each trial ended when the rat sucessfully found its way to the safe side. He was allowed to remain there for 30 seconds before the next trial began. Training was concluded when the rat performed ten consecutive trials correctly.

After the rats were trained, they were kept in the home cage for a variable period before being injected with physostigmine (0.4 mg/kg). The different intervals between the initial training and

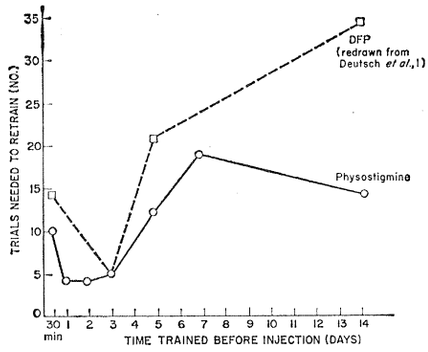

Fig. 1. Mean number of trials needed for retraining of animals injected with physostigmine compared with those required by animals injected with DFP.

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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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