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6. Tissues used in this study were collected during the summer months and may not be representative of the growth phase. It is probable that the growth rate is severely restricted because of the narrow peduncle described above. Therefore, the tumor is self-limiting and nonlethal.

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Alternation as Function of Preliminary Training and Type of Deprivation

Abstract. Laboratory rats trained upon a table top with randomly positioned reinforcement containers showed significantly a greater tendency to alternate in a Y-maze than animals trained on a straight runway. The type of deprivation, either food or water, did not have an effect. The differences in alternation disappeared with repeated trials on the Y-maze.

Alternation has attracted interest among behavioral scientists because of the observed tendency of many organisms to make a different choice on one trial of a series than that made on the preceding trial even though the two choices appeared to require the same degree of effort and to be equally rewarding. Previous studies (*1*) have shown that alternation appears in a variety of similar experimental situations —for example, human beings guessing the outcomes of coin tossing (*2*)—and that there is a persistent tendency to alternate even though one of the choices may be continuously reinforced (*3*).

Explanations of alternation have tended to consist of postulating that the organism has an attribute with properties necessary to account for the observed results. For example, reactive inhibition (*4*) has been a favorite explanation because it has been postulated that reactive inhibition is generated as a positive function of effort and dissipates with time. Presumably responses requiring a greater degree of effort would generate more reactive inhibition with a consequent greater tendency to avoid response repetition temporarily. However, experiments which have separated the degree of effort required to make a response from the time required to make the response have not supported the hypothesis that effort is a variable determining alternation.

Another type of construct links the process producing alternation to perception rather than to responses by postulating a perception-produced decrement in the perceptual process, for example, "stimulus satiation" (*1*). Such response-inferred constructs, however, tend to be circular unless the variables which control them are delineated, in which case they are redundant. An alternative approach, which has not been seriously attempted (*3*), is to examine the extent to which alternation is an effect dependent upon transfer of training. For example, rats in their laboratory living quarters may have to move around to find food; consequently, when a piece of food is consumed in one place, the animals learn to look elsewhere for other pieces.

Conceivably, similar but more complex explanations can be invoked to account for the avoidance by human beings of recently made responses. In fact, a learning explanation of the human tendency to avoid repeating guesses seems to be more plausible than an explanation involving perceptual decrement.

To test the hypothesis that alternation behavior is learned, 38 experimentally naive hooded rats, from the colony maintained at Indiana University by the psychology department, were run in a two-variable factorial design. At the start of each experimental and training session each animal was under 18-hour food or water deprivation. The animals all received 13 days of preliminary training. For 20 of the animals the preliminary training consisted of six reinforced—that is, rewarded with food or water—runs a day on a 4-foot straight runway. Each of the other 18 animals was placed for 3 minutes a day upon a 2½- by 4-foot table top on which were six randomly positioned reinforcement containers with either food or water. No animal failed to find and consume the reinforcements within the 3-minute period. The food reward was a pellet of commercial rat food weighing 45 mg. The water reward was two drops of water from a standard medicine dropper. The reinforcements were presented in small depressions drilled in 1½-inch-square aluminum pieces cut from 3/16-inch stock.

After preliminary training the animals were tested for 15 consecutive days on a Y-maze with 2-foot legs. Each animal was given two pairs of reinforced runs a day. Each pair of runs was separated by the time required to run the other animals in the group receiving the same treatment conditions. An alternation was counted if, on the second run of a pair, an animal chose the side opposite to the side chosen on the first run of the pair.

Because there is less tendency to alternate with longer time between trials (*1*), a time score based on running time in the second run of each pair of runs was computed for each animal. It might be expected that the faster animals would alternate more because they would have a shorter interval between the first and second runs of each pair. Analysis indicates that the running time for the animals trained on the straight runway was significantly shorter ($p < .01$) than that for the animals trained on the table top. The average times were, respectively, 9.99 and 15.05 seconds. If alternation depended only on the time between choices, the animals trained on the straight runway would be expected to alternate more than the others. It was found, however, that the animals trained on the table top alternated significantly more than those trained on the straight runway ($p < .05$). The type

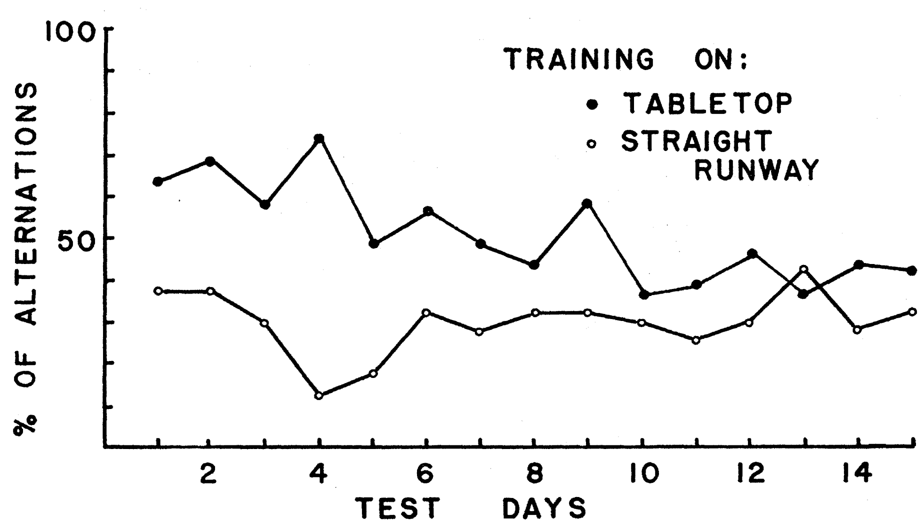

Fig. 1. Percentage of trials on which alternation occurred as a function of days with two pairs of test trials per day. Note the initially greater percentage of alternation of the animals trained on the table top.

of deprivation did not have a significant effect; therefore, the data for the animals deprived of food and water were combined (Fig. 1). A feature of the data is that the animals trained on the table top exhibit a relatively high percentage of alternation at the outset, but alternate progressively less with trials. We interpret these results to mean that the effects of preliminary training were temporary (*5*).

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3 January 1961

Electron Microscopy and Autoradiography

Abstract. The combined techniques of electron microscopy and autoradiography were used for the purpose of differentiating radioactive from nonradioactive particles collected on membrane filters. Newer methods of processing the membrane filters and applying the nuclear emulsion have resulted in an improvement in the qualitative nature of the procedure.

A method for differentiation of radioactive and nonradioactive particles collected on membrane filters and examined with the electron microscope was reported in an earlier paper (*1*). Continual application of this method during the past year has resulted in a number of refinements and the development of a reliable and reproducible method for processing membrane filters used in the sampling of radioactive aerosols. The objective of the present report is to describe in detail the method of processing membrane filters for examination with the electron microscope, and to discuss those modifications of the technique which have improved its qualitative nature.

Previous workers have reported on application of the combined techniques of electron microscopy and autoradiography for various purposes (*2*). The technique described in the present report was developed for the purpose of obtaining a more accurate analysis of aerosols that contain radioactive particles. The aerosols in this particular case were used in a study on the effects of the inhaled radioactive particles. The same technique could be applied to a number of other studies involving the collection and analysis of air samples containing or potentially containing radioactive particles. Application of the present technique is limited to alpha-emitting particles. Further studies are presently being conducted to test its usefulness for beta-emitting particles.

In the technique under discussion, membrane filters (type AA; plain, Millipore Filter Corp., Bedford, Mass., 25-mm diameter), which are an integral part of closed chambers used to expose the lungs of animals to radioactive dusts, are processed for examination with the electron microscope in the following manner. The membrane filter containing the collected aerosol sample is positioned with the collecting side upward on a 2- by 2-in. piece of Teflon. While the filter is steadied with a dissecting needle or similar instrument, a small disk is cut out of the central portion of a membrane filter with a 3-mm brass cork bore. The small disk thus cut out of the filter is placed with the collecting side down on the surface of a Formvar and carbon-coated electron microscope grid. The grid(s) thus prepared are supported in a petri dish by a medium-pore, sintered-glass filter stick beneath which are three sheets of No. 41 Whatman filter paper. The filter solvent of choice (acetone in this case) is then carefully poured into the petri dish until the filter paper is completely saturated, but not covered, by the acetone. The petri dish is then covered with its lid and allowed to stand at room temperature until the acetone has evaporated and the specimen grid has dried. This usually requires approximately 16 hours.

Dissolving the filters in this manner is a distinct departure from previous methods, and in our experience it has resulted in a more uniform distribution pattern of the collected aerosol sample. The commonly used rapid method for dissolving samples of membrane filters (*1*, *3*) resulted in loss of the smaller particles and aggregation of the larger particles on or along the grid wires. After dissolution of the membrane filter, the dried specimen grids can be examined with the electron microscope and, if desired, selected areas can be photographed. The grids with the sample side up are then secured for application of the nuclear emulsion to one end of small glass pegs 3 mm in diameter by means of double-coated Scotch tape. The pegs, with the grids in an upright position, are then inserted into holes of slightly larger diameter drilled into a block of stainless steel. The depth of the drilled holes is equal to one-half the length of the glass pegs. All subsequent steps in the process of autoradiography are carried out in a darkroom under light filtered through a Wratten, Series OA, filter. Alpha-sensitive Eastman Kodak NTA emulsion in gel form (*4*) is heated at 45°C until fluid, and then 2 ml are diluted 1:1 with distilled water. One drop of a dilute solution (0.05 percent) of sodium lauryl sulfate is added to the diluted emulsion. In previous work the emulsion was more dilute (1:2) and the detergent was omitted. This resulted in an uneven distribution of silver grains over the specimen grid. The modification in the preparation of the emulsion has resulted in a uniform and even distribution of the silver grains over the entire specimen grid aperture. Figure 1 shows the distribution pattern of undeveloped silver grains applied by the above method.

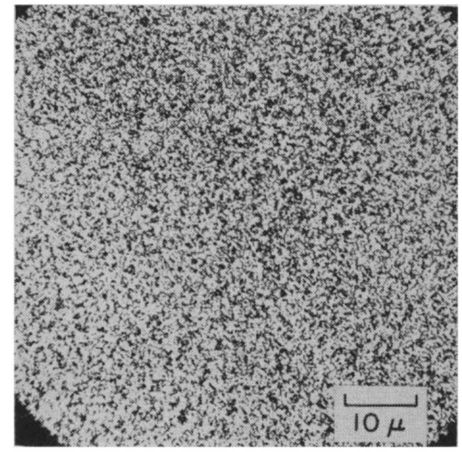

Fig. 1. Electron micrograph showing the distribution pattern of undeveloped silver grains over a specimen grid aperture.

The specimen grids secured to the glass pegs in the stainless steel block are then coated by means of a fine wire loop which is dipped into the emulsion

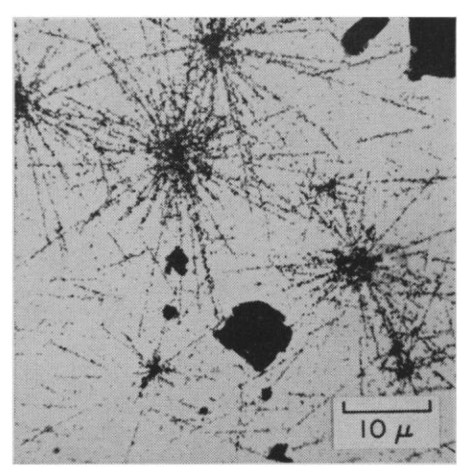

Fig. 2. Electron micrograph showing the differentiation of radioactive and nonradioactive particles.