tilation was in the order of 6 to 10 liter/min, depending on the size of the dog. The catheter electrode was introduced through the carotid artery or femoral artery and was located in the descending aorta. A second sampling catheter was inserted to the same place through which blood samples for the calibration in our in vitro polarograph could be withdrawn.

Figure 2 shows a plot of the readings from the Visicorder tracing against the absolute values of PO_2 in millimeters of mercury as obtained from the samples by the in vitro polarograph—that is, an in vivo calibration curve.

The tracings also show oscillations synchronous with the heart beat, the amplitude of which increase with rising inspiratory oxygen concentrations. Further animal and also model experiments are planned in order to assess the nature of these conditions. In accord with the observations by Sproule *et al.*, we are rather inclined to attribute this phenomenon to a mechanical pulse pressure effect (*8, 9*).

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7. These experiments were carried out partly in collaboration with Dr. T. R. Watson, Jr., and Dr. M. L. Heller of the Cardiopulmonary Laboratory, Hitchcock Foundation, Mary Hitchcock Memorial Hospital, Hanover, N.H. We thank these colleagues for their kind cooperation.
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8 August 1958

Quantitative Technique for Analysis of Radiation-Induced Tumorization in Fern Prothalli

Tumors occur spontaneously at a very low rate in the in vitro culture of the prothalli of the bracken fern, *Pteridium aquilinum* (*1*). Various nontumorous abnormalities also occur, largely as a result of environmental variations, but these are quite readily distinguished from the tumors by several criteria (*1*). The tumors have been observed to undergo a characteristic nuclear behavior leading from the original haploid prothallial condition to a subsequent polyploidy-aneuploidy by means of endomitotic reduplication and other mitotic abnormalities (*2*). This nuclear change is further characterized by a change in nuclear DNA levels from a stable 1C to 2C range to an extremely variable and heterogeneous distribution of values from the normal to several times that amount (*3*). It was also discovered that the rate of occurrence of the tumors could be greatly increased by applying ionizing radiations to young actively growing prothalli or to dormant spores (*4*). The study of this phenomenon seemed promising if it could be put on a quantitative basis. This is a report on the development of such a technique and a preliminary report of some results obtained (*5*).

The season's supply of field-collected spores (*6*) is cleaned, pooled, and thoroughly mixed. This constitutes the stock from which all experimental materials are drawn during the year. Weighed amounts of spores are taken from the supply. Spores in the experimental lots are x-rayed, while the controls are untreated. Subsequently they are handled in the sterile transfer room, and always in an ascending order with respect to the amount of radiation administered.

The spores are placed into a 15-ml centrifuge tube and wet with 0.5 ml of a 0.1 percent solution of Tween-80. The tube is shaken gently until the spores are no longer clumped. Then 12 ml of a filtered 5 percent solution of Pittchlor (*7*) is added, and the capped tube is shaken vigorously for 2 minutes. The spores are then spun down at moderate speed for about 2½ minutes, and at the end of a total exposure of 5 minutes in the Pittchlor solution the supernatant is quickly poured off, leaving a pellet of spores at the bottom of the tube. These are resuspended in 12 ml of sterile water and spun down again. The water is poured off quickly and the tube is left inverted for a moment to drain the spore pellet completely. The spores are then put into true suspension by adding a measured volume of a medium which matches their density.

Since any appreciable osmotic value of the suspending medium would be objectionable, a large water-soluble molecule that is biologically inert is required. For this purpose the highest available molecular weight of polyethylene glycol (Carbowax 20M) was found satisfactory. Although any momentary density of dormant fern spores newly placed into water is a highly transient state, and is not exactly the same throughout the sample, for the purposes of the present technique a 5 percent solution of Carbowax 20M is a satisfactory approximation of the average spore density during that stage of hydration prevalent at the time of inoculation. A portion of this

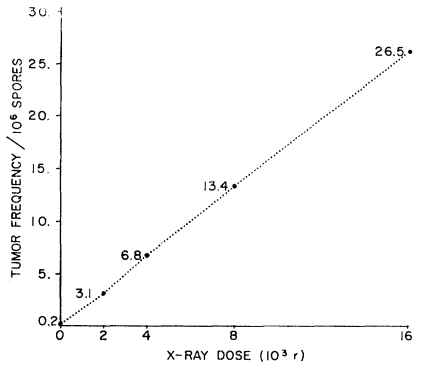

Fig. 1. Dosage-response relationship of ionizing radiations applied to dormant spores of *Pteridium aquilinum,* with the frequency of tumors scored after 6 weeks in culture. The tumor frequency per million spores is plotted against the x-ray dose in thousands of roentgens.

suspension is drawn up into a sterile pipet, and the inoculations are made drop by drop. The surface of the agar medium in the petri plate is pre-wetted by pouring sterile water into the dish and pouring it off before inoculation. This leaves a surface film of water in which the inoculum disperses readily with no clumping, and greatly facilitates subsequent studies of the cultures.

The number of spores in a single drop of the inoculum can readily be determined by the application of blood-counting techniques. The size of the drop and the number of spores that it contains have been found to be very reproducible in any one lot of spores. Approximate uniformity from one lot to another can be obtained by starting with weighed amounts of spores and standardizing procedure throughout.

When the spores have germinated and are of a convenient size—for example, when they are in the two- or three-cell stage, the total numbers of viable spores in single plates are determined. Since we work with large inocula, the counting is most readily done on an enlarged shadowgraph of the entire culture. The 60-mm dish is placed without its cover upon a glass negative carrier in an enlarger, and the image of the culture is projected onto an 8- by 10-in. enlarging paper. This can then be readily counted at a convenient time with the aid of a magnifier.

By using these quantitative techniques, various cultural conditions and other factors such as the safe or tolerable limits of the sterilization process were first investigated and defined more precisely than was earlier possible.

However, the primary objective has been the further analysis of the process of tumorization in the fern gametophyte and especially its increased frequency of occurrence after ionizing radiations. One of the first approaches has been to determine the rate of this occurrence, and to

establish a possible dose-response relationship. Unfiltered 136 kv x-rays were administered to dormant spores at the rate of 1000 r/min for total doses of 2000, 4000, 8000, and 16,000 r, with an unirradiated control. The total numbers of recognizable tumors of all sizes were counted after a 6-week culture period. The frequency of tumors per million spores of the inocula for the various doses is shown in Fig. 1. Approximately 28,000,000 spores were analyzed for the five points shown.

There is as yet no definitive evidence concerning the nature of the basic cellular alteration which occurs in the spores and is eventually expressed as a tumor. It would therefore be premature to attempt to conclude beyond our present data. However, for the sake of a working hypothesis we are inclined to consider this a mutation, or possibly a group of similar mutations. Dormant spores of *Pteridium* are quite radio-resistant, the survival curve extending beyond the limits of the present data by approximately a factor of 10 (*4*). Considering that this is a haploid organism, the type of linearity shown in the dose-response curve (Fig. 1) might be expected of a mutation in this dosage range. Also, at this relatively low frequency of occurrence, an exponential response can be linear (*8*). That dormant spores can be irradiated, kept in continued dormancy for at least another year, and still produce tumors would indicate that the causal event is not transient. It might be said, then, that although there is no evidence which points directly to this being a mutation, neither does any of the evidence at hand contradict this hypothesis. In any case this system and the present approach do present rather unique opportunities for the quantitative analysis of a tumorization process at the cellular level.

The limits of the present experiments were determined by the practical limits of the equipment at hand. Current work is being directed, among other things, toward an extension of these data through the entire viability range to determine both the tumor frequency and the complete survival curve.

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25 July 1958

An Olfactometer for the Rat

This report describes an apparatus and operant conditioning procedure (*1*) for studying olfactory discrimination in the rat (*2*). A bar-pressing apparatus and dipper mechanism to provide water reinforcement is mounted in a cylindrical glass "wind tunnel." A stream of deodorized air flows continuously through the tunnel at a known velocity. This can be odorized by the addition of known volumes and concentrations of odorized air. The animal is trained to face into the air stream when pressing the bar so that all body odor or odorant adsorbed on the animal's fur is blown out to the rear of the response chamber. The injection of the odorized air is the signal to stop bar-pressing.

The apparatus shown schematically in Fig. 1 shows the response chamber (*b*), containing the bar-press apparatus (*i*) in the cylindrical "wind tunnel" (*j*, *k*), 4 in. in diameter and 12 in. in length. A rotary-vane laboratory pump forces a continuous stream of air through a calcium chloride drying agent and the charcoal and silica gel deodorizing column at a rate of approximately 40 lit. per minute, as monitored by flowmeter *a*. This is the main air stream to the response chamber (*b*).

A small fraction of the air is diverted at *c* through the flowmeter (*d*), via the manifold and stopcocks, to one of four odor bottles (e_1, e_2, e_3, e_4). The odor stream passes over the surface of odorant *f* and returns to the main air stream just in front of the response chamber. Two stationary stainless-steel vanes (*h*) mix the odorized and pure air streams. The

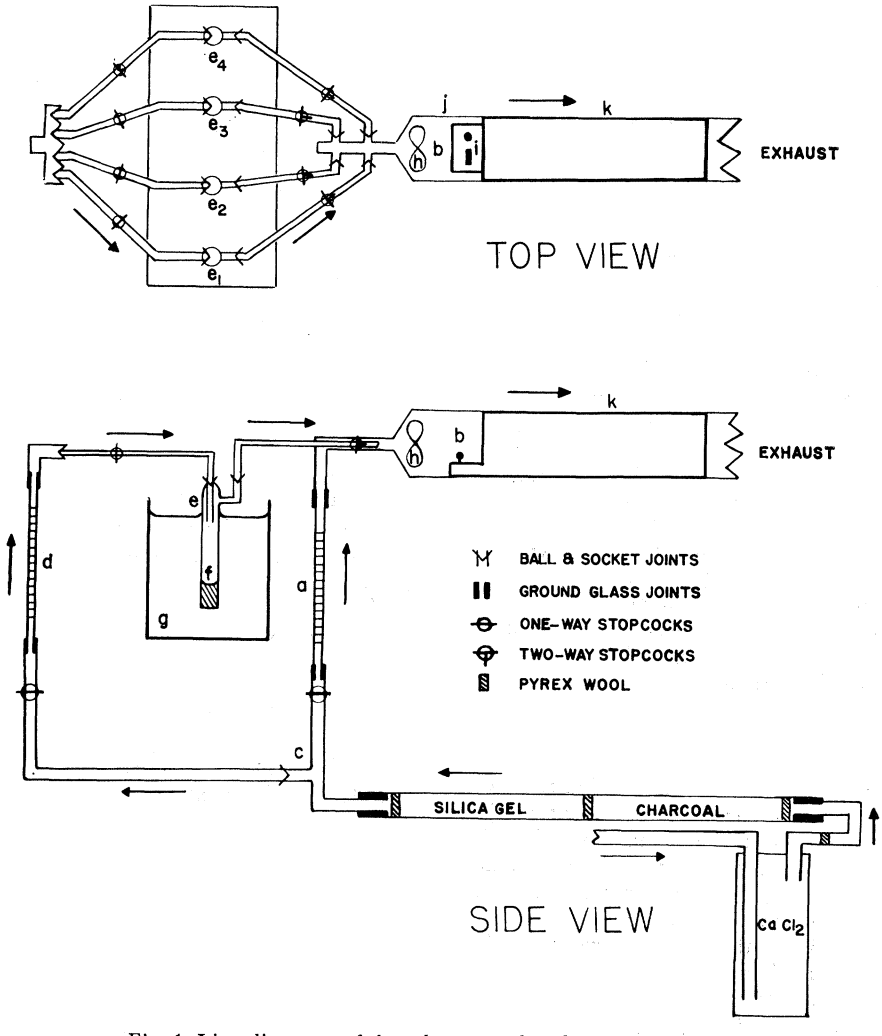

Fig. 1. Line diagrams of the odor control and response chamber.