driven at a rate of approximately 120/min; at *b* the stimulator is turned off and the record shows the absence of ventricular contraction; at *c* the animal exhibits a tonic convulsion; at *d* the stimulator is turned on again, the ventricles are stimulated, and the convulsion ceases.

Figure 2*B* shows an electrocardiographic record taken from the same dog 3 days later. At *a* the ventricles are being stimulated; at *b* the stimulator is turned off and the ventricles stop contracting (only the P wave is visible on the record); at *c* the ventricles begin to contract spontaneously, first slowly, then faster, reaching, at *d*, a rate of approximately 66 per minute; at *e* the stimulator is turned on again.

Animals whose ventricular rate had dropped, after surgical block, below 60 were maintained at normal rates by remote stimulation for periods as long as 8 days (*3*).

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2 June 1958

Method of Polarographic in vivo Continuous Recording of Blood Oxygen Tension

Abstract. A catheter-type PO_2 electrode has been developed which permits the polarographic continuous recording of the blood oxygen tension in vivo. The electrode has been tested in model experiments in vitro and applied in animal experiments lasting several hours. It yielded a continuous tracing of PO_2 with good reproducibility.

The development of a new type of oxygen electrode by Clark (*1*) has given a fresh impetus to the polarographic determination of the oxygen tension in the blood by means of platinum electrodes. In a first step of our studies, we incorporated this electrode into a procedure for measuring the blood oxygen tension in vitro (*2*). This method was thoroughly tested (*3*) by comparing the results with (i) adjusted tonometer equilibrations with determination of the oxygen in the gas phase by the Van Slyke technique and (ii) data obtained from determinations by the Riley technique (*4*). The method is now in routine use for clinical purposes (*5*) and was also used for the calibrations in the new procedure described in this report (*6*).

We have constructed catheter-type PO_2 electrodes for the continuous recording of the blood oxygen tension in vivo by ordinary catheterization. The basic principle is similar to that employed in Clark's electrode (*1*). The catheter consists of Intramedic polyethylene tubing PE 160 (inside diameter, 0.045 in.; outside diameter, 0.062 in.) or PE 200 (inside diameter, 0.055 in.; outside diameter, 0.075 in.) of any desired length. Another thinner polyethylene tubing containing the platinum cathode and its connecting wire was surrounded by the silver anode wire and inserted into the catheter proper; the silver wire at the same time kept the inner tubing concentrically in place. The catheter was filled with saline free of air bubbles and, on the proximal end, was covered with Teflon membrane of thickness 0.001, 0.0005, or 0.00025 in.; the latter was fixed by a specially made very thin ring of stainless steel. The distal end of the catheter was plugged with Seal-All. For the application in animals, the whole catheter-electrode was coated with a thin coat of Velvasil; this procedure effectively prevented any coagulation in the vessel over experimental periods of several hours and did not result in any appreciable loss of sensitivity. The electrodes were freshly assembled and checked in vitro before every animal experiment; this enabled us to service the electrode parts thoroughly every time—that is, to polish the platinum surface and the silver wire.

The current developed by these electrodes was of the order of 1 to 4 μa, depending on the thickness of the Teflon membrane employed (that is, it was similar to or slightly higher than, the current in the case of the Clark electrode) for air at a voltage of 0.6 volts. It was amplified by a General Radio Company type 1230-A d-c amplifier (input resistance 10^4 ohm) and recorded by a Honeywell 906 Visicorder. The amplifier scale permitted immediate readings during the experiment, and the values from the continuous record yielded another set of data for the same conditions.

The performance of these electrodes was always first tested in vitro by taking the calibration curve in various gas mixtures at room temperature several times. Figure 1 shows such a calibration curve (Teflon membrane 0.0005 in. thick). With proper construction of the electrode, we always get straight lines which never go quite through zero but which intersect the ordinate at a rather low reading (the helium reading amounts to about, or less than, 15 percent of the air reading in vitro).

Model experiments were performed in order to explore the effect of various rates of flow and of varying static pressures on the electrode readings. With increasing rates of flow the readings go up slightly and become constant at and above a linear velocity of about 7 cm/sec. The readings remain constant for a range of static pressures between 0 and 120 cm of water. The standard deviation both in vitro and in vivo was never more than 3 percent for any reading, in most cases much less. The response time of these electrodes was about 1.5 sec for 95 percent amplitude and roughly 7 sec for approximately 100 percent amplitude. It depends very much on proper construction of the unit.

A number of dog experiments were carried out in order to test these electrodes in vivo (*7*). The dogs were anesthetized with Nembutal (30 mg/kg). The trachea was intubated or cannulated in order to administer various inspiratory oxygen mixtures. Ventilation was kept constant throughout the experiment, which lasted 2 to 5 hours, by means of an Etsten ventilator (checked by a Bennett ventilation meter) or by a Starling pump; the minute volume of ven-

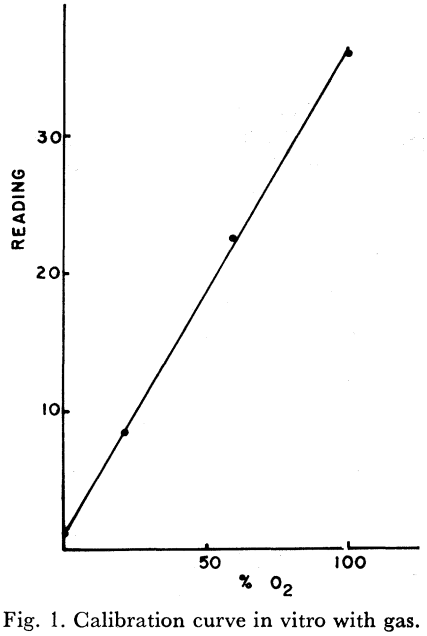

Fig. 1. Calibration curve in vitro with gas.

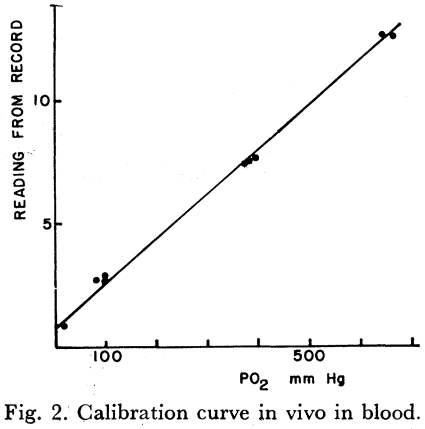

Fig. 2. Calibration curve in vivo in blood.

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

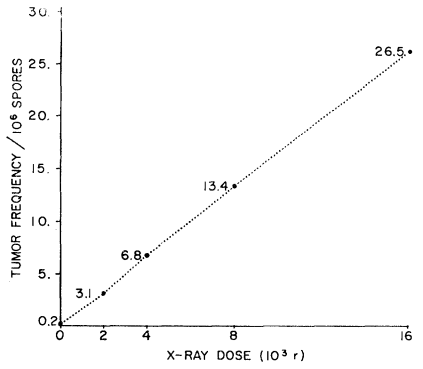

Fig. 1. Dosage-response relationship of ionizing radiations applied to dormant spores of *Pteridium aquilinum,* with the frequency of tumors scored after 6 weeks in culture. The tumor frequency per million spores is plotted against the x-ray dose in thousands of roentgens.

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