

It required 125 mg per kg of vitamin D₂ to raise the serum calcium to a level of 12 mg%. This level was also reached after a dose of 10 mg per kg of vitamin D₃, but not after a dose of 5 mg per kg. The ratio of effectiveness in producing hypercalcemia may therefore be set as approximately D₃ : D₂ = 125 : 7.5 or 16 : 1. This ratio is significantly different from the generally accepted ratio of antirachitic effectiveness of 30 or 40 to 1, although we must consider the fact that the nature of these experiments does not permit an evaluation of better than $\pm 25\%$.

Dihydrotachysterol is surprisingly ineffective in elevating serum calcium in the chick. It required 40 mg per kg to produce a level of 13 mg%. This is, on a weight basis, about 3500 times the usual human daily dose.

The ratios of quantities of the activated

sterols required to produce equivalent hypercalcemic effects (as to peak values) in the chicken contrast markedly with those required by the dog, which are:⁹ D₂, 5 mg per kg; D₃, 5 mg per kg; and dihydrotachysterol, about 0.8 mg per kg. They also contrast markedly with those required by the albino rat, which are:¹⁰ D₂, 12.5 mg per kg; D₃, 12.5 mg per kg; and dihydrotachysterol 6.25 mg per kg. These figures are not intended to be those which would produce the same peak value of 12 mg% in all species, but are simply those which produced the same peak in that species, whatever it might be, under the experimental conditions.

⁹ McChesney, E. W., and Messer, F., *Am. J. Physiol.*, 1942, **135**, 577.

¹⁰ McChesney, E. W., and Kochev, H., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 156.

14066

Physarum as a Radiation Test Object.

SHIELDS WARREN AND OLIVER K. SCOTT.

From the Laboratory of Pathology of the Harvard Cancer Commission and of the New England Deaconess Hospital.

One of the problems in experimental radiation has been to find a satisfactory test object which is on the one hand responsive to radiation and on the other hand sufficiently simply organized to make possible an analysis of the changes which occur. *Physarum polycephalum* partially fulfills this need. This slime mold is readily cultivated, macroscopically visible, rapidly growing, and continual streaming of its protoplasm gives an index of the direction in which it is growing as well as of the vigor of its growth in any given direction. In addition, we have found it to be sensitive to certain types of radiation.

Method. The plasmodium of *Physarum polycephalum* was grown in glass refrigerator dishes on paper towels sprinkled with oatmeal. The towels were kept just above the bottom of the dishes by pieces of glass, and the bottom of the container was covered with tap water. If the plasmodium is kept at a temperature

below 20°C, there is little tendency to form fruit bodies; if the temperature falls below about 12°C, the cultures grow only slowly.

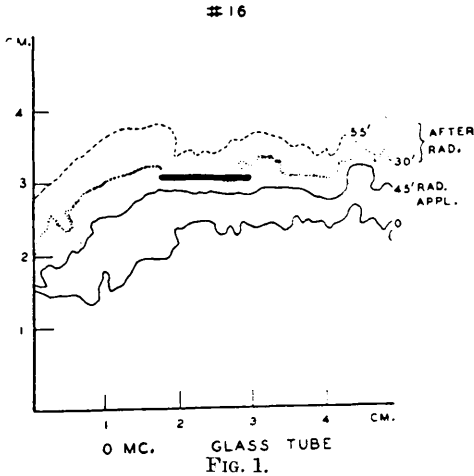
For testing, pieces of paper towel covered with plasmodium were transferred to one end of a refrigerator dish in the bottom of which there was an agar medium which was thin, flat, and translucent. A sheet of paper ruled to square millimeters was placed under this dish so that at any time the extent of the growth of the plasmodium could be accurately recorded by tracing the frontal edge on a similarly lined piece of paper.

In some cases many of the indentations were left out of the tracing if they were not important as it took too much time to go into such detail with a rapidly shifting contour. The radiation tubes were placed on the agar a short distance in front of the growth where it was growing best and fastest. This method of tracing has the disadvantage that it does

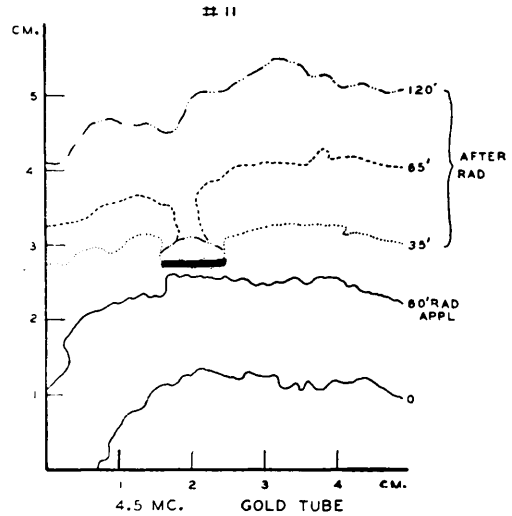
not show the thickness of growth or direction of protoplasmic streaming at a given spot but shows only the change in lateral growth. The changes in volume may be as striking as the changes in position.

Results. The growth of the plasmodium streams over a spent glass or gold radon tube (0.3 mm wall thickness). If sufficiently inactive the plasmodium will stream over it with only very slight slowing of growth due to

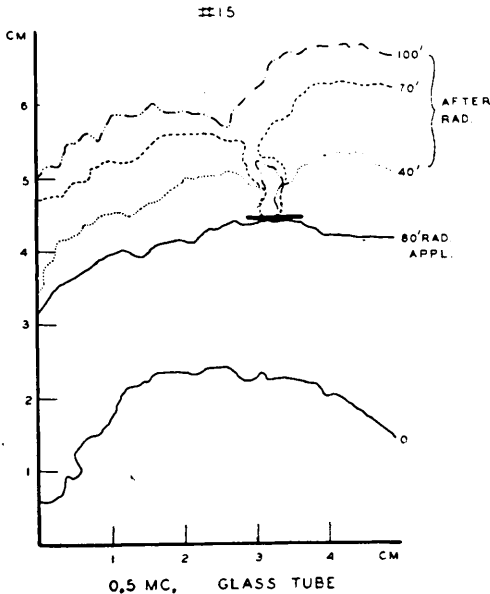
mechanical obstruction, as in experiment No. 16 (Fig. 1). This glass radon tube was about 18 months old. With a slight amount of radon present (0.5 to 5 mc), the plasmodium will not overgrow the tube but will stream close around it, even touching it (Fig. 2 and 3.) If the radon in a glass tube is strong, 20 mc or above, the growth will change course and not grow within 2 cm or more of the tube (Fig. 4). The distance varies with the strength of the



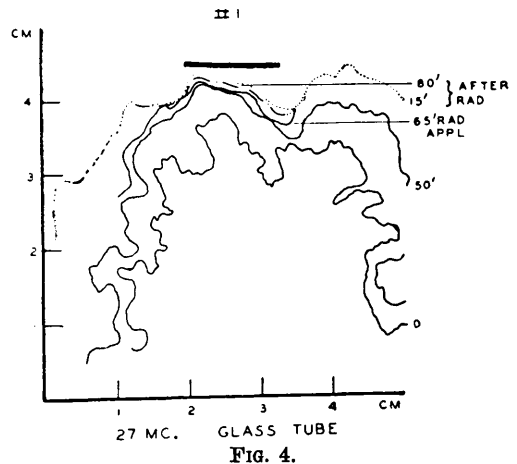
The heavy black line represents a glass tube used for radon previously. Assaying by usual methods of measurement showed no radioactivity.



Retardation of growth induced by a 4.5 millicurie gold radon tube. Note that the retardation is but little more than is brought about by $\frac{1}{16}$ th the amount of unfiltered radon in a glass tube.



Retardation of growth induced by an 0.5 millicurie glass radon tube.



The marked growth-inhibiting effect of a 27 millicurie glass tube on Physarum. The only significant growth is taking place 2 or more centimeters away.

tube. In contrast, if the radon is in a gold tube, the growth will change course only a little and will grow within 3 to 4 mm of the tube (Fig. 5).

When the plasmodium is confronted by a strong glass tube, the whole front close to the tube retracts about 1 mm (Fig. 6). Then a

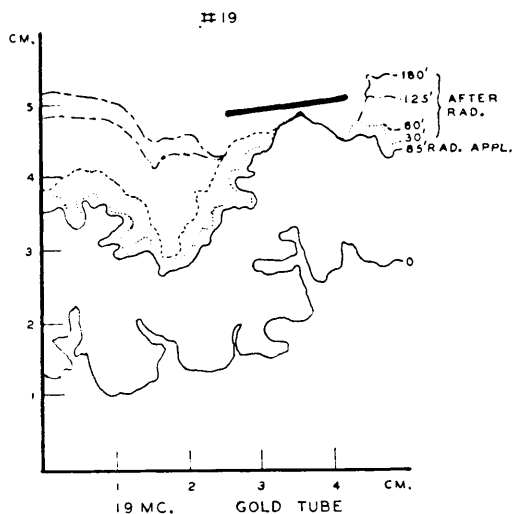


FIG. 5.

Represents a gold radon tube with a strength of 19 millicuries, showing the appreciably reduced inhibiting effect as contrasted with the glass radon tubes shown in Fig. 4 and Fig. 6.

process sets in by which the part nearest the tube grows least and that which is far enough away grows most. There is a continual shifting of the plasmodium so that eventually all of it is outside the area of noxious stimulus. This is particularly well shown in Fig. 6 where there was plainly forward growth fairly close to the tube up to one-half hour after the tube was in place, but after another 30 minutes there was no growth, and an hour later hardly any plasmodium remained.

The effect of X-ray radiation was difficult to demonstrate; first, because it was hard to prevent drying of the culture and at the same time to cover it with material which did not cause much scatter; and second, because the culture did absorb enough X-rays to warm it up, and warming above 25 to 30°C has a

deleterious effect on the growth. After several attempts, 2 recordings were made which seem to show little or no effect from X-rays. The agar medium was placed in cellulose acetate dishes, made by taking the emulsion off X-ray films, and wrapped in cellophane. The extent of the growth was then recorded for an hour before and an hour after X-ray irradiation. The X-ray was delivered at 140 kv at a rate of 106 r per minute; thus a total of 6,360 r was given.

As the gold tube very largely cuts out beta radiation, one may suppose that the plasmodium is sensitive to beta rays, and hence the difference in action between the glass and gold radon tubes. The indifference of the plasmodium to X-ray corroborates this view. The fact that even small amounts of radiation in gold or glass tubes do have an effect on the plasmodium could be explained by the great

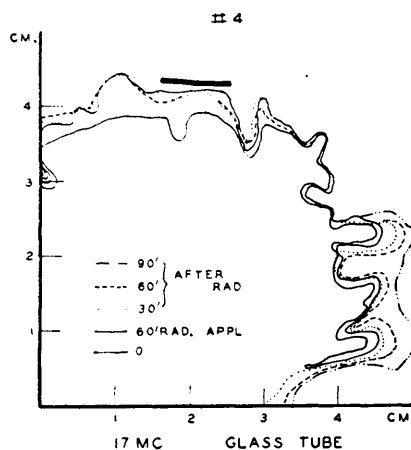


FIG. 6.

The marked growth inhibitory effect produced by a glass tube containing 17 millicuries of radon. Note that the entire direction of growth of the organism has been changed.

sensitiveness of the growth to the beta radiation which must exist in small concentration at the surface of even the gold tube.

The possibility of alpha ray effect is very slight as the effect on protoplasmic streaming is apparently exerted at too great a distance from the source for alpha radiation to be an important factor.