

solution was acidified and the precipitate centrifuged, washed, redissolved in sodium hydroxide, and reprecipitated. This procedure was repeated, and then the methone-formaldehyde reaction product was recrystallized twice from acetone-water. A sample of the purified methone-formaldehyde product was counted.

The residue from the first distillation containing the formic acid was acidified to pH 1 and distilled *in vacuo*. The distillate was titrated to phenolphthalein end point with a saturated barium hydroxide solution after flushing with CO₂ followed by nitrogen. The precipitate of barium carbonate which formed was centrifuged off. The supernatant containing the barium formate was evaporated to approximately 0.5 ml and while warm was treated with absolute ethyl alcohol, which precipitated crystalline barium formate. This was redissolved in water and recrystallized in this manner four times.

A fraction of original solution, which had been removed for iron determination, was acidified with 6 N sulfuric acid and titrated with standard solution of potassium permanganate. A second fraction of this solution was reduced with sulfur dioxide and titrated with potassium permanganate after the excess sulfur dioxide was removed by boiling. The Fe³⁺ concentration in the target solution was calculated from the difference in titer. In Table 1, Bombardments 1, 2, and 3 were made using the all-glass cell. Bombardment 4 was made using the cell having a 1-mil platinum window. With this cell, the helium ion beam incident on the solution had an energy of 35.8 mev. The number of ion-pairs produced in Bombardments 1, 2, and 3 were calculated, assuming that the ion-pair yield for ferric ion oxidation obtained in Bombardment 4 was also obtained using the all-glass target cells. This assumption is considered reasonable since the energy losses in the glass and platinum windows were of the same order of magnitude.

To insure that HC¹⁴OOH and HC¹⁴HO were actually produced by helium ion bombardment, the following additional control experiments were performed: (1) A sample of the original unbombarded target solution containing C¹⁴O₂ and FeSO₄ was retained at approximately 30° C for 1 week and then processed in a manner identical with that used in separating the HCOOH, HCHO and CH₃OH fractions in the bombarded samples. No C¹⁴ activity could be detected in these fractions from the unbombarded solution, indicating that reduced C¹⁴ compounds were not present in the original solution or formed by a metabolic process involving mold or other organisms. (2) A blank bombardment (#5) was made without added C¹⁴O₂; the isolated HCOOH and HCHO carriers were inactive. (3) Mass absorption curves run on active barium formate produced in the radiation reduction of C¹⁴O₂ were identical with those obtained using known samples of active barium formate prepared chemically and having the same specific activity and counting geometry. (4) No decay could be detected in the activity of the radiation produced HC¹⁴OOH and HC¹⁴HO.

An examination of Table 1 demonstrates unequivocally that it is quite possible to reduce appreciable quantities of carbon dioxide to formic acid by means of water through the agency of radiation. In fact, it appears that approximately one fourth of the dissolved carbon dioxide was reduced in Expt 2. Whether the formic acid is further reduced to formaldehyde or whether the formaldehyde has its origin in a direct reduction of carbon dioxide still remains to be demonstrated, but formaldehyde can also be produced from carbon dioxide and water under the influence of radiation.

The actual ion-pair yield is certainly not optimal even in Expt 2 in view of the large excess of the number of ion-pairs produced over the number of molecules of carbon dioxide in the solution. Presumably this reduction is achieved by means of the secondary hydrogen atoms resulting from the ionization. The actual amount of reduction observed is clearly still only the resultant of the reduction and oxidation reactions. The oxidation reaction is presumably minimized by the destruction of the hydroxyl radicals by their reaction with ferrous ion (8-10).

Whether carbon-carbon bonds and carbon-nitrogen bonds can be formed and more highly organized structures created under the influence of high-energy radiations is at present under investigation.

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Fungus Fruiting in Submerged Culture

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Although several species of ascomycetes have been grown in submerged liquid culture, the writer has been unable to discover any reports of perithecial formation under these conditions. Burkholder and Sinnott (1) studied the morphogenesis of about 150 species of fungi, including a number of ascomycetes, in shake cultures in three different nutrient solutions. In no case were perithecia reported, although this may have been due to the use of too short a culture period or to the possibility that none of these media was conducive to fruiting.

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Among the species grown by Burkholder and Sinnott was a strain of *Sordaria fimicola* Ces. and De Not. We wish to report the formation of mature and apparently normal perithecia by a strain of this species when grown under "deep vat" conditions. Our observation would seem to open the possibility of undertaking an experimental analysis of the intrinsic and extrinsic factors which control the onset of fruiting and the production of ascospores. Submerged cultures, as shown by Burkholder and Sinnott, take the form of small, discrete mycelia, which can be counted, transferred intact to Warburg respirometers, and otherwise manipulated as units for the purposes of experimentation.

The medium used was essentially that of Westergaard and Mitchel (2), without agar, and modified to contain 8–10 µg of biotin per liter and 5% sucrose. The pH range before inoculation was 4.0 to 4.5, and the temperature of incubation was about 25° C. Inoculations were made by adding an aliquot of an ascospore suspension prepared in a loosely fitting Potter homogenizer as suggested by Burkholder (3). The culture vessels were tubes about approximately 50 × 400 mm. Some of the tubes had an air inlet to the bottom ring-sealed into the top and a side-arm air outlet also sealed to the tubes; others were provided with rubber stoppers carrying an air inlet and outlet. Air, sterilized by passing through cotton, and humidified by passing through sterile water, was passed into the cultures at a rate that caused constant vigorous bubbling.

After about 4 days, the medium began to darken somewhat and to foam in a way characteristic of protein solutions, and soon thereafter the fungus apparently began to grow more rapidly and the mycelium took on a light-brown color. After about 2 weeks, young to fairly mature perithecia were formed on the newer part of the mycelium. Eventually some of these matured, and the ascospores were shed into the medium. At the time of fruiting, the cultures were very thick and dark, and the mycelium was a tan color under the microscope. It should be noted, however, that the individual colonies here were of a much looser texture than the colonies of this fungus studied by Burkholder and Sinnott, and also that they were not perfectly regular in shape. The largest colonies were about 1 cm in diameter, and had larger, more mature, and greater numbers of perithecia than did the smaller colonies.

On lower concentrations of sucrose and on unfavorable carbon sources such as inulin, young perithecia can be seen on the very scanty mycelium in 4–6 days, and very little further mycelial growth occurs.

Apparently strictly aerobic conditions are not necessary for the formation of the perithecia of this fungus. This was also noted by Edwards *et al.* (4) for this species and by Denny (5) for *Neurospora crassa*.

We have also noted the formation of submerged perithecia of *Sordaria* in standing liquid cultures containing low concentrations (less than 1%) of sucrose and other carbon sources. Formation of immature

perithecia of *N. crassa* (15,300 A × 15,300 a) occurred in standing liquid cultures containing the nonnitrogenous mineral constituents of the Westergaard and Mitchel medium and 0.2% Difco yeast extract but lacking sugar. These perithecia failed to mature.

The ascospores of this strain of *S. fimicola*, which normally seem to germinate only to the extent of about 5% in sugar-containing media, may be germinated to advantage in the above-mentioned culture vessels, using 0.5–1.0% sodium acetate instead of sucrose in the Westergaard and Mitchel medium. At least 60% germination may be secured in 18 hr at 25° C; after this length of time, further counts are not feasible.

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The Rate of Mutation of the Gene Responsible for Retinoblastoma in Man

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Retinoblastoma is a highly malignant ocular neoplasm that characteristically appears in children ranging in age from a few months to four years. Unless effective surgical or irradiation therapy is initiated before the lesion has spread beyond the confines of the globe of the eye, the outcome is almost invariably fatal. Most children with the disease have no similarly affected relatives. The literature contains numerous reports, however, of families in which individuals surviving the disease have transmitted it to one or more of their children. There are also reports of several affected children being born to normal parents (1–4).

Current genetic theory (3, 5, 6) holds that the disease is due to a dominant gene. The apparently isolated cases are attributed to mutation. In a state of nature the genes responsible for retinoblastoma would for the most part tend to be eliminated by natural selection in the same generation that they arose through mutation. When, however, either because of appropriate medical treatment or in consequence of a very rare spontaneous regression of the tumor, an affected individual survives, he may transmit the gene to half his children, who in turn may develop the disease. It is further postulated that occasionally, for unknown reasons, an individual who possesses this gene fails to develop a retinoblastoma. He will still transmit the

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