(A film of the residue left by the broth after the drying probably covers the bacteria.)

The effect of radiation on a frozen bacterial-broth suspension was of the same order of magnitude as the effect in experiments with the bacteria in dry state. Here, as expected, the indirect action[2] of radiation must have been inhibited to a great extent.

Irradiation of *E. coli* in broth solution and in the dried state showed that the results observed with *P. aeruginosa* can be obtained also with another organism. For example, at 3,000 r the number of survivors in the irradiated broth suspension is approximately one-fourth the number of bacteria surviving after an aliquot of the same broth suspension was dried and irradiated.

The experiments show that bacteria in a dry state are much less affected by irradiation than bacteria in solution. This observation cannot be explained on the purely physical basis of X-ray absorption. The indirect action of radiation on dried organisms must be greatly reduced, but it has not been shown to be the sole reason for the high protective effect of dehydration.

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[2] The definition of indirect action of radiation in this case covers only lethal events due to absorption of radiation outside the bacteria.

FURTHER USE OF DIPHENYLAMINE FOR THE STUDY OF CAROTENOID BIOSYNTHESIS IN MYCOBACTERIUM PHLEI

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It has been demonstrated that carotenoid production in *Mycobacterium phlei* is strongly inhibited by diphenylamine at concentrations which do not affect growth (Turian, G., Helv. Chim. Acta, **33,** 1988, 1950). There is good reason for thinking that diphenylamine acts as an antioxidant, directing metabolism toward the formation of the less oxidized representatives of the C_{40}-polyene series. In *M. phlei* this inhibition was found to be most marked at the terminal synthetic steps, i.e., apparent conversion of neutral hydrocarbons into acidic compounds (keto-enol polyenes such as chrysoflein). More recently it has been reported by T. W. Goodwin (Biochem. J., **49,** xxiii, 1951) that in *Phycomyces* grown in the presence of diphenylamine the observed decrease in colored polyenes is accompanied by an increase in hydrogenated C_{40}-polyenes such as phytofluene and phytoene. Similar results have been obtained by the authors in *Neurospora* inhibited by diphenylamine or grown in the absence of light (unpublished observations).

From the nature of its inhibitory action it was felt that diphenylamine might

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be employed as a tool in the detection of intermediate hydrogenated polyenes not ordinarily accumulated during carotenogenesis. This might prove particularly illuminating when applied to the bacteria where, to our knowledge, the existence of such polyenes has not been established and, hence, a unique mechanism of carotenoid formation remains a possibility.

For this purpose, *M. phlei* was grown in the presence of 1/40,000 diphenylamine on Ingraham and Steenbock's liquid medium with the addition of 1 per cent glycerol but no iron (diphenylamine antagonist). After 7 days' growth at 37 C the pigment from the nearly colorless bacterial mass (23 g dry weight) was extracted with acetone, saponified, and transferred to petroleum ether. A chromatogram of the epiphasic polyenes on "MgO-celite" (1:1) revealed the presence of two zones not previously observed in extracts from uninhibited bacteria. Both were absorbed below the wider, orange-yellow zone of leprotin, the major carotenoid usually present in *M. phlei* (Takeda, Y., and Ohta, T., Hoppe-Seyl. Z., **258,** 6, 1939). Our leprotin fraction gave the β-carotene spectrum with absorption maxima at 425, 452, 480 mμ in petroleum ether (b.p. 30 to 60 C) but showed a stronger adsorbability. Nonidentity was verified by a mixed chromatogram with β-carotene from carrots.

Of the two new zones, the first, a well defined yellow zone, contained a pigment which was epiphasic to 95 per cent methanol and exhibited maxima at 384, 403, 426, and 454 mμ in petroleum ether, as determined with the Beckman spectrophotometer. The position of the absorption maxima indicates the presence of a shorter chromophoric group than in the isomeric carotenes and, together with the adsorption behavior (found above β-carotene on MgO), is reminiscent of flavacin (Tischer, J., Hoppe-Seyl. Z., **251,** 109, 1938; **260,** 257, 1939).

Second, and less strongly adsorbed, was a colorless zone which displayed an intense greenish-grey fluorescence under ultraviolet light and was easily recovered in the filtrate by washing the column with 1 per cent acetone in petroleum ether. This fluorescent compound exhibited spectral maxima at 332, 348, and 367 mμ in purified hexane. The position of the spectral maxima, the slight adsorbability, and the typical fluorescence of this compound correspond to the characteristic properties of phytofluene, a colorless C_{40}-polyene first described in detail by Zechmeister and Sandoval (J. Am. Chem. Soc., **68,** 197, 1946) and of common occurrence in higher plants. Nevertheless, certain identification of the fluorescent compound as phytofluene has not yet been made; the purest preparation obtained gave an absorption curve which was distorted by colorless impurities. Estimated as phytofluene, the compound was present in concentrations of about 0.05 mg per 100 g dry weight of bacteria. The aforementioned unsaponifiable impurities absorbed strongly below 320 mμ and precluded a search for more saturated polyenes such as phytoene.

By the use of diphenylamine inhibition it has been possible to detect in *M. phlei* two polyenes which have not been reported previously present in cultures grown under normal conditions. These may represent intermediate compounds in the biosynthesis of carotenoids which, in the untreated bacterial cell, do not accumulate in sufficient quantity for easy detection.