evident in column 4, the weights of the control birds have been gradually increasing. For example, in 1950–51 certain groups of White Leghorn cockerels weighed 330 g at 5 wk of age, whereas in 1953–54 the same breed and strain of birds, on the same ration and in the same batteries, weighed more than 400 g. During the same period, the 4-wk weights of New Hampshire chicks that had antibiotics in their ration increased from 385 g to 406 g. Thus, both control and antibiotic-fed birds increased in weight during the last 4 yr, but the former gained at a much greater rate than the latter. The apparent decreased effectiveness of the growth promotants, therefore, is caused by a relatively greater increase in the rate of growth of birds fed the control ration.

These data, thus, do not furnish support for the theory that the continued use of antimicrobial agents in feeds results in the proliferation of resistant strains of organisms that eventually cause these agents to become ineffective. Rather, the data can be interpreted from an entirely different viewpoint—that is, that their continuous use over a long period produces an environment with a lowered germ load or disease potential. After long-time usage, these growth promotants are still capable of suppressing the germ load, but less of a challenge is presented. Thus, birds that are grown on the premises where birds have or have had antibiotics or arsonic acids in their feeds benefit to some extent from the improvement in environment.

The reduction in the mortality of chicks that we have observed during the last 4 yr adds support to the postulation that a reduced germ load develops. As can be seen in column 5, prior to the inclusion of antibiotics and arsenicals in chick rations, the mortality of battery-raised chicks up to 6 wk of age was about 8.5 percent, whereas the mortality of birds in these same batteries in recent years has declined to less than 3 percent. In addition, fewer cull chicks and less variability in growth rate within pens are now being noted.

From the practical point of view, this study indicates that antibiotics and arsonic acids would be of greatest value in promoting growth (i) when first used in a poultry-feeding enterprise and (ii) under the environmental conditions usually surrounding farm feeding. If once used and then discontinued, feeding performance may still be somewhat improved, because the contamination level has been lowered. Continued use of these substances at low levels, however, would help to insure maximum results, because an "infected" area can be reestablished through disuse (*5*). Indications are that such use of antibiotics and arsonic acids in feeds will not build up a resistant microflora that future poultry populations will have difficulty to live with.

From the point of view of the laboratory that attempts to evaluate antibiotics and arsonic acids for growth promotion, it would be desirable to be able to create at will a controlled level of contamination so that these substances can be tested under conditions more comparable to those in the field. Investigations along these lines are under way.

References and Notes

1. This paper is Journal article 1714, Michigan Agricultural Experiment Station.
2. T. L. Jukes, *Feedstuffs* (11 Sept. 1954) ; D. V. Frost and H. C. Spruth, Symposium on nontherapeutic uses for antibiotics, 124th meeting Am. Chem. Soc., Chicago, Ill. (1953).
3. P. E. Waibel *et al., Poultry Sci.* **33**, 1141 (1954).
4. P. Gyorgy, *Ann. N.Y. Acad. Sci.* **57**, 925 (1954).
5. M. E. Coates, *Chemistry & Industry* 1333 (1953).

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Straight-Line Function of Growth of Microorganisms at Toxic Levels of Carbon Dioxide

Richard D. Durbin

Department of Plant Pathology, University of California, Los Angeles

Although CO_2 is essential for the growth of many heterotropic organisms (*1*), at high concentrations it becomes toxic (*2–4*). Such toxic action may, in part, be upon the CO_2-fixation mechanism itself (*5*).

Studies have been conducted to determine the influence of various concentrations of CO_2 upon the growth of several fungi (*2–4*). It has been found that these data will fit a straight-line dosage-response curve when they are plotted on a log-log scale, examples of which are shown in Fig. 1. Bateman's (*6*) method was employed by plotting the logarithm of the CO_2 concentration versus the logarithm of the

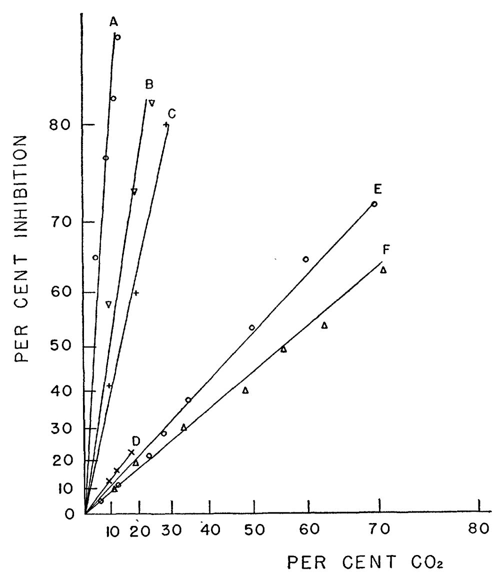

Fig. 1. Inhibition of growth at various levels of CO_2 for (*A*) *Penicillium nigricans*, (*B*) *Alternaria grossulariae*, (*C*) *Cladosporium herbarum*, (*D*) *Ophiobolus graminis*, (*E*) *Fusarium oxysporum*, and (*F*) *Fusarium solani* f. *eumartii.*

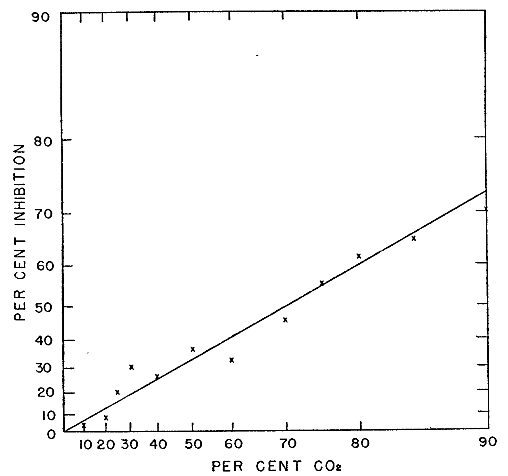

Fig. 2. Inhibition of growth at various levels of CO_2 for *E. coli*.

percentage inhibition of growth at that concentration. The slope of the CO_2-response curve depends on the temperature and the organism's response to CO_2. As the temperature departs from the optimum for growth, the response slope becomes steeper as a result of increased growth inhibition at lower temperatures for any given CO_2 level (*2, 4*). The effect of temperatures higher than the optimum has not been studied.

Experimental studies were carried out at 25°C to determine the amount of growth of *Escherichia coli* under different CO_2 concentrations. Test tubes containing equal amounts of bacteria in nutrient broth were placed in sealed 1-lit flasks containing the desired atmospheric mixture and placed on a shake machine for 24 hr. The oxygen concentration was maintained at 20 percent, except at the 85- and 90-percent CO_2 levels, where the oxygen concentration was 15 and 10 percent, respectively. Growth was expressed as a function of the amount of turbidity produced in the test tubes. The results plotted on a log-log scale are presented in Fig. 2.

The fit of the data, both experimental and from the literature, would seem to justify the validity of this method. The use of this type of representation has several advantages: (i) it makes possible direct comparisons between organisms; (ii) errors in collected data become immediately apparent; (iii) a large number of data may be presented graphically without confusion; and (iv) it makes possible the experimental determination of only a few growth points, with the others derived from the graphic representation. An inherent drawback, however, is that data derived from two organisms grown at the same temperature may not be strictly comparable if the optimum growth temperatures lie far apart.

It can be seen from Fig. 1 that the curves of organisms (*D, E,* and *F*) that are capable of growth in the lower soil depths are more gently sloping than surface or aerial types (*A, B,* and *C*). This means that at any particular CO_2 concentration, their growth is inhibited less than the growth of organisms that are usually confined to upper soil leaves or aerial environments. This tolerance to CO_2 probably plays an important part in their occurrence in a subterranean environment where concentrations of CO_2 normally range from 3 to 10 percent. Their ability to grow at lower soil levels allows them to escape competition with the many microorganisms that exist at or near the soil surface.

References

1. G. P. Gladstone, P. F. Fildes, G. M. Richardson, *Brit. J. Exptl. Pathol.* **16**, 335 (1935); J. W. Hes, *Nature* **141**. 647 (1938); A. Rippel and F. Heilmann, *Arch. Mikrobiol.* **1**, 119 (1930); G. E. Rockwell and J. H. Highberger, *J. Infectious Diseases* **40**, 438 (1927).
2. W. Brown, *Ann. Botany (London)* **36**, 257 (1922).
3. A. Burges and E. Fenton, *Trans. Brit. Mycol. Soc.* **36**, 104 (1953); H. Fellows, *J. Agr. Research* **37**, 349 (1928); N. S. Golding, *J. Dairy Sci.* **23**, 891 (1940); J. P. Hollis, *Phytopathology* **38**, 761 (1948); D. C. Neal and R. E. Wester, *ibid.* **22**, 917 (1932).
4. R. G. Tomkins, *J. Soc. Chem. Ind. (London)* **51**, 261 (1932).
5. J. W. Foster and J. B. Davis, *Arch. Biochem.* **21**, 135 (1949).
6. E. Bateman, *U.S.D.A. Tech. Bull.* *346* (1933).

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Communications

Carbon-14 Dates from Ellsworth Falls, Maine

Through the kindness of H. R. Crane and the Radiocarbon Laboratory, Michigan Memorial-Phoenix Project, the ages of two samples submitted by us have been obtained. These samples were taken from the Smith Farm, Ellsworth Falls, Maine, during a program of excavation covering the summers of 1947–50, inclusive, under the auspices of the Robert Abbe Museum of Bar Harbor, Maine, and the Robert S. Peabody Foundation for Archaeology. They yielded the first radiocarbon dates for the archaic in eastern New England.

Sample M-89 was taken from a pit that appears to equate with middle levels of the deposit including archaic remains of Red Paint character. Above it was a deposit comprising floors in which sherds of Vinette I pottery and later Point Peninsula pottery types were found. Although two pits containing Vinette I sherds were at comparable levels, they could be traced upward to these floors and are not connected in any way with sample M-89. Two runs of this sample produced the following: first run, 4150 ± 450 yr; second run, 3800 ± 400 yr. The average age is therefore 3959 ± 310 yr, or 2005 B.C.