centrates containing "folic acid."[4, 5, 6, 7] Severe leucopenia and granulocytopenia, similarly produced, have been cured promptly by the administration of very small doses of a crystalline folic acid or of vitamin B_c.[8]

The exceedingly low content of "folic acid" in milk[9] suggested that suitable reinforcement of its content of vitamins and minerals and its supplementation with a poorly absorbed sulfonamide should provide a simple diet for the production of "folic acid" deficiency. Accordingly, powdered whole milk (Klim) was fortified as follows (per 100 grams): $FeSO_4 \cdot 7H_2O$, 25 mg; $CuSO_4 \cdot 5H_2O$, 7.8 mg; thiamine chloride, 0.8 mg; riboflavin, 1.6 mg; pyridoxine hydrochloride, 0.8 mg; nicotinic acid, 4.0 mg; calcium pantothenate, 4.4 mg; choline chloride, 100 mg; and a source of vitamins A, D and E, 100 mg [corn oil, 82 mg; A and D concentrate, 14 mg (6300 units A and 1250 units D); alpha-tocopherol, 4 mg]. Assays with *Lactobacillus casei* ε have shown that such a diet contains from 0.7 to 1.8 μg of "folic acid" per 100 gm; typical highly purified diets have contained from 0.5 to 1.4 μg of "folic acid" per 100 gm; assays with *Streptococcus lactis R* have given similar results.

The inclusion of succinylsulfathiazole in the dried milk diet, in amounts as large as 10 per cent., in contrast to the effects produced by levels of only 1 or 2 per cent. in highly purified diets, caused no evidence of nutritional deficiency in rats during a period of 14 weeks following weaning. The growth rate of the animals given the milk-sulfonamide ration was not inferior to that of animals on the milk ration alone, and leucopenia did not develop. At the end of the period of feeding, total leucocyte counts of 5,000 to 22,000 were observed, while on the milk diet alone the counts ranged from 7,000 to 13,000.

Assays for "folic acid" in the tissues of these and other rats showed that considerably larger amounts of microbiologically active material were present in the hepatic tissue of animals fed a whole milk ration than were found in the liver of rats given a highly purified diet "contaminated" with a comparable amount of "folic acid." In each case the addition of succinylsulfathiazole to the diet caused a marked reduction in the "folic acid" content of the liver; however, the reduction was notably greater in the case of animals given the sulfonamide in highly purified diets.

The above observations suggest that one (or more) of the components of milk may be utilized by the rat for growth and other purposes in lieu of material possessing the microbiological activity of "folic acid." Whether the material in milk is structurally related to microbiologically active factors (the various folic acids,[10] vitamin B_c,[11] and the factor of Keresztesy, *et al.*[12]) or whether it is in some manner concerned with the metabolism of "folic acid" is now under investigation.

It is readily apparent that the use of various microorganisms for the assay of foods and other natural materials may fail to measure their total content of various factors having vitamin activity in animals.

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THE HERBICIDAL ACTION OF 2,4 DICHLOROPHENOXYACETIC AND 2,4,5 TRICHLOROPHENOXYACETIC ACID ON BINDWEED[1]

THE use of growth-regulating substances on plants has been directed mostly towards improving their performance in terms of usefulness, such as increasing the set of fruit, preventing the premature dropping of fruit, speeding the rootings of cuttings and developing fruits which are seedless. It is well known, however, that growth substances may be toxic to plants in concentrations greater than those used to secure these desirable responses.

Kraus,[2] and more recently Mitchell and Hamner,[3] have suggested the possibility of growth-regulating substances as selective herbicides, since species and varieties of plants show wide differences in the degree to which they react or respond to the various compounds. Some of the more potent of these compounds are the substituted phenoxy compounds.[4]

[4] Folic acid, as defined (H. K. Mitchell, E. E. Snell and R. J. Williams, *Jour. Am. Chem. Soc.*, 63: 2284, 1941) refers to a factor essential for the growth of *Streptococcus lactis R.* Since there appear to be several entities with activity for that organism, we have used the term folic acid to include factors with microbiological activity for *L. casei* ε, as well as *Strep. lactis R.*

[5] L. D. Wright and A. D. Welch, SCIENCE, 97: 426, 1943.

[6] H. D. West, N. C. Jefferson and R. E. Rivera, *Jour. Nutr.*, 25: 471, 1943.

[7] L. D. Wright and A. D. Welch, *Jour. Nutr.*, 27: 55, 1944.

[8] F. S. Daft and W. H. Sebrell, U. S. Pub. Health Repts., 58: 1542, 1943.

[9] R. J. Williams, V. H. Cheldelin and H. K. Mitchell, The Univ. of Texas Publication No. 4237: 97, 1942.

[10] B. L. Hutchings, E. L. R. Stokstad, N. Bohonos and N. H. Slobodkin, SCIENCE, 99: 371, 1944.

[11] J. J. Pfiffner, S. B. Binkley, E. S. Bloom, R. A. Brown, O. D. Bird, A. D. Emmett, A. G. Hogan and B. L. O'Dell, SCIENCE, 97: 404, 1943.

[12] J. C. Keresztesy, E. L. Rickes and J. L. Stokes, SCIENCE, 97: 465, 1943.

[1] Journal Paper No. 596 of the New York State Agricultural Experiment Station, Cornell University.

[2] E. J. Kraus, correspondence, August, 1941.

[3] J. W. Mitchell and C. L. Hamner, *Bot. Gaz.*, 105: 474–483, 1944.

[4] P. W. Zimmerman and A. E. Hitchcock, Contrib. Boyce-Thompson Institute, 12: 321–343, 1942.

On July 14, 1944, one of these, 2,4 dichlorophenoxyacetic acid at a concentration of 1,000 ppm in water was applied as a spray to two 100-foot rows of apple nursery stock infested with bindweed (*Convolvulus arvensis* L.)—just enough to wet the leaves lightly. Before the chemical was introduced into the spray tank of water it was dissolved in .5 per cent. Carbowax 1500 as described by Mitchell and Hamner.[3] The diurnal temperatures for several days both preceding and following the application were approximately 80 to 85 degrees Fahrenheit by day and 55 to 60 degrees by night. No rain fell for several days either before or after application, and general field conditions were what would be termed "dry."

The sprayed plants showed change within a few hours following application. They appeared wilted. There was a slight upward folding of the leaves along the midrib and they were somewhat stiff to the touch. These symptoms were strongly evident within 24 hours of application, the plants becoming dull green in color and lying flat to the ground. Petals of unopened flowers failed to open and the stamens were arrested in development. No terminal growth of shoots was observed. The plants became progressively more harsh and woody to the touch during succeeding days. By the fifth day following application of the spray, the basal leaves were yellow, and at ten days the above-ground parts were dry and dead.

The etiolated, below-ground parts five days after spraying were spongy, water-soaked and enlarged to twice the diameter of similar parts of unsprayed plants. The outer layers showed longitudinal splitting and sloughing off.

Buds, which typically arise from the underground stems of the plant and which are responsible in large part for the difficulty of its eradication and for its noxiousness as a weed, were checked and failed to develop as shoots. Many small roundish budlike swellings appeared at nodes and rubbed off easily.

Sections of treated and untreated roots and underground stems were placed in a propagation frame in order to study bud development. Shoots arose from underground roots and stems of untreated plants, but not from treated plants. Within five days of placing in the frame, the roots and underground stems from treated plants were entirely dead.

On July 24, a second series of applications prepared in the same way with Carbowax were made to three 600-foot rows at concentrations of 1,000 ppm, 500 ppm and 100 ppm. Day temperatures were 80 to 85 degrees Fahrenheit; a rainy period immediately preceded application. The concentrations of 1,000 ppm and 500 ppm were equally effective and plant response was similar to that from the previous treatment at 1,000 ppm made on July 14. The concentration of 100 ppm also produced a definite response but of reduced intensity. A third set of applications at 1,000 ppm, 500 ppm and 100 ppm, followed within 15 minutes by a rain, were also of reduced intensity.

Miscellaneous applications of 2,4 dichlorophenoxyacetic acid at 1,000 ppm to Canada thistle, dewberry, broad-leaf plantain, dandelion, red raspberry, wild carrot, poison ivy, burdock, milkweed, sorrel and wild lettuce resulted in varying responses, such as severe curvature and chlorosis.

Preliminary observations on treatments with 2,4,5 trichlorophenoxyacetic acid indicate that this material also may be effective as an herbicide. Development of growing points of bindweed were not only arrested but also browned and killed at concentrations of 1,000 ppm, 500 ppm and 100 ppm, prepared with Carbowax 1500.

The method of killing by the use of growth-regulating substances seems of special significance with such a plant as bindweed which is deeply rooted and which regenerates so readily not only from seed but also from shoots arising from underground stems and roots. Not only is the foliage destroyed but also portions of the plant are affected at some distance from the point of application. It is possible that the effectiveness of the materials may be increased by applying them in warm solutions or as aerosols.[5]

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[5] C. L. Hamner, H. A. Schomer and L. D. Goodhue, SCIENCE, 99: 85, January 28, 1944.

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MODIFIED TECHNIC OF CUTTING THE EGG SHELL FOR VIRUS CULTURE

THE culture of virus on the chorio-allantoic membrane of the developing chick embryo according to the method of Woodruff and Goodpasture,[1] as well as the modifications by Burnet[2] and Burnet and Faris,[3] requires that the egg shell be cut. This procedure is accomplished by the use of a small electrically operated rotary drill or the small vibrating cutter,[4] which recently appeared on the market. The latter instrument, which makes 120 vertical strokes per second, has

[1] A. M. Woodruff and E. W. Goodpasture, *Am. Jour. Path.*, 7: 209, 1931.

[2] F. M. Burnet, *Jour. Path. Bact.*, 37: 107, 1933.

[3] F. M. Burnet and D. D. Faris, *Jour. Bact.*, 44: 241, 1942.

[4] Examined through the courtesy of Simeon Trenner.