frequently show two buds (*B*, Fig. 1). The yeasts do not seem to ferment glucose with development of carbon dioxide.

Reimplanted into "sterilized" insects from culture, the yeast cells reestablished themselves normally in the mycetomes. This reinhabitation was achieved by various methods, of which smearing the surface of eggs or feeding sterilized insects with yeast culture proved the most successful. Indeed, yeast from *Lasioderma* settled apparently normally in *Stegobium*, and from *Stegobium* in *Lasioderma*, without altering their characteristic appearance and physiological function. It is evident, from a comparison of the results in Table 1, that with *Lasioderma* the effect of yeast from *Lasioderma* is, in most cases of individual vitamin deficiencies, superior to that of yeasts from *Stegobium*, and the same holds, though to a lesser degree, for yeasts from *Stegobium, mutatis mutandis*. Altogether, it seems that yeasts from *Lasioderma* may contain larger amounts of certain factors, especially thiaman, pyridoxin, pantothenic acid, biotin, and pteroylglutamic acid, than those from *Stegobium*. Inositol is not required by either species, and *Stegobium* is little affected by a deficiency in choline.

It is shown in Table 1 that "sterilized" insects, in the presence of all 9 vitamins, grow about as well as normal insects. In the absence of the pure vitamins, normal growth ensues with the addition of either 2.5% dried brewers' yeast, or 2.5% dried *Lasioderma* yeast from pure culture. This again clearly shows *Lasioderma* yeast to be a good source of all the known vitamins of the B complex.

TABLE 2

GROWTH OF LARVAE OF *Lasioderma* AND *Stegobium* ON THE BASIC DIET (Table 1) IN THE PRESENCE AND ABSENCE OF SYMBIONTS AND WITH AND WITHOUT THE ADDITION OF CHOLESTEROL

| | *Lasioderma* | | | | *Stegobium* | | | |
|---|---|---|---|---|---|---|---|---|
| | With symbionts | | Without symbionts | | With symbionts | | Without symbionts | |
| | No. | Period, days | No. | Period, days | No. | Period, days | No. | Period, days |
| With cholesterol | 15 | 30–46 | 15 | 30–36 | 13 | 38–57 | 14 | 41–62 |
| Without cholesterol | 14 | 33–43 | 7 | 33–41 | 7 | 35–47 | 2 | 50–62 |
| Wheat bran + 5% yeast | 19 | 25–29 | 17 | 25–34 | .. | ..... | .. | ..... |

The yeasts in our two species also serve for their hosts as sources of sterols. It has been shown for many insects, including *Lasioderma* and *Stegobium* (*4*), that a sterol is a necessary constituent of the diet. Omission of cholesterol from the synthetic diet has little effect on the normal larva of *Lasioderma* and some effect on the normal larva of *Stegobium*. In the "sterilized" larvae, however, the sterol-free diet becomes relatively more inferior (Table 2).

A full report of these observations will appear elsewhere.


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## Some New Plant-Growth Inhibitors[1]


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It is intended in this report to describe briefly the inhibitory action on the growth of healthy and tumor tissue of higher plants of certain compounds that have shown promise as chemotherapeutic agents in the treatment of cancer (*2–5*). The growth-inhibiting action of 57 different compounds has been assessed in this laboratory. As test object in the preliminary survey, small (5-mm) fragments of stem tissue of the garden chrysanthemum var. Golden Treasure were employed. These were more uniform in their response than were the carrot fragments used in an earlier study (*1*). The fragments were inoculated with crown-gall bacteria (strain B.P.) and cultured for 3 days *in vitro* in small tubes containing 3 ml sucrose-mineral agar. An aqueous solution of the substance to be tested (conc, 1 mg/ml) was then applied to the surface of the developing tumor. Inhibition of tumor growth generally became evident 3 days later. Seven sulfonamides, 6 antibiotic substances, 9 plant-growth hormones, and 4 purine and pyrimidine derivatives proved inactive. The most active growth inhibitors belonged to the group of analogues of pteroylglutamic acid. Certain nitrogen mustards, 8-azaguanine (guanazolo), and cortisone also exerted an inhibitory action on the growth of the crown-gall tumors.

The action of these compounds on tumor and healthy plant tissue was compared in a series of *in vitro* tests. Various concentrations of the substances to be tested were added to suitable nutrient media in which fragments of bacteria-free crown-gall tumor tissue of sunflower, excised tomato roots, or excised sunflower embryos were cultured aseptically. The tissue fragments were weighed at the beginning and end of the 4-week culture period, and their percentage weight increases were calculated.

The most powerful growth-inhibiting action was possessed by 4-amino-$N^{10}$-methylpteroylglutamic acid, (A-methopterin), 4-amino-9-methyl P.G.A., (A-ninopterin), and 4-amino-9, $N^{10}$-dimethyl P.G.A., (A-denopterin). These compounds completely suppressed the growth of excised tomato roots in a concentration of 1 μg/litre. The growth of sunflower tumor tissue and embryos was only slightly inhibited at this low concentration but was almost completely inhibited by concentrations of 10–100


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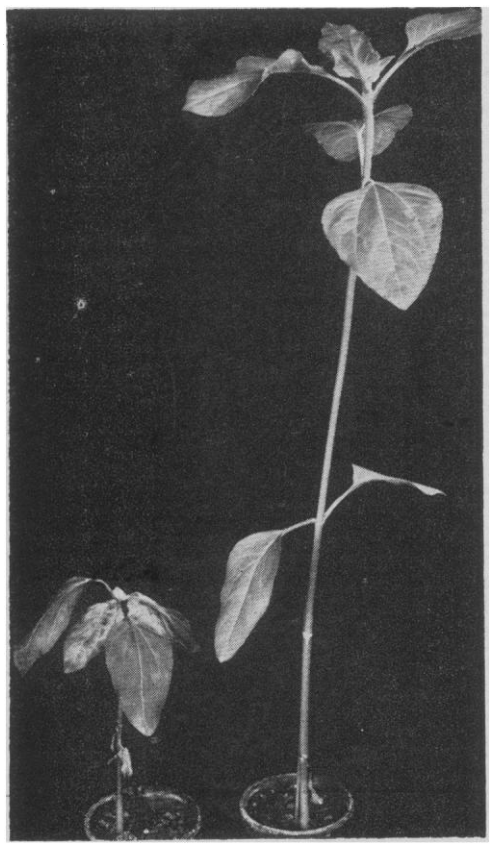

FIG. 1. Sunflower plant on left was sprayed once with a solution of 0.1 mg/ml A-methopterin. It has not died, but its growth has been almost completely inhibited. Plant on right was sprayed with water only.

μg/litre of the active compounds. 4-Amino-P.G.A., (Aminopterin), 4-aminopteroylasparatic acid (Amino-anfol), and 4-amino-α-glutamyl-α-glutamyl glutamic acid (Amino-teropterin) exerted an inhibitory action about one-tenth as powerful as that of the first 3 compounds. No reversal of the inhibition could be obtained by the addition of pteroylglutamic acid to the medium containing A-methopterin. Pteroylglutamic acid itself inhibited the growth of these tissues at concentrations above 10 mg/litre.

A solution of A-methopterin (0.1 mg/ml) applied locally to young crown-gall tumors on sunflower plants completely inhibited growth of the tumors without damaging the plant, provided the tumors were located on mature tissue. Serious damage resulted when this substance was applied at this concentration to tumors on young, growing stems. Sunflower plants sprayed with this concentration of A-methopterin grew at a greatly diminished rate (Fig. 1) but did not die. Onion roots grown in 0.1 mg/litre A-methopterin were devoid of mitoses during the first 24 hr after exposure to this compound. After 48 hr mitoses were again observed. It seemed probable that these compounds acted by interfering with cell division.

The nitrogen mustard, bis-ß-chloroethylmethyl amine, totally inhibited the growth of excised tomato roots exposed for 1 hr to a concentration of 1 mg/litre. Tumor and embryo tissue of sunflower was less affected by this agent. The growth-inhibitory action of guanazolo was detectable at concentrations of 1 mg/litre in all 3 tissues. Cortisone strongly inhibited the growth of bacteria-free crown-gall tissue of sunflower at a concentration of 1 mg/ml. The tissue fragments recovered and grew at their normal rate as soon as the cortisone was removed.

None of the substances tested exerted a specific inhibitory effect on tumor as opposed to healthy tissue. Tissue of excised tomato roots proved more sensitive to the inhibitory action of these agents than did the other tissues tested. It would appear that all these materials, with the possible exception of cortisone, act by interfering with the process of cell division, but that they affect tumor and healthy cells equally.

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## An Antigenically Distinct Subtype of Influenza Virus A Which is Virulent for Mice in Primary Passage of Allantoic Fluid[1]


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Influenza virus, when introduced nasally in mice, following primary growth in the chick embryo, usually produces no clinical disease or histological change. The virus has been shown to multiply in the lungs of these apparently normal mice (*2*, *6*). After several passages in mice, they are killed by lung suspensions containing virus in high dilution.

Kalter (*3*) recovered one strain of influenza virus in 1947 which was strikingly different from another influenza virus isolated at the same time, in that it was invariably fatal for white mice in 3–5 days. The lungs showed complete consolidation, typical of influenza virus infection. There was no explanation for this behavior, but Kalter stated that the possibility of a toxic substance had not been eliminated.

Payzin and Okkan (*5*) recovered 3 influenza virus strains directly from cases in mice which occurred during an epidemic in Ankara in 1949. The strains attained high mouse virulence after 5 serial passages in mice. The


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