period of environmental contamination. The data further suggest that the amount of calcium involved decreases with age, which is in keeping qualitatively with classic concepts of bone physiology. If a similar fraction of the skeletal calcium of growing subjects is involved in exchange plus remodeling, then the strontium-90 levels in children would be proportionally higher than the curve based on skeletal calcium accretion alone. This indeed appears to be the case and indicates that the major factors have been considered in constructing the model.

If the fraction of remodeling and exchange for children's bones is similar to that observed for adults, then the accretion curve below age 20 should be raised proportionately, to give the over-all apparent fraction of equilibrium strontium-90/calcium ratio as a function of age (dashed line, Fig. 1). This curve permits the use of adequate bone data from any age group to predict the average maximum equilibrium strontium-90 bone level and indicates an average maximum equilibrium level of 0.9 μμc/g of calcium at the end of 1955.

The strontium-90 content of skeletons of stillborns (*2*) during 1955 averaged about 0.5 μμc/g of calcium, which gives an average maximum equilibrium level of 1.0 when the placental discrimination factor of 0.5 (*6*) is considered. Bryant *et al.* (*7*) in England reported analyses of 28 bone samples from subjects of all ages collected about January 1956. Eight samples from persons ranging from 3 months to 3½ years old (average 1⅓ years) averaged 0.9 μμc of strontium-90 per gram of calcium, and 11 subjects ranging from 20 to 65 years of age (average 36 years) averaged 0.07 μμc of strontium-90 per gram of calcium, after all rib results had been divided by 2. The predicted average maximum strontium-90 equilibrium level about January 1956, based on these age groups, is 1.0 and 0.9 μμc/g of calcium, respectively.

Since the strontium-90 environmental contamination level continued to rise during 1956, the predicted average maximum strontium-90 equilibrium level of new bone as of the first part of 1957 is about 1.8 μμc/g of calcium. The frequency distribution patterns have been reported for strontium-90 (*8*), stable strontium (*5*), natural radium (*9*), and cesium-137 (*10*). All these nuclides show essentially normal distributions with standard deviations of about 35 percent, which suggests that the range ($\pm 3\sigma$) of strontium-90 equilibrium levels as of the first part of 1957 for the world population will be between about 0.3 and 4 μμc/g of calcium.

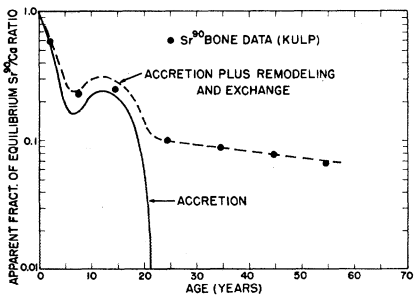

Fig. 1. Apparent fraction of equilibrium strontium-90/calcium ratio as a function of age.

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27 May 1957

Chemically Induced Cell Proliferation and Its Inhibition by a Naturally Occurring Antiauxin

There are several types of cellular abnormality which can be induced in plants by a properly chosen kind or concentration of chemicals, including animal carcinogens. Among these are (i) proliferation of mature cells, as when callus tissue forms from pith or cortical cells after wounding (Fig. 1); (ii) unorganized division of cells in meristematic regions, as with 2,4-dichlorophenoxyacetic acid (*1*, Figs. 4–7); (iii) hypertrophy of cells in certain areas; (iv) inhibition of apical dominance (Fig. 2); and (v) cell elongation, producing epinasty (*2*, Figs 1–3). Chemical stimulation can also produce hyponasty, as with maleic hydrazide and taller plants with gibberellic acid (*3*). These various types of cell abnormality and the resulting morphologic irregularities can be inhibited by the use of the naturally occurring antiauxin previously reported (*1, 2, 4*).

The callus type of proliferation was used as the assay technique in the present study (*5*). Callus forms by rapid division of mature pith and cortical cells. A slit about ¾ inch long was made in the third to fourth internodes from the tips of cocklebur plants (*Xanthium* sp. native to western Wisconsin) having several mature leaves. Moist spaghnum moss was maintained about the slit stems to provide a humid environment favorable for callus development.

The poor solubility of antiauxin in solvents suitable for application to plants led to the practice of placing crystals in the slits when its effect on callus production was being tested. Presumably, antiauxin is soluble in the lipoidal phase of the plant and therefore would be transported through this medium.

This practice of implanting crystals was continued when chemicals were tested for their tumor-producing potentiality. Approximately 0.5 mg or less of the crystals was used, and, in the case of liquids, one drop.

A record of the presence and extent of formation of callus was made at 5 to 7 days after treatment. The tumorous tissue deteriorates soon after this time because it is isolated from the plant by the rapidly forming wound periderm. It is the formation of a "cork cambium" under a wound that produces the short life of callus tissue, rather than any inability of the proliferating tissue to continue to regenerate (as in neoplasia of animal tissues).

More callus tissue than normal was formed when the following chemicals were applied to the plants (Fig. 1): acetates of ammonia, barium, calcium, copper, iron, lead, magnesium, mercury, potassium, silver, sodium and zinc; 1-percent acetic acid; acetone; adenine sulfate; adipic acid; androstenediol; androstenedione; arginine monohydrochloride; benzanthracene; benzene (axillary bud injury and callus formation in the leaf axis); 1,2-benzopyrene; 3,4,-benzopyrene; beryllium; beta alanine; betaine hydrochloride; carboxymethylcellulose; cephalin; chloromelamine; chloropromazine hydrochloride; cholesterol; cholic acid; choline; copper tartrate; croton oil; cyclopentanone; deoxycholic acid; dibenzanthracine; diethylstilbestrol; 4,4-dihydroxydiphenyl; diosgenin acetate; disodium malonic acid; estradiol; ethyl carbamate; ethylenedinitrilotetraacetic acid; folic acid; guanine hydrochloride; hecogenin; hexamethylenediamine; histamine; histidine dihydrochloride; hydrocortisone acetate; 3-hydroxy-2-butanone; 5-hydroxyindoleacetic acid; 8-hydroxyquinoline; iron tartrate; isopropyl maleimide; kynurenine; 1-kynurenine sulfate; malic acid; melamine; methylcholanthrene; morpholine; naphthyl maleimide; oleic acid; oxapentamethylenediethylenethiophosphoramide (OPSPA); pentadecylcatochol; quinal-

dic acid; sarsasapogenin; serotonin creatinine sulfate; serotonin hydrochloride; sesame oil; smilagenin and its acetate; sodium oleate; sodium tartrate; sodium trichloroacetic acid; stigmasterol acetate; tartaric acid; testosterone; testosterone propionate; thioacetamide; thiourea; tobacco tar extract "C"; trichloroacetic acid; trimethanolamine; triethylenemelamine; uracil; uric acid; vinyl cyclohexane; and xanthine.

It should be assumed that more extensive tests with different dosages of the chemicals on trial would result in reclassifications of the activity of some of them.

Antiauxin inhibited the stimulatory effect of nearly all of the afore-mentioned materials. There was limited inhibition in the cases of adipic, deoxycholic, folic, malic, and oleic acids.

Following are chemicals which were found neither to increase nor to reduce callus tissue formation beyond the normal at the dosages used and under the experimental conditions of the trials: acetylaminofluorene; acetylcholine chloride; *n*-acetyl-1-kynurenine; acetylpodophyllotoxin pyridinium chloride; adenosine triphosphate; *o*-aminohippuric acid; ammonium carbonate, nitrate, oxalate, phosphate, and tartrate; androsterone; anthracene; anthranilic acid; antimycin A; antistine; ascorbic acid; azaguanine; benzoylcarbinol; caffeine; calciferol; 3-chloroisopropyl carbamate; chrysene; citric acid; corn oil; cortisone acetate; coumarin; deoxycortisone and its acetate; diamino purine; digitonin; dimethylazoaniline; dimethylnaphthylazoaniline; diosgenin benzoate; disodium malonic acid; ethyl acetate; histone; hydrocortisone; 3-hydroxy-*dl*-kynurenine; 8-hydroxyquinaldic acid; 5-hydroxytryptamine; kynurenic acid; lecithin; 6-mercaptopurine; *dl*-methionine; *n*-methyl-2-pyridone-5-carboxamide; mineral oil; myristil choline chloride; naphthyl acetamide; niacin; nicotine; nicotinic acid; nortestosterone; olive oil; phenanthrene; phenothiazine; phytol; piperazine estrone sulfate; podophyllin; podophyllic acid sodium salt; polyvinylpyrrolidine; Reichstein's substance "S" and its acetate; riboflavin; saponin; *dl*-serine; sitosterol; sphingomyelin; stearone; succinic dehydrogenase; thiamine hydrochloride; thiobromine; thiophyllin; thymidine; tolylazotoluidine; triphenylmethane; l-tryptophan; urea; and xanthuronic acid-8-methyl ether.

Free cortisone inhibited callus formation in a manner similar to that resulting from implanting antiauxin. Cortisone has a keto-alcohol structure. Antiauxin is also a keto-alcohol as indicated by chemical (*6*) and infrared (*7*) analyses.

A peculiar side effect was obtained with cortisone. Areas in the stem above the point of its application showed hypertrophy and accompanying necrosis of the cells. This condition was also induced by the implanting of testosterone, which is in effect the cortisone molecule minus the keto-alcohol structure.

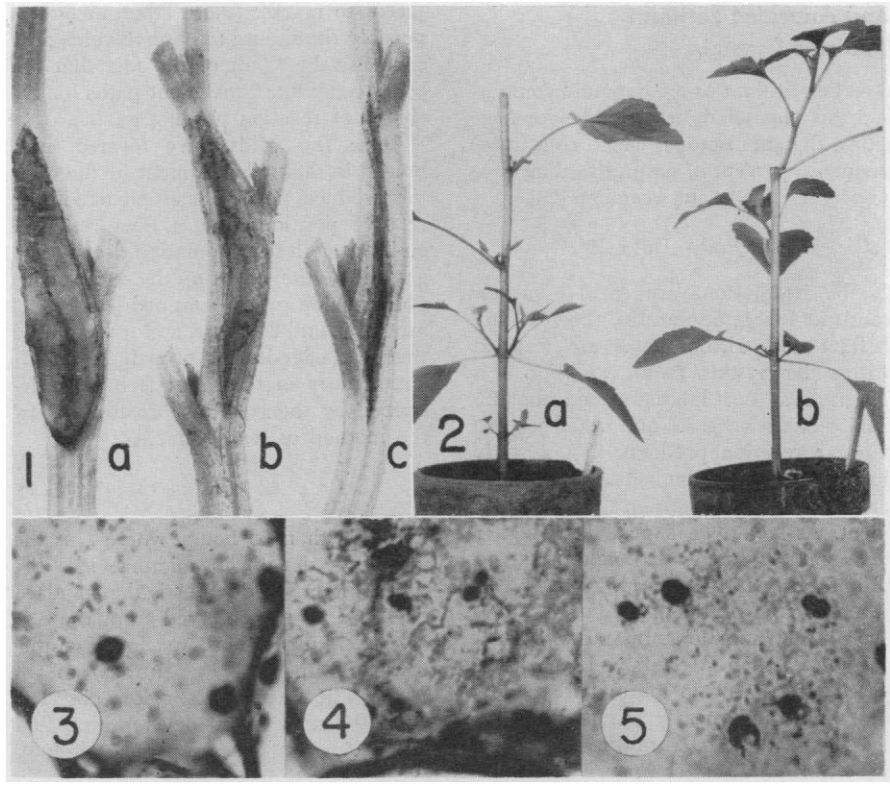

Fig. 1. Typical "callus" formation in slit stems of *Xanthium*; (*a*) large tumor 5 days after implantation of magnesium acetate; (*b*) small tumor growth in untreated slit; (*c*) no tumor was formed when antiauxin was applied. Fig. 2. Decapitated *Xanthium* plants; (*a*) inhibition of upper shoots by maleic hydrazide, 50 ppm; (*b*) same as *a* but also treated with antiauxin to neutralize maleic hydrazide effect, giving normal apical dominance. Figs. 3–5. Mitochondria in pith cells of stems of cocklebur. Fig. 3. Spherical or globular mitochondria in untreated plant. Fig. 4. Rod-shaped or filamentous forms of mitochondria in tissue treated with tumor-inducing substance. Fig. 5. Normal appearance of mitochondria when antiauxin was applied together with the stimulating agent.

The chemicals which stimulated tumor tissue development beyond that present in untreated plants are predominately fat soluble. Antiauxin, which inhibits proliferation, is also fat soluble. This suggests that callus or tumor growth is associated with a disturbed lipoidal metabolism. The mechanism involved would logically be, at least in part, cell or membrane permeability. Several chemicals, such as choline, caused bleeding from the implanted slits. This bleeding did not occur if antiauxin was also added.

The mitochondria in the pith cells had a different appearance in plants which had been chemically treated to produce cell proliferation. On the other hand, they appeared normal in plants that were kept from proliferating by the use of the antiauxin hormone, applied with the chemical (*4*, *8*) (Figs. 3–5).

The fact that the tissue is predominantly normal suggests the presence of a physiological mechanism having this function. It may be that antiauxin belongs to such a system. At least, it appears to be an aid to any natural host defense mechanism since it inhibits several types of chemically induced cell abnormality and the accompanying abnormal morphology. In addition, it has a role in producing maturation of erratically dividing plant tissue (*1*).

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5. This article is published with permission of the director of the Agricultural Experiment Station. This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.
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16 May 1957