materials by 55 ml. of water, and the compounds were usually removed in about the same amounts. The relatively soluble $NH_4$2,4-D was not removed in greater quantities than 2,4-D, which is of much lower solubility. It seems probable that in a soil 2,4-D or a relatively insoluble 2,4-D salt might be converted to soluble forms by ammonium, sodium, or other ions present in the soil solution.

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Granulosa Cell Tumors in Intrapancreatic Ovarian Grafts in Castrated Mice[1]

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Biskind and Biskind (*1*) reported that granulosa cell tumors have developed in ovaries transplanted into spleens of three castrated female rats. Our previous experiments showed the formation of granulosa cell tumors and luteomas in intrasplenic ovarian grafts in castrated male and female mice (*3*). These studies were based on two principles: (1) the capability of the liver to inactivate ovarian hormones when the hormones circulate through the hepatic portal system, and (2) the increase of pituitary gonadotropins subsequent to castration. It was assumed that the prolonged stimulation, by augmented amounts of gonadotropic hormones, of intrasplenic ovarian grafts was responsible for the neoplastic growths. More recent investigations (*2*) revealed that the development of ovarian tumors in intrasplenic ovarian grafts was inhibited by administration of estradiol benzoate and testosterone propionate. The malignancy of the induced granulosa cell tumors was indicated by the ability to metastasize and to transplant in new hosts. The present experiment, using intrapancreatic ovarian transplantation in castrated mice, demonstrates that splenic tissues do not play a direct role in the pathogenesis of ovarian tumors arising in the grafts.

Male and female mice of A, C_3H, CBA, and C_{57} strains and hybrid mice were used. These were castrated and received, at the same time, an autoplastic or homoplastic ovarian graft in the pancreas. Among the first group of 5 experimental animals, two granulosa cell tumors and one pretumorous growth were found 168 days after grafting. No tumor was noted in two grafts with vascularized adhesions that permitted drainage through other than the hepatic portal system. One tumorous graft in a male mouse (C_3H strain) was 7 x 8 x 10 mm. in diameter; the other, which developed in a female hybrid mouse (AC_3), measured 10 x 11 x 13 mm. in diameter. The uterus of the latter animal weighed 75 mg. at autopsy. The pretumorous graft occurred in a male mouse of the A strain.

Microscopically, the granulosa tumor cells were arranged in a folliculoid pattern showing numerous mitotic figures. Some of the folliculoid structures contained hemorrhagic cavities. Luteinized cells and small necrotic areas were present, and a spicule of bone was observed at the periphery of one tumorous ovarian graft. Major portions of the tumors were separated from the pancreatic tissue by bursa-like spaces lined by germinal epithelium. No metastasis was observed in the liver. The pretumorous graft showed masses of tubular ingrowths from the germinal epithelium, and the transformation of some of the epithelial cells into granulosa tumor cells was noted. Thus, the morphology of granulosa cell tumors induced in the pancreatic site resembled that of the tumors developed in intrasplenic ovarian grafts. The present experiments are interpreted to substantiate further the assumption that overaction of gonadotropic hormones is responsible for the development of the ovarian tumors in mice.

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Differential Phytotoxicity of Metabolic By-Products of *Helminthosporium victoriae*[1]

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The "*Helminthosporium* blight" of oats caused by *H. victoriae* Meehan and Murphy has developed so rapidly that it has attained the proportions of a major plant disease within two years after its discovery. Susceptibility is apparently limited to oat varieties and selections that possess the "Victoria-type" resistance to crown rust (*Puccinia coronata avenae* (Corda) Eriks. & E. Henn.). The unusually fast build-up of the disease has been facilitated by the widespread planting of large acreages to susceptible varieties.

The means by which *H. victoriae* causes necrosis has been the subject of some speculation. In a previous article (*2*) the suggestion was made that the pathogenic action of this fungus involves the production of a toxic substance. Inoculation tests with sterilized mycelium and filtered extracts from cultures have given evidence that a very potent toxin is secreted by the fungus, which is responsible for the characteristic longitudinal foliar striping or discoloration. Data regarding its production and effects are briefly summarized in this paper.

Evidently the basal infection of the oat plant is the only direct manifestation of parasitic action by *H. victoriae*, since the organism has not been isolated from the blighted leaves until after complete necrosis of the tissue. It may be that this

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fungus is too weak a parasite to establish infection in healthy tissue even of susceptible oat varieties without the help of toxic secretion in advance. Otherwise, it would be expected that a fungus that grows vigorously in culture would progress rapidly in the plant from the basal portions to the leaves.

Preliminary tests showed that the toxic substance or substances were readily formed in cultures grown on media containing either organic or inorganic nitrogen. In a typical experiment, cultures of *H. victoriae* were grown for 30 days at room temperature (24–28° C.) in flasks, each containing 100 ml. of Richard's solution, filtered through a Büchner funnel to remove the hyphal mass, and the filtrate then passed twice through a Berkefeld filter to render it aseptic. Bocne and Clinton oat seedlings were grown for one week in nutrient

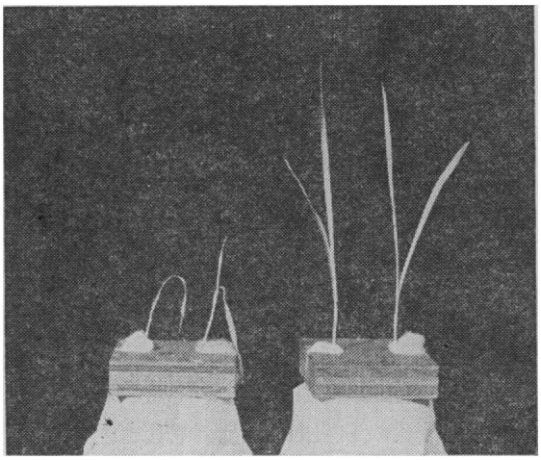

FIG. 1. Oat plants of varieties Boone (left) and Clinton (right) grown in nutrient solution, 90 hours after toxic extract, 1 part in 45 parts of water, had been added.

water culture, after which time the nutrient solution was replaced by the filtrate in a series of dilutions ranging from 1:15 to 1:1,800 in tap water. Observations made at 4-hour intervals showed the following reactions in the susceptible Boone variety: at dilutions of 1:90 or less the leaf blades became rigid and inflexible within 40 hours, and after 48 hours these leaves showed a slight twisting. A more critical indication of phytotoxicity was obtained at dilutions of 1:45 or less: the healthy green color of normal leaves changed to a dull grayish-brown after 52 hours. This color change preceded the death and drying of the leaves. The seedlings of the resistant variety, Clinton, were unaffected by the filtrate in these dilutions. Since it was found that Richard's solution alone, minus the amount of sugar equivalent to that used by the fungus in growth, was harmless to the susceptible plants, it may be concluded that a substance was formed as a metabolic by-product of the growth of *H. victoriae* that was toxic to the susceptible variety of oats. The color reaction is considered most reliable for bioassay technique, since it was obtained consistently at the same dilution range in a series of tests.

The toxin occurs in the cells of *H. victoriae* as well as in the nutrient medium, as shown by the fact that leaves of susceptible oat varieties were killed when sprayed with a water suspension of sterilized blended mycelium containing no culture substrate.

The toxic principle in the culture extract is relatively stable, as it was not destroyed by autoclaving for 20 minutes at 15 pounds pressure. Lee's work (*1*) with *H. sacchari* (Breda de Haan) Butler showed this fungus to have a strong capacity for reducing inorganic nitrates to nitrites which were assumed to be responsible for the toxicity of this organism to sugar-cane leaves. The production of toxin by *H. victoriae* on media containing only organic sources of nitrogen is evidence that nitrite formation is not the cause of this toxic action.

Some tapering-spored species of *Helminthosporium* have been found to produce characteristic intracellular chemical compounds (*3*) of the polyhydroxyxanthone series, such as *ravenelin* (3 methyl-1,4,8-trihydroxyxanthone) in *H. ravenelii* Curt. Further studies will be required to determine whether comparable materials are present in the toxic solutions from *H. victoriae*.

H. victoriae is primarily a facultative soil- and seed-borne saprophyte that possesses a low order of phytopathogenicity. It causes severe leaf blight in addition to basal stem and root necrosis without invading the plants extensively. The limited progress it does make may depend largely upon direct injury to plant tissues by the toxin. Varieties of oats such as Clinton, that are highly resistant to, or immune from, attack by the fungus itself, are likewise not injured by the toxic secretions. The extreme susceptibility to the fungus exhibited by the Victoria derivatives might be reasonably expected, since the resistance shown by these varieties to crown rust is dependent upon a hypersensitive reaction to the rust fungus, an obligate parasite.

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Application of "Metabolite Antagonism" to Cancer Research

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The concept that a compound structurally related to an essential metabolite may interfere with the function of that metabolite has been attracting widespread attention (*5*). The initial stimulus in establishing the "metabolite antagonist" concept has come from the work of Wood and Fildes (*6*), who demonstrated the antagonistic effect of p-aminobenzoic acid on the action of sulfanilamide. Subsequent investigations from other laboratories (see Roblin, *5*) have attempted to find synthetic substances which, as in the case of sulfanilamide, would be incapable of duplicating the physiological action of the metabolite, but would possess great affinity for the same enzyme system and/or other cell constituent with which the metabolite reacts. These attempts have been successful in some instances. In view of the more rapid growth of tumor tissue as compared with normal tissue, it seems plausible to us that it may be possible to interfere with the growth of the malignant tissue to a greater degree than with normal tissue by making use of an appropriate