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

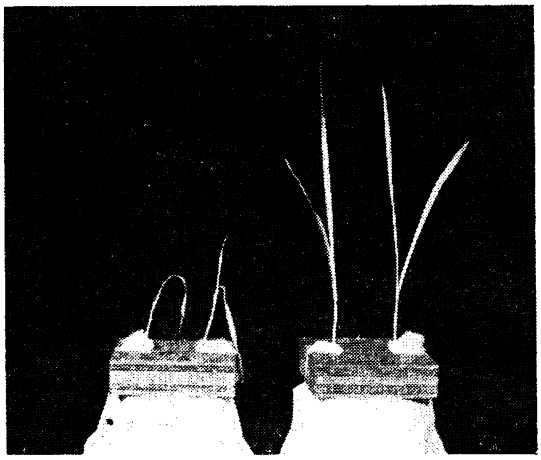

FIG. 1. Oat plants of varieties Boone (left) and Clinton (right) grown in nutrient solution, 90 hours after toxic extract, 1 part in 45 parts of water, had been added.

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.

References

1. LEE, ATHERTON. *Plant Physiol.*, 1929, **4**, 193–212.
2. MEEHAN, FRANCES, and MURPHY, H. C. *Science*, 1946, **104**, 413–414.
3. RAISTRICK, H., ROBINSON, R., and WHITE, D. E. *Biochem. J.*, 1936, **30**, 1305–1314.

Application of "Metabolite Antagonism" to Cancer Research

DAVID M. GREENBERG and MARTIN P. SCHULMAN

Division of Biochemistry, University of California Medical School, Berkeley

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

metabolite antagonist. Thus, regression of a tumor might be affected. Burk and Winzler, in a recent review article (*2*), have proposed a somewhat similar approach to cancer studies, utilizing competitive vitamers to produce differential vitamin deficiencies in growing and nongrowing tissues.

Since amino acids serve as building blocks in the formation of proteins of normal as well as neoplastic tissue, and further, since proteins are intimately concerned with the functions of protoplasm, our attention was first focused on potential amino acid antagonists (see Fig. 1). It is the purpose of this

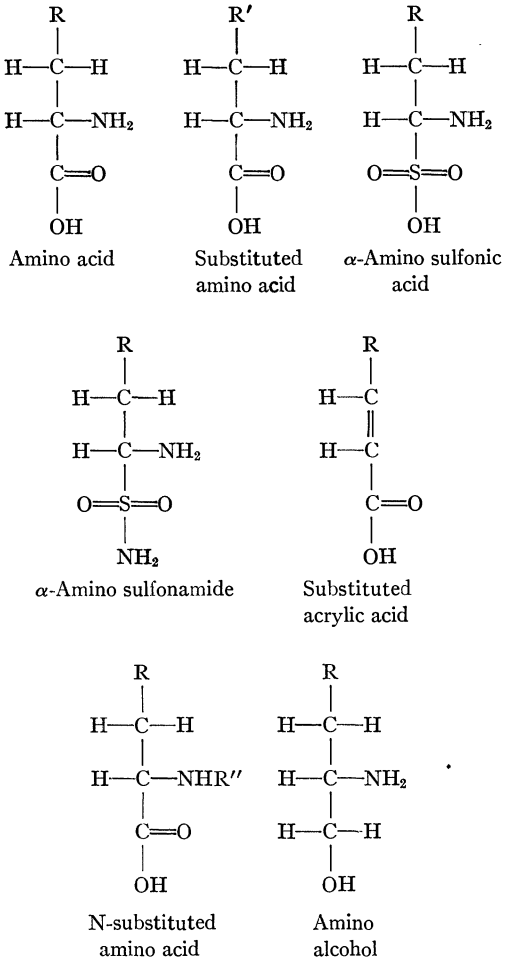

FIG. 1. Potential metabolite antagonists of amino acids.

report to furnish a brief account of preliminary experiments concerning the effects of several amino acid analogues on tumor growth. Future work will deal with other types of potential metabolite antagonists and their application to metabolic studies in normal and cancerous tissues.

Because of the relative ease of preparation of α-amino-sulfonic acids as contrasted with other potential antagonists, these were used exclusively in this study. Aminomethane sulfonic acid, the compound resembling glycine, was prepared according to the directions of Raschig and Prahl (*4*); α-amino-ethane sulfonic acid, the sulfonic acid analogue of alanine, according to Backer and Mulder (*1*); α-aminoisobutane and α-aminoisopentane sulfonic acids, analogues of valine and leucine, respectively, according to McIlwain (*3*); and α-amino, β-phenylethane sulfonic acid, the analogue of phenylalanine, was synthesized in this laboratory. For simplicity, the sulfonic acid corresponding to the amino acid will be referred to as the S-amino acid. Thus, α-amino,β-phenylethane sulfonic acid is S-phenylalanine, while α-aminoisobutane sulfonic acid will be called S-valine.

Highly inbred "A" mice of both sexes were used. The diet of the animals consisted of Purina Laboratory Chow plus twice-weekly supplements of lettuce. The tumor tissue, Sarcoma A274,[1] was inoculated subcutaneously into the right groin by means of a trochar. Eight days after implantation, the animals were matched according to tumor size. Then, 36 animals with equal-sized tumors (about 1 cm.) were divided into 6 experimental groups of 6 animals each. One of these groups was a control series for all groups, and these 6 mice received no therapy. Each of the remaining 5 groups consisted of 4 test and 2 control animals, the former receiving daily intravenous injections of the S-amino acid while the latter were injected with the equivalent weight of the corresponding dl-amino acid. The groups were: (I) S-glycine, (II) S-alanine, (III) S-phenylalanine, (IV) S-leucine, and (V) S-valine. In all cases the compounds were administered in a total volume of 0.2 ml., the pH of each solution previously being adjusted to 7.4 with 0.2 N sodium hydroxide. Preliminary trials for toxicity level were carried out on other animals, and in all cases daily intravenous doses several times those employed here were well tolerated and no toxic symptoms were observed. After one week, intravenous administration was abandoned, and subcutaneous injections (0.2 ml.), twice daily, were employed. The injections were made at a site far removed from the region of the tumor implantation, namely, into the midscapular region.

The final growth of the sarcoma was not affected appreciably by the administration of the S-amino acid or even by the corresponding dl-amino acid. The experiment, which was terminated 19 days after tumor implantation because several test and control animals succumbed, is being repeated with variations of the experimental conditions and with additional analogues.

References

1. BACKER, H. J., and MULDER, H. *Rec. trav. chim.*, 1934, **53**, 1120.
2. BURK, D., and WINZLER, R. J. *Vitamins and hormones.* (Vol. 2.) New York: Academic Press, 1944. P. 305.
3. MCILWAIN, H. *J. chem. Soc.*, 1941, 75.
4. RASCHIG, F., and PRAHL, W. *Annalen*, 1926, **448**, 265.
5. ROBLIN, R. O. *Chem. Rev.*, 1946, **38**, 255; WELCH, A. D. *Physiol. Rev.*, 1945, **25**, 687; WOOLLEY, D. W. *Advanced enzymology.* (Vol. 6.) New York: Interscience, 1946. P. 129.
6. WOODS, D. D., and FILDES, P. *J. Soc. chem. Ind.*, 1940, **59**, 133; WOODS, D. D. *Brit. J. exp. Path.*, 1940, **21**, 74.

[1] This sarcoma originated at the Wistar Institute. It is a vigorous tissue, palpable three days after implantation and causing death in about three weeks. Sarcoma A274 has been maintained in "A" mice for many generations, 146 transplants having been made since the tumor first appeared. Spontaneous regressions have not been observed to date.