Effect of Acetic Acid Hydrazide on Mammary Carcinoma 755 in C57 Black Mice.* (21234)

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Hydrazine derivatives are of theoretical interest in the chemotherapy of experimental tumors. As early as 1876 Fischer(1) noted the ease with which hydrazine compounds conjugate with carbonyl groups. These derivatives may conceivably upset biological oxidation systems important for the promotion of tumor growth, such as the citric acid cycle. Sixty-six hydrazine derivatives were previously reported by the authors as having no therapeutic effect on mouse leukemia(2). In the present experiments mammary carcinoma 755 was selected for study. This tumor is sensitive to the vit. B_6 antagonist, 8-azaguanine [5-amino-7-hydroxy-1H-v-triazolo(d)-pyrimidine](3). The inhibitory effect of acetic acid hydrazide [$CH_3CONHNH_2$] on this tumor is reported below.

Materials and methods. The drug was prepared in this laboratory. C-57 black mice averaging 20 g in weight were implanted subcutaneously with mammary adenocarcinoma 755. Therapy was started 24 hours later. Dosage was the maximum tolerated with slight weight loss. Acetic acid hydrazide was dissolved in water and thoroughly mixed with ground Purina Laboratory Chow, to make a final concentration of 0.15%. Each experiment of 7 to 20 mice was controlled by an equal number of animals which were given the same diet without the drug. After 11-14 days the surface area of the tumors was calculated from measurements of two diameters. The tumors and organs were examined for gross pathological and histological changes. All animals were weighed at the start and end of the experiment. As a positive control, 8-azaguanine,‡ 40 mg/kg was given daily intraperitoneally.

TABLE I. Inhibition of Growth of Mouse Tumor 755 by Acetic Acid Hydrazide.

Compound	Surface area tumor relative to controls, %	Ratio wt gain, treat/controls	Ratio living, treat/controls
Acetic hydrazide	28	−1.7/+1.0	7/ 7
	27	−2.7/+2.2	7/ 7
	18	−2.0/+2.2	9/ 9
	15	−2.5/+1.3	19/20
8-Azaguanine	8	− .9/+3.8	7/ 8
	17	−1.7/+ .9	6/ 7

Results. The results are summarized in Table I. The surface area of the treated tumors was approximately 20% of the untreated control tumors. Inhibition produced by 8-azaguanine was more pronounced. No permanent effect was obtained; a week after the end of therapy the tumors had resumed growth. The mice showed approximately 10% weight loss; however only one animal died out of 45 treated. It is doubtful that this moderate amount of debilitation was the main cause of the tumor inhibition. The spleens and thymus glands were small compared to the tumor controls. The average weight of the spleen of the treated mice was 77 mg and the thymus 58 mg, which were 57% and 64% respectively, of the same organs in the untreated controls. Antopol, Glaubach, and Graff(4) have recently observed the spleens of mice inoculated with tumor 755 to be enlarged. Acetic hydrazide in the present experiments prevented this splenomegaly. Histological examination showed atrophic changes in the spleen and thymus. Otherwise no abnormal pathological or histological changes were observed in the tumors or organs of the treated animals. The absence of histological

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changes in the treated tumors suggests that the mode of action of acetic hydrazide on tumor inhibition is not a direct toxic action on the tumor cells. Three mechanisms of action have been considered. The hydrazide portion of the molecule may combine with a carbonyl group and remove an essential compound from the citric acid cycle, or the acetyl moiety can manifest itself through interference with active acetate formation. Busch(5) in experiments on the metabolism of acetate-1-C^{14} in tissues of tumor-bearing rats showed that utilization of the acetate molecule in the tumors was markedly diminished. Acetic hydrazide may further depress this utilization of acetate. In addition acetic hydrazide might, conceivably, interfere with the activity of some amidases.

Summary. Acetic acid hydrazide caused moderate growth inhibition of transplanted mammary carcinoma 755. Possible modes of action are discussed.

1. Fischer, E., *Ber.*, 1876, v9, 880.
2. Freedlander, B. L., and Furst, A., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 638.
3. Gellhorn, A., Engleman, M., Schapiro, D. M., Graff, S., and Gillespie, H., *Cancer Research,* 1950, v10, 170.
4. Antopol, W., Glaubach, S., and Graff, S., *Proc. Am. Assn. for Cancer Research,* 1954, v1, 2.
5. Busch, H., *Cancer Research,* 1953, v13, 789.

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Fluorescent Antibody Studies with Agents of Varicella and Herpes Zoster Propagated *in vitro*.* (21235)

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Investigation of varicella and herpes zoster viruses in the laboratory has been hampered by the lack of a susceptible experimental animal. An alternative approach was indicated by the observation(1) that specific cytopathic changes followed inoculation of roller tube cultures of human tissues with vesicle fluid materials from cases of varicella or herpes zoster. Serial propagation *in vitro* of agents apparently derived from these cases was accomplished, as demonstrated by the regular appearance in subcultures of cytopathic changes that were focal in nature and associated with the presence of intranuclear inclusion bodies. A peculiarity of the agents in the culture system employed was the apparent failure of infectious material to appear in the fluid phase; therefore, tissue suspensions from infected cultures were utilized as inocula for passage *in vitro*. Initially this peculiarity posed technical problems in obtaining immunologic evidence of their identity. The fluorescent antibody technic developed by Coons and coworkers(2,3) was then applied in an effort to clarify the immunologic relationship of the agents under investigation.

This technic was first applied to problems dealing with the growth of viruses *in vitro* by Watson(4) in a study of mumps virus. She employed fluorescein-conjugated antimumps serum, and in general heretofore conjugates have been prepared with each type of antibody under investigation. Recently, one of us (AHC) prepared a fluorescein conjugate with antihuman gamma globulin rabbit serum for the purpose of detecting any specific antibody of human origin after combination with

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