Retardation of Growth of Implants of Carcinoma 755 in Cortisone-Injected Mice. (21099)

WILLIAM ANTOPOL, SUSI GLAUBACH, AND SAMUEL GRAFF.

From the Joseph and Helen Yeamans Levy Foundation, Beth Israel Hospital, and Francis Delafield Memorial Hospital, College of Physicians and Surgeons, Columbia University, New York City.

Heilman and Kendall(1) reported that 17-hydrocorticosterone prevented the growth of implanted undifferentiated lymphosarcoma in mice. Earlier, Murphy and Sturm(2,3) noted a similar adrenocortical control of lymphoid tumors. Wooley(4) reported that cortisone reduced the rate of growth of lymphoid tumors when given orally, and reduced less when administered subcutaneously. Gottschalk and Grollman(5) reported the inhibition of a transplanted mouse mammary carcinoma by large doses of cortisone, and Baserga and Shubik(6) showed that the growth rate of a transplanted mouse adenocarcinoma was temporarily inhibited by the administration of cortisone. It was of interest to investigate the effect of cortisone* on growth of a transplantable epithelial tumor, carcinoma 755 in C57 mice and to study the mechanism of its action.

Method. Approximately 200 mice, 4-5 months of age, of the C57 strain inbred for many generations were used in these experiments. Animals in a single experiment received implants in the axilla, by trocar, of approximately equivalent fragments of carcinoma 755 from a single, fresh, dissected tumor free of necrosis. One group of the tumor implanted mice were injected subcutaneously once weekly with 2.5 mg cortisone in 0.1 cc vehicle starting at various periods after transplantation. Another group received 0.1 cc of the vehicle without cortisone or 0.1 cc saline weekly and some received no additional treatment after tumor transplantation. The tumor growth was followed daily by palpation and measurement of the tumor. The animals were sacrificed at intervals, complete autopsies were performed, the tumors weighed, and tissue examined histologically.

Results. A) *Growth of tumor.* Fig. 1 is a composite of a representative experiment in which the mice received 2.5 mg cortisone once weekly, starting 24 hours after transplantation. Controls received 0.1 cc saline or 0.1 cc cortisone vehicle. The mice were sacrificed 28 days later. Each vertical column represents weight of tumor from one mouse. The average tumor weight in the saline control was 3.14 g, in the vehicle control 3.02 g, and in the cortisone-treated mice 0.39 g. In the saline controls the tumor became palpable 13 days after implantation (average), in the vehicle controls 13.3 days, and in the cortisone-treated after 19.9 days. A similar result was obtained in female mice in which the first cortisone injection was administered 6 days after tumor implantation. These animals were sacrificed 33 days later. The tumors from 7 cortisone-treated animals averaged 0.764 g, while those of 8 untreated controls averaged 5.74 g.

B) *Tissue studies.* 1) *Gross appearance.* The tumor fragment in control mouse is seen distinctly within the formalin fixed subcutaneous tissue while in the cortisone treated mouse it is difficult to identify the tumor particle during the first 2 weeks because it is sur-

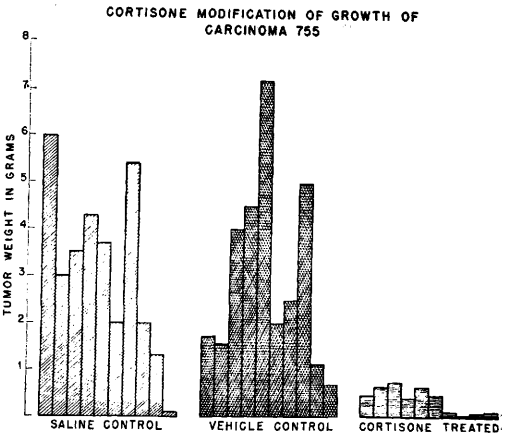

FIG. 1.

* Cortagen—Schering.

rounded by a dense, opalescent, mucoid fluid.

2) *Histology.* In the untreated mouse a moderate number of polymorphonuclear leukocytes are present about the trocar tract. In 72 hours new capillary channels begin to appear in the tissue surrounding the tumor. An occasional short, isolated strand of degenerate collagen is seen about the trocar tract. In 4-7 days, buds of the tumor grow into the adjacent areolar tissue. In 10 days there is considerable invasion by the tumor into the surrounding tissue.

In the cortisone treated mouse there are fewer polymorphonuclear leukocytes about the tumor than in the control. A discontinuous intensely eosinophilic membrane resembling "degenerate" or "necrotic collagen" is present in the tissue adjacent to the site of the tumor fragment and to the tract made by the trocar. (In the controls this is only occasionally seen and is considerably less in amount than in the cortisone-treated animals.) Only a few polymorphonuclear leukocytes are present about this tract. In the first week, very few if any new capillaries are seen in the tissues about the tumor implant. Fluid often appears in the blind end of the trocar tract. The tumor, rather than budding into and invading the adjacent tissues, seems to progress along the surface of the sinus tract and extend centripetally into the accumulated fluid as though growing into a tissue culture medium. This growth is slight. At this stage tumor growth is not seen in the areas lined by the eosinophilic membrane. Budding of the tumor into adjacent tissue is not evident until after 2 weeks.

Discussion. In studying the effect of various substances on transplantable tumors it is necessary to consider 1) the survival of the tumor transplant and retention of its growth potentialities before the establishment of a blood supply from the host; 2) the effect of the substance on the host tissues, particularly as it effects the immediate milieu of the tumor and the establishment of a blood supply, and 3) the response of the transplant after a blood supply from the host has been established. In the case of cortisone administered in the first few days after transplanting carcinoma 755, the tumor remains viable but a strong inhibiting effect on growth of the tumor occurs. Histological studies show that during this period cortisone has a profound effect on the immediate environment of the tumor transplant. Fluid often appears in the blind end of the trocar tract, and the tumor, rather than bud into the surrounding tissue, seems to extend along the surface of the sinus tract skipping for the most part the areas lined by the membrane of "degenerate collagen." No new capillaries are seen in the first week after tumor transplantation. These environmental changes in the host produced by cortisone, administered in the first days after transplantation, could account for the cortisone effect on the tumor, *i.e.*, retardation in growth due to altered milieu and delay in capillary formation. This latter effect is not entirely unexpected since it has been demonstrated that cortisone inhibits new vessel formation in granulation tissue(7-12). Furthermore, the formation of the eosinophilic membrane about the trocar tract appears to be unfavorable to growth and spread of the tumor. This may be considered as the result of an exaggerated reaction of the collagen to the trauma of the trocar, and would correspond to the pronounced degenerative changes produced by noxious agents in animals receiving cortisone (13-16). Viability of the transplant during this stage is probably dependent upon nourishment from the fluid adjacent to the tumor. Once an ample capillary bed is formed, providing a blood supply from the host, the tumor starts to grow and the inhibitory effect of cortisone is abolished. In the later stages of tumor growth, cortisone does not exert an inhibitory effect but in some instances there was a tendency to enhance tumor growth after cortisone administration.

In this communication we did not include the effect of cortisone on permeability of the host's capillaries, particularly in the period immediately following tumor implantation (17,18).

Since infections are not infrequently encountered in cortisone-treated mice(13) and cortisone produced visceral changes which, in some respects, are similar to those caused by inanition(14), these factors must be eliminated as contributing elements in the car-

cinostasis. In our experiments smears and cultures were taken from liver, kidney, and tumor of cortisone-treated mice, particularly in those in which the tumor growth was inhibited. With very few exceptions no bacteria were found on smears or in cultures. Because of this, infection as a contributory factor was ruled out. The fact that the animals appeared well nourished and continued to gain weight spoke against inanition as a factor in producing the retardation of tumor growth.

Summary. 1. Cortisone, when administered during the first few days after transplantation of carcinoma 755 in C57 mice, has a profound inhibitory effect on growth of the tumor but the tumor remains viable. 2. These effects of cortisone appear to be due to the change in the environment and to the delay in new blood vessel formation about the tumor transplant.

1. Heilman, F. R., and Kendall, E. C., *Endocrin.*, 1944, v34, 415.

2. Murphy, J. B., and Sturm, E., *Science,* 1933, v98, 568.

3. ———, *ibid.*, 1944, v99, 303.

4. Wooley, G. W., *Trans. N. Y. Acad. Sci.*, 1950, v13, 64.

5. Gottschalk, R. G., and Grollman, A., *Cancer* Res., 1952, v12, 651.

6. Baserga, R., and Shubik, P., *ibid.*, 1954, v14, 12.

7. Taubenhaus, M. and Anromin, G. D., *J. Lab. Clin. Med.*, 1950, v36, 7.

8. Ducommun, P., and Mach, R. S., *Semain Hop. Paris,* 1950, v26, 3170.

9. Selye, H., *Brit. M. J.*, 1949, v2, 1129.

10. Spain, D. M., Molomut, N., and Haber, A., *Am. J. Path.*, 1950, v26, 710.

11. Shapiro, R., Taylor, B., and Taubenhaus, M., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 854.

12. Rosenberg, M. Z., and Liebow, A. A., *Arch. Path.*, 1954, v57, 89.

13. Antopol, W., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 262.

14. Antopol, W., Glaubach. S., and Quittner, H., *Rheumatism,* 1951, v7, 187.

15. Antopol, W., and Quittner, H., *J. Mt. Sinai Hosp.*, 1952, v19, 91.

16. Turner, T. B., and Hollander, D. H., *Bull. Johns Hopkins Hosp.*, 1950, v87, 505.

17. Menkin, V., PROC. SOC. EXP. BIOL. AND MED., 1942, v51, 39.

18. ———, *Am. J. Physiol.*, 1951, v164, 294.

Received May 10, 1954. P.S.E.B.M., 1954, v86.

Accumulation of Astatine^{211} by Thyroid Gland in Man.* (21100)

JOSEPH G. HAMILTON, PATRICIA W. DURBIN, AND MARSHALL W. PARROTT.

From the Division of Medical Physics, Experimental Radiology and Medicine, Crocker Laboratory, University of California, Berkeley and San Francisco.

Astatine^{211}, which is the last member of the halogen group of elements, has been found to be accumulated by the thyroid glands of experimental animals to a degree similar to that of iodine^{131}(1-4). Since the average energy of the alpha-particles arising from the disintegration of At^{211} is 6.8 Mev and the range in tissue is but 70μ, the degree of specific ionization produced as compared to the beta-particles from I^{131} is much greater. The maximum energy of I^{131} beta-particles is 0.6 Mev and the range is 2,000 μ. The motivation for these extensive studies with At^{211} has been the consideration that the short range and high specific ionization of the alpha-particles of At^{211} as compared to the much longer range and more weakly ionizing beta-particles from I^{131} might have clinical applications in treatment of thyroid disorders, and in particular hyperthyroidism.

For several years this laboratory has been engaged in a study of the rate and degree of accumulation of both I^{131} and At^{211} in the thyroid gland and other tissues and organs of experimental animals. The acute and chronic effects of relatively large amounts of these two radiohalogens has also been investigated(2-4). The results of these experiments

* This work was performed under contract of the University of California under the U. S. Atomic Energy Commission.