difference between the two colorant systems, that different colorants must be used and therefore an exact match is not obtainable" (3).

REFERENCES

(1) Hollidge, K. B., talk presented at western regional meeting of the Pharmaceutical Mfgs. Assoc., 1961.
(2) Kibbe, W., *Drug Cosmetic Ind.*, **88**, 170(1961).
(3) Saltzman, M., *SPE Tech. Papers*, **8**, (1962).
(4) Saltzman, M., *Offic. Dig. Federation Soc. Paint Technol.*, **35**, 245(1963).
(5) Raff, A. M., THIS JOURNAL, **52**, 291(1963).
(6) Davidson, H. R., and Hemmendinger, H., paper presented at the Fifth International Color meeting, Dusseldorf, Germany, 1961.
(7) Davidson, H. R., and Hemmendinger, H., *SPE Tech. Papers*, **8**, (1962).
(8) Davidson, H. R., and Hemmendinger, H., *J. Opt. Soc. Am.*, **48**, 281(1958).
(9) Kubelka, P., and Munk, F., *Z. Tech. Physik.*, **12**, 593 (1931).
(10) Kubelka, P., *J. Opt. Soc. Am.*, **38**, 448, 1067(1948).
(11) Fink-Jensen, P., Congres' FATIPEC III, Belgium, 1955.
(12) Billmeyer, F. W., Jr., Beasley, J. K., and Sheldon, J. A., *J. Opt. Soc. Am.*, **50**, 70(1960).
(13) Billmeyer, F. W., Jr., *ibid.*, **50**, 137(1960).
(14) Alderson, J. V., Atherton, E., and Derbyshire, A. N., *J. Soc. Dyers Colourists*, **77**, 657(1961).
(15) Saltzman, M., *Dyestuffs*, **43**, 57(1959).
(16) Duncan, D. R., *J. Oil Colour Chemists' Assoc.*, **32**, 296 (1949).
(17) Judd, D. B., "Color in Business, Science and Industry," John Wiley & Sons, Inc., New York, N. Y., 1951, p. 331.
(18) Committee on Colorimetry, Opt. Soc. Am., "The Science of Color," Thomas Y. Crowell Co., New York, N. Y., 1953.
(19) Committee on Colorimetry, Opt. Soc. Am., *J. Opt. Soc. Am.*, **33**, 552(1943).
(20) *Federal Register*, **28**, 317(1963).
(21) Munsell, A. H., "A Color Notation," 9th ed., Munsell Color Co., Baltimore, Md., 1941.
(22) Commission Internationale de l'Eclairage, Proceedings of the 8th Session, Sept., 1931, Cambridge University Press, Cambridge, England, 1932.
(23) Hardy, A. C., "Handbook of Colorimetry," The Technology Press, Cambridge, Mass., 1936.
(24) MacAdam, D. L., *J. Opt. Soc. Am.*, **25**, 249(1935).
(25) *Ibid.*, **25**, 361(1935).
(26) Vickerstaff, T., *Proc. Phys. Soc. (London)*, **57**, 15 (1945).
(27) Atherton, E., and Peters, R. H., Congres' FATIPEC III, Belgium, 1955.
(28) Raff, A. M., Robinson, M. J., and Svedres, E. V., THIS JOURNAL, **50**, 76(1961).
(29) Davidson, H. R., and Imm, L. W., *J. Opt. Soc. Am.*, **39**, 942(1949).
(30) Murray, H. D., "Colour in Theory and Practice," Chapman & Hall, Ltd., London, England, 1952.
(31) Davidson, H. R., *J. Opt. Soc. Am.*, **45**, 216(1955).
(32) Nickerson, D., *Am. Dyestuff Reptr.*, **39**, 541(1950).
(33) Saltzman, M., *Dyestuffs*, **44**, 217(1960).
(34) Evans, R. M., "An Introduction to Color," John Wiley & Sons, Inc., New York, N. Y., 1948.
(35) Davidson, H. R., and Hemmendinger, H., *J. Opt. Soc. Am.*, **51**, 1459; abstract WB17, (1961).
(36) Clement, L. A., *Color Eng.*, **1**, 10(1963).

Effect of Cortisone on the Minimal Carcinogenic Dose$_{50}$ of Methylcholanthrene in Albino Mice

By RALPH T. MANCINI, RONALD F. GAUTIERI, and DAVID E. MANN, JR.

The administration of 0.4 mg. of cortisone acetate per 30 Gm. of body weight, injected subcutaneously five times a week over a 16-day period (12 injections) during the initial phase of the experiment, caused a slight decrease in the incidence of methylcholanthrene-induced tumors. The CF-1 albino mice used in this experiment also exhibited a definite retardation or even a significant decrease in body weight during the 16-day period of cortisone acetate administration. However, when cortisone acetate was withdrawn, the mice gained weight rapidly. At the termination of the experiment, the groups of mice which had received cortisone (Groups B and C) exhibited a mean weight approximately equal to that of the control groups.

THE ISOLATION of cortisone from the adrenal cortex by Kendall, *et al.* (1), Reichstein (2), and Wintersteiner and Pfiffner (3) in 1936 was followed by the extraction and identification of approximately 45 additional steroids, five of which—besides cortisone—exhibited significant physiological activity: corticosterone, 11-desoxycorticosterone, 11-dehydrocorticosterone, 17-α-hydroxycorticosterone (hydrocortisone), and 17-hydroxy-11-desoxycorticosterone. Of these, cortisone has been subjected to the most thorough clinical evaluation in such diverse conditions as arthritis, leukemia, Addison's disease, allergic states, skin diseases, and ophthalmic disorders. In addition, the influence of the steroid on carcinogenesis has been studied.

One of the earliest experiments to determine the effects of cortisone on the growth of malignant tumors in mice was conducted in 1944 by Heilman and Kendall (4). They showed that the administration of cortisone in drinking water inhibited lymphosarcomas, while withdrawal caused the initiation of carcinogenesis. However, growth was not reversed by the subsequent readministration of cortisone. Other investigations have demonstrated that cortisone exerted a temporary inhibition of ependyomas (5), lymphosarcomas (6), adenocarcinomas (7), and rhabdomyosarcomas (8).

In a study conducted by Baserga and Shubik (9), it was observed that cortisone reduced the

Received June 3, 1963, from the School of Pharmacy, Temple University, Philadelphia, Pa.
Accepted for publication July 19, 1963.
Abstracted from a thesis presented by Ralph T. Mancini to the Graduate School, School of Pharmacy, Temple University, in partial fulfillment of Master of Science degree requirements.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.

TABLE I.—COMPOSITE RESULTS OF PART 1 AND PART 2

Group	Total MCA Admin., mcg.[a]	Effective Total Mice/Group	Tumorous Mice/Group	Tumor Incidence, %	Male	Female	Incidence of Multiple Tumors	Male	Female
A	336–504	58	29	50.0	17	12	11	9	2
B	312–504	55	26	47.3	14	12	5	3	2
C	288–504	53	18	33.9	9	9	3	2	1
D	360–504	59	26	44.0	12	14	9	5	4

[a] The first column of numbers refers to the smallest quantity of methylcholanthrene (expressed in micrograms) needed to induce the formation of a tumor in at least one CF-1 albino mouse in that group. The second column of numbers represents the total amount of methylcholanthrene (expressed in micrograms) applied to all animals during the 10.4 week period of the experiment. MCA-methylcholanthrene.

TABLE II.—PART 1.—QUANTITY (MCG.) OF METHYLCHOLANTHRENE APPLIED (AS A 0.12% ACETONE SOLUTION) UNTIL ONSET OF TUMORS IN MICE

Groups	Sex	Tumors, No.	Applications, No. Range	Applications, No. Av.	Total Admin., mcg. Range	Total Admin., mcg. Av.	Induction Time, Days Range	Induction Time, Days Av.
A	M	7	16–21	19	384–504	456	56–71	66
	F	4	14–21	19	336–504	462	49–72	66
B	M	8	15–21	18	360–504	426	54–72	62
	F	7	17–21	20	408–504	483	60–72	70
C	M	4	13–21	17	312–504	408	46–72	59
	F	3	15–21	17	312–504	416	53–72	60
D	M	8	15–21	18	360–504	441	53–72	65
	F	8	15–21	19	360–504	450	53–72	65

tumor incidence in mice receiving topical applications of methylcholanthrene.

Sugiura, *et al.* (10), noted, however, that cortisone was inconsistent in temporarily inhibiting different types of tumors. Using cortisone acetate, they observed a marked inhibition of rat sarcoma R-39, mouse lymphosarcoma, and mouse osteogenic sarcomas, while the hormone only slightly inhibited Lewis sarcoma T-241 and exerted no effect on sarcoma 180 or mammary adenocarcinoma E-0771.

Contrary to the results obtained in the foregoing experiments, which indicated that cortisone inhibited neoplastic growths to varying degrees, other investigators have compiled evidence that cortisone treatment caused a definite increase in the tumor incidence (11–14).

Possibly conflicting results have occurred because investigators have failed to employ a valid dose/response relationship for the carcinogen used. With the establishment of the minimal carcinogenic dose$_{50}$ (MCD$_{50}$) of methylcholanthrene (15), the elucidation of tumor enhancement or inhibition by an agent can be determined. The purpose of this investigation was to discern the effects of cortisone on methylcholanthrene-induced skin carcinogenesis in mice.

MATERIALS AND METHODS

In this study the experiment was performed twice to verify and confirm the MCD$_{50}$ of methylcholanthrene described in a previous paper (15).

A total of 240 CF-1 albino mice approximately 10 to 11-weeks-old—four groups of 60 mice each (30 males and 30 females)—were used in this experiment. The cages employed, diet, methylcholanthrene solution, hair trimming procedure, weighing, manipulation of the mice, recording of data, preparation of tumors for pathological study, and the method of application of the carcinogen are described in a previous paper (15).

The cortisone acetate[1] used in this experiment was a saline suspension containing 25 mg. of cortisone acetate per milliliter. Two milliliters (50 mg.) of cortisone acetate suspension was diluted in a 25-ml. volumetric flask with normal saline solution. The diluted suspension, containing 2 mg. of cortisone acetate per milliliter, was used for the subcutaneous administration of cortisone acetate to CF-1 mice according to body weight (0.4 mg. per 30 Gm. of body weight).

In this experiment the four groups of mice were *Group A* (control group), received 504 mcg. through 21 bi-weekly applications of 0.02 ml. of a 0.12% solution of methylcholanthrene in acetone. This is the MCD$_{50}$ of methylcholanthrene. *Group B* (cortisone group) received the MCD$_{50}$ of methylcholanthrene with the concomitant subcutaneous administration of cortisone acetate suspension three times a week (Monday, Wednesday, and Friday) alternately in the left and right inguinal area over a 16-day period from the day of the initial application of methylcholanthrene. *Group C*, (cortisone group) was the same as *Group B*, except that the cortisone acetate was administered five times a week (Monday through Friday). *Group D* (normal saline control group) received the MCD$_{50}$ of methylcholanthrene with the concomitant subcutaneous administration of normal saline solution five times a week, equivalent in volume to the injections of cortisone acetate suspension (based on body weight).

EXPERIMENTAL

The hands were protected by heavy neoprene gloves when handling the mice or the apparatus used for the application of the carcinogen. The mice were grasped with the index finger and the thumb at the base of the tail during the applications. When the mice were held in this manner, the solution of

[1] Marketed as Cortone Acetate by Merck Sharp and Dohme, Philadelphia, Pa.

TABLE III.—PART *2*.—QUANTITY (MCG.) OF METHYLCHOLANTHRENE APPLIED (AS A 0.12% ACETONE SOLUTION) UNTIL ONSET OF TUMORS IN MICE

Groups	Sex	Tumors, No.	Applications, No. Range	Av.	Total Admin., mcg. Range	Av.	Induction Time, Days Range	Av.
A	M	10	14–21	17	336–504	413	48–71	59
	F	8	18–21	19	432–504	459	62–72	66
B	M	6	14–21	20	336–504	432	48–72	62
	F	5	13–21	17	312–504	403	44–71	57
C	M	5	12–21	17	288–504	418	41–72	60
	F	6	18–21	20	432–504	476	62–72	68
D	M	4	15–21	19	360–504	450	51–72	64
	F	6	18–21	20	432–504	476	63–72	68

TABLE IV.—MEAN WEIGHT AND STANDARD DEVIATION IN WEIGHT OF EACH GROUP AT ONSET OF EXPERIMENT, AT THE END OF CORTISONE ADMINISTRATION, AND AT THE TERMINATION OF THE EXPERIMENT

Group (30 Mice/ Group)	Part *1* Mean Wt., Gm.	Part *1* S.D.	Part *2* Mean Wt., Gm.	Part *2* S.D.
A[a]	20.25	±1.81	25.49	±2.73
A[b]	24.09	±2.06	26.33	±2.93
A[c]	30.05	±3.16	30.40	±2.87
B[a]	20.22	±1.60	23.26	±3.57
B[b]	20.80	±2.18	22.25	±2.99
B[c]	30.32	±2.85	29.00	±3.74
C[a]	20.54	±1.85	23.18	±2.53
C[b]	19.79	±1.77	21.21	±2.24
C[c]	29.41	±2.99	29.57	±3.30
D[a]	20.54	±1.95	23.92	±3.25
D[b]	23.94	±1.92	25.64	±3.36
D[c]	31.41	±2.45	29.98	±3.15

[a] Initial weight. [b] Weight at end of cortisone therapy. [c] Termination weight.

methylcholanthrene in acetone was applied by placing the interscapular area beneath the tip of the micropipet. A 2-cm. square area of hair in the interscapular region was trimmed with a pair of nickel-plated scissors to enable the solution of methylcholanthrene in acetone to spread evenly by capillarity to all parts of the area of application.

The recording of tumors was initiated at the appearance of the first macroscopically observed tumor. After the appearance of the initial growth, the interscapular area was examined at least three times a week with a Stanley 701-A magnifying glass. The mice that died before the recording of the first tumor were not included in the effective total.

The surviving mice were sacrificed with chloroform at the termination of the experiment. Tumors were randomly excised from each of the four groups of animals and placed in a 10% solution of formaldehyde for histopathological study.

All data obtained from Parts *1* and *2* of this study were compiled in four tables. Only those mice exhibiting tumors measuring 1 mm. or more in one dimension (width *versus* height) were recorded. Table I shows the composite results of Parts *1* and *2* obtained after 21 bi-weekly applications of 0.02 ml. of a 0.12% solution of methylcholanthrene in acetone to the control mice (Groups *A* and *D*) and to the cortisone groups (Groups *B* and *C*). Tables II and III exhibit the quantity in micrograms of methylcholanthrene applied until the onset of tumors in each part of the experiment. The induction time for the occurrence of at least one epidermal tumor in each group is also indicated. Table IV exhibits the mean weight and standard deviation in the weight of each group of mice at the onset of the experiment, at the end of cortisone administration, and at the termination of the experiment.

DISCUSSION

The results of this investigation indicated that the subcutaneous administration of cortisone acetate five times weekly (Group C) decreased the incidence of methylcholanthrene-induced tumors. The control mice (Group A) exhibited a confirming MCD_{50} for methylcholanthrene, as previously shown by Gautieri and Mann (15) in CF-1 albino mice.

These results are in agreement with those obtained by Baserga and Shubik (9), who demonstrated that methylcholanthrene-induced carcinogenesis was inhibited by the subcutaneous administration of 0.5 mg. of cortisone acetate, daily, 6 days a week for 19 weeks.

Table II (Part *1*) and Table III (Part *2*) show that in both phases of the experiment the first tumor was observed at approximately the same time. In Part *1*, the first tumor appeared 46 days after the initial application of methylcholanthrene; in Part *2* the first tumor occurred 41 days after the first methylcholanthrene application. These data also agree with previous studies (15).

Epilation was initiated 5 to 7 days after the first application of a 0.12% solution of methylcholanthrene in acetone. The epidermis of the interscapular area of the control mice (Groups A and D) appeared hyperemic with the application of the methylcholanthrene solution. However, similar applications of methylcholanthrene to the skin of the interscapular area of mice treated with cortisone acetate suspension did not cause a comparable degree of hyperemia. The number of epidermal lesions appearing in the interscapular area of the control mice (Groups A and D) seemed to increase as the experiment progressed. Approximately 1 week after the termination of cortisone therapy, a mild vasodilation became apparent in the mice of the cortisone groups (Groups B and C). Skin lesions appeared in the interscapular area in some of the cortisone-treated mice toward the end of the experiment.

A resurgence of hair growth in the control mice (Groups A and D) occurred 16 to 20 days after the beginning of the application of methylcholanthrene. This same resurgence of hair growth in the mice receiving cortisone acetate suspension (Groups B and C) did not commence until 33 to 36 days after the initial application of methylcholanthrene. The resurgent hair did not have the glossy appearance and natural conformation to the body contours that was characteristic of normal hair; it was stiff and difficult to cut with a pair of scissors.

Table IV shows that the cortisone-treated mice (Groups B and C) in *Parts 1 and 2* of this study had a definite retardation or even a significant decrease in mean body weight at the termination of the cortisone injections, while the control mice (Groups A and D) exhibited a definite increase in mean body weight during the same period.

SUMMARY

The administration of 0.4 mg. of cortisone acetate per 30 Gm. of body weight, injected subcutaneously five times a week over a 16-day period (12 injections) during the early phase of the experiment, caused a slight decrease in the incidence of methylcholanthrene-induced tumors.

When administered subcutaneously, cortisone acetate suspension decreased the skin irritation and subcutaneous vasodilation caused by the application of a 0.12% solution of methylcholanthrene in acetone to the interscapular region. However, this effect was noticeable only during the interval of cortisone therapy and for a short period thereafter.

The CF-1 albino mice used in this experiment also showed definite retardation or even a significant decrease in body weight during the 16-day period of cortisone acetate administration. However, when cortisone acetate was withdrawn, the mice gained weight rapidly. At the termination of the experiment, the groups of mice which had received cortisone (Groups B and C) exhibited a mean weight nearly equal to the mean weight of the control groups (Groups A and D).

The administration of cortisone acetate to the mice of Groups B and C delayed the resurgence of hair growth approximately 2 weeks beyond the resurgence of hair growth in the control groups.

REFERENCES

(1) Kendall, E. C., Mason, H. L., and Meyers, C. S., *J. Biol. Chem.*, **114**, 613(1936).
(2) Reichstein, T., *Helv. Chim. Acta*, **19**, 1107(1936).
(3) Wintersteiner, O., and Pfiffner, J. J., *J. Biol. Chem.*, **116**, 281(1936).
(4) Heilman, F. R., and Kendall, E. C., *Endocrinology*, **34**, 416(1944).
(5) Bizustowicz, R. J., Svien, H. J., Bennett, W. A., *Proc. Staff Meetings Mayo Clinic*, **26**, 121(1951).
(6) Emerson, G. A., Wurtz, E., and Zanetta, M. E., *Federation Proc.*, **9**, 357(1950).
(7) Agosin, M., Christen, R., Badinez, O., Gasic, G., Neghme, A., Pizarro, O., and Jarpa, A., *Proc. Soc. Exptl. Biol. Med.*, **80**, 128(1952).
(8) Higgins, G. M., Woods, E. A., and Bennett, W. A., *Cancer Res.*, **10**, 203(1950).
(9) Baserga, R., and Shubik, P., *ibid.*, **14**, 12(1953).
(10) Sugiura, K., Stock, C. C., Dobringer, K., and Rhoads, C. P., *ibid.*, **10**, 244(1950).
(11) Herrmann, F., Morrill, S. D., Sherwin, R. W., Rothstein, M. J., and Sulzberger, M. B., *J. Invest. Dermatol.*, **25**, 423(1955).
(12) Sherwin-Weidenreich, R., Herrmann, F., and Rothstein, M. J., *Cancer Res.*, **19**, 1150(1959).
(13) Herrmann, F., Sherwin, R. W., Morrill, S. D., Rothstein, M. J., and Sulzberger, M. B., *J. Invest. Dermatol.*, **25**, 403(1955).
(14) Morrill, S. D., and Herrmann, F., *ibid.*, **37**, 243(1961).
(15) Gautieri, R. F., and Mann, D. E., Jr., THIS JOURNAL, **47**, 350(1958).

Effect of Sodium Cobaltinitrite on the Minimal Carcinogenic Dose$_{50}$ (MCD$_{50}$) of Methylcholanthrene in Albino Mice

By RAYMOND F. ORZECHOWSKI, RONALD F. GAUTIERI, and DAVID E. MANN, JR.

The bi-weekly, intraperitoneal administration of sodium cobaltinitrite in doses of 50, 60, and 70 mg./Kg. in mice subjected to the MCD$_{50}$ of methylcholanthrene resulted in a reduction of tumor incidence to 31, 31, and 25 per cent, respectively, compared to 47 per cent for the controls.

FROM THE MANY diverse factors that have been shown to modify experimentally induced carcinogenesis, an intriguing concept has arisen which stresses the need for a deeper understanding of the role played by oxygen in the inception and regulation of the cancerization process. According to Warburg (1), a normal cell becomes cancerous because of irreversible damage to its respiratory mechanism. Cells which are unable to compensate immediately to the abrupt change in intracellular respiration die, while others survive only by adjusting to a fermentation type of respiration, thus becoming undifferentiated and cancerous. In short, the cancer cell derives energy from fermentation which fulfills its metabolic demands as adequately as intracellular respiration meets the requirements of the normal cell. On the other hand, Weinhouse (2) presented experimental evidence that the respiration of tumor slices is approximately equal to the oxidative metabolic activity of normal cells. In further support of these findings, isotope studies performed in 1949 (3) have shown that tumor cells can oxidize glucose to carbon dioxide at rates comparable to those of normal cells. Therefore, it

Received June 3, 1963, from the School of Pharmacy, Temple University, Philadelphia, Pa.
Accepted for publication July 19, 1963.
This investigation was supported by Grant CA-06239 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.
The authors are indebted to Drs. Marion W. McCrea and William R. Sabes, Henry Isaiah Dorr Department of Histopathology, Temple University, for their valuable assistance.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.