Effect of Cobalt Chloride and Sodium Cobaltinitrite on the Growth of Established Epithelial Tumors Induced by Methylcholanthrene

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Abstract □ Previous investigations showed that when cobaltous chloride or sodium cobaltinitrite is administered in conjunction with the topical application of the carcinogen, methylcholanthrene, the tumorigenic response is significantly decreased. The experimental design of this study involved the administration of each cobalt compound, following completion of the minimal carcinogenic dose$_{50}$ (MCD$_{50}$) regimen of methylcholanthrene when tumorigenesis was firmly established, to ascertain whether these agents could retard the growth of formed tumors in addition to inhibiting those during the period of tumorigenesis. It was demonstrated that cobaltous chloride and sodium cobaltinitrite have no influence on the growth of established epithelial tumors induced by methylcholanthrene and that the antitumorigenic capacity of these compounds is restricted solely to the incipient stages of cancerization.

Keyphrases □ Tumors, epithelial, induced by methylcholanthrene—effect of cobalt chloride and sodium cobaltinitrite on growth □ Methylcholanthrene-induced tumors—effect of cobalt chloride and sodium cobaltinitrite on growth □ Cobalt chloride, sodium cobaltinitrite—effect on growth of methylcholanthrene-induced tumors

Since the observation by Kennaway and Heiger (1) during the early thirties that carcinogenesis could be initiated following repetitive stimulation by a single chemical entity, vindictive efforts to elucidate the nature of this exceedingly complex disease have been more multitudinous than fruitful. One presumptive concept pertaining to the origin of the cancerization process concerns the appearance of abnormal cells in response to stimuli that have caused delicate growth control mechanisms to go awry (2–5). Setala (6) recently called attention to the theory, postulated almost 100 years ago by Cohnheim, that suggests an embryologic accident as being the primary etiologic factor in initiating cancer in higher mammals. According to this supposition, during embryonic development, not all of the dividing cells become differentiated and mature but remain for some unknown reason undifferentiated and dormant. Later when challenged by certain chemical agents, carcinogens, they respond at the level of susceptibility they enjoyed during that much earlier time when embryonic arrest occurred. Certain similarities between teratogens, agents that act upon young cells to cause malformations in later life, and carcinogens, which induce cancer at all ages, become even more comparable when the Cohnheim hypothesis is considered. A gross dissimilarity cannot escape unnoticed, however, between the two types of stimuli. Teratogens generally interfere with cellular development to cause growth retardation (inhibition), while carcinogens inevitably precipitate uncontrolled growth (stimulation).

Because this laboratory has been involved in both teratogenic and carcinogenic studies in recent years, with a special interest in the inhibitory influence of certain cobalt compounds on the development of cortisone-induced cleft palate in mice (inhibition of an inhibition) and the prevention of methylcholanthrene-induced carcinogenesis (inhibition of a stimulation), it became the ultimate purpose of this investigation to determine whether or not these cobalt compounds could inhibit the growth of established tumors that had responded to methylcholanthrene administration in mice. If the administration of cobalt, after the tumors formed, failed to affect their developmental course, it might indicate that these cells have been basically changed to types that are no longer susceptible to the action of cobalt and indeed might suggest that methylcholanthrene transformed those latent immature embryonic cells into an adult type of proliferating cell.

Table I—Tumor Response of Individual Groups

Group	Effective Total Mice per Group	Tumorous Mice	Tumor Percent	Number of Males with Tumors	Number of Females with Tumors
A	57	28	49.1	15	13
B	60	30	50.0	17	13
C	58	29	50.0	16	13
D	58	34	58.6	17	17
Total	233	121	51.9	65	56

Table II—Mortality Rate of Individual Groups during Cobalt Therapy

Group	Number of Deaths	Deaths with Tumors	Deaths without Tumors
A	3	2	1
B	7	3	4
C	5	3	2
D	11	8	3
Total	26	16	10

EXPERIMENTAL

Two hundred and forty CF-1 mice were divided into four groups of 60, each containing 30 males and 30 females. Cages employed, diet, handling, shaving, and application procedure were discussed in a previous paper (7). All groups were initially subjected to the minimal carcinogenic dose$_{50}$ (MCD$_{50}$) of methylcholanthrene

Table III—Response of Individual Groups to Cobalt Therapy

Group	Number of New Tumors	Percent of New Tumors	Number of Regressions	Percent of Regressions	Percent Tumor Incidence at End of Therapy
A	11	20.4	4	7.4	61.1
B	11	21.2	5	9.6	63.5
C	11	21.2	3	5.8	65.4
D	5	11.1	8	17.8	51.1

Table IV—Comparison of Animals with Multiple Tumors to Total Number of Animals with Tumors before and after Cobalt Therapy

Group	Treatment Regimen Period, days 1–70	76–108	Status of Tumor Incidence on Day 72: Number with Tumors	Number with Multiple Tumors	Percent with Multiple Tumors	Status of Tumor Incidence on Day 111: Number with Tumors	Number with Multiple Tumors	Percent with Multiple Tumors
A	MCA[a]	No drug	28	9	32.1	33	23	69.7
B	MCA	$CoCl_2$ (25 mg./kg.)	30	9	30.0	33	21	63.6
C	MCA	$Na_3Co(No_2)_6$ (50 mg./kg.)	29	8	27.6	34	22	64.7
D	MCA	$Na_3Co(NO_2)_6$ (60 mg./kg.)	34	6	17.6	23	12	52.2

[a] Methylcholanthrene in the minimal carcinogenic dose$_{50}$(MCD$_{50}$).

(504 mcg.), applied through 21 biweekly applications of 0.02 ml. of a 0.12% solution of methylcholanthrene in acetone. Two days after the final administration of the carcinogen, all animals were examined closely, and those with growths measuring 1 mm. or greater (width *versus* height) were recorded. Table I shows the tumorigenic responses of each of the four groups at the end of the 10.4-week application period. On the 6th day after the cessation of methylcholanthrene applications, cobalt administration was commenced. Group A received no additional treatment and served as a control group; Group B received 25 mg./kg. of cobaltous chloride; Group C received 50 mg./kg. of sodium cobaltinitrite; Group D received 60 mg./kg. of sodium cobaltinitrite. All agents were administered intraperitoneally on Monday afternoons and Friday mornings for a period of 5 weeks (10 administrations). On the 3rd day after the final administration of the cobalt compounds, the animals were again closely examined for additional tumors and/or regressions.

RESULTS

Epilation was noted in the majority of animals approximately 10 days after the initial application of the carcinogen. This condition persisted for about 2 or 3 weeks and was characterized by an almost total loss of hair in the intrascapular region of most animals. A period of increased hair growth followed, which lasted for the remainder of the MCD$_{50}$ applications.

In Table I, the tumorigenic response of each group to the methylcholanthrene can be seen. The percent tumor incidence is in good agreement with all previously published data and serves, once again, to confirm the MCD$_{50}$ dose of methylcholanthrene in acetone (7) in this strain of mice. Table II shows the mortality in each group during cobalt therapy, the majority of deaths appearing in the group that received 60 mg./kg. of sodium cobaltinitrite (Group D). Most of these deaths can be attributed to the drug, because they occurred within a 2-hr. period following drug administration in which all of the animals exhibited marked hypoxia. In Table III the group receiving 60 mg./kg. of sodium cobaltinitrite (Group D) had twice as many regressions and less than half the amount of new tumor formations as compared to those of the control group. This finding, however, does not represent a significant change (*i.e.*, $\chi^2 < 3.84$). Table IV indicates the percentage of animals with tumors actually showing more than one recordable growth (multiple tumors) both before and after cobalt therapy. Again, there is no apparent difference between the control group and the treated groups, because the percent of animals with multiple tumors increased in all groups by approximately the same factor.

DISCUSSION

Cobaltous chloride and sodium cobaltinitrite have no significant effect on the fate of tumor development when these compounds are administered during the posttumorigenic period (after the application of the MCD$_{50}$ of methylcholanthrene in acetone for 10.4 weeks), although each compound is capable of decreasing the tumorigenic response when given concurrently with the MCD$_{50}$ of methylcholanthrene (8, 9). It appears that these cobalt compounds are only effective during the early stages of carcinogenesis, thus supporting the theory that cancer cells arise from embryologic cellular rests or cells that have failed to mature and differentiate (6). Therefore, during the early stages of carcinogenesis, before these cells have differentiated into a cancerous type, cobalt compounds are effective antitumor agents because the cells maintain their susceptibility to the inhibitory action of cobalt which is inherent in embryologic tissue. However, once the growths become firmly established and differentiated into cancerous types, they are resistant to the inhibitory action of cobalt because they are no longer, in effect, embryologic or undifferentiated cells.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 8, 1970, from the *Department of Pharmacology, School of Pharmacy, Temple University, Philadelphia, PA 19140*

Accepted for publication September 30, 1970.

Presented to the Pharmacology and Biochemistry Section, APhA Academy of Pharmaceutical Sciences, Washington, D. C. meeting, April 1970.

Abstracted in part from a thesis presented by G. P. O'Hara to the Graduate School, Temple University, in partial fulfillment of the Master of Science degree requirements.

The authors thank Mr. James C. Tatnall for his technical assistance.

* Fellow of the American Foundation for Pharmaceutical Education.