Effect of Cobaltous Chloride on the Minimal Carcinogenic $Dose_{50}$ of Methylcholanthrene in Albino Mice

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The biweekly intraperitoneal administration of cobaltous chloride in doses of 10, 25, 30, and 40 mg./Kg. in mice subjected to the minimal carcinogenic $dose_{50}$ (MCD_{50}) of methylcholanthrene resulted in a percentage inhibition of the tumor incidence by 49, 60, 58, and 71, respectively, compared to the controls.

RECENTLY, Orzechowski *et al.* (1) have shown that sodium cobaltinitrite, when administered intraperitoneally to mice subjected to the minimal carcinogenic $dose_{50}$ (MCD_{50}) of methylcholanthrene, elicited an antitumorigenic response, presumably through the production of methemoglobinemic hypoxia. However, although a reduction in the amount of oxygen available to tissues has been shown by others to decrease tumor incidence (2–5), additional studies (6) appeared to refute the original concept that methemoglobinemic-induced hypoxia was a primary inhibitory factor in methylcholanthrene tumorigenesis, for sodium nitrite and *p*-aminopropiophenone, which cause variable degrees of hypoxia by this means, failed to elicit corresponding decreases in tumor incidence. Therefore, attention was directed toward a portion of the cobaltinitrite molecule—*viz.*, the cobalt moiety, which perhaps was implicated more directly in the inhibitory phenomenon. To verify this new hypothesis, a relatively simple cobalt compound, cobaltous chloride, was administered intraperitoneally to mice which were subjected concurrently to the MCD_{50} of methylcholanthrene.

EXPERIMENTAL

In each of the two trials, 240 CF-1 albino mice were divided into four groups of 60 mice (30 males and 30 females). Each group was labeled according to the particular dose of cobaltous chloride that was administered as follows: group B, 10 mg./Kg.; group C, 25 mg./Kg.; group D, 50 mg./Kg.; group F, 25 mg./Kg.; group G, 30 mg./Kg.; group H, 40 mg./Kg.; one group per trial (groups A, E) served as a control.

Cages employed, diet, handling, shaving, and method of preparation and application of the carcinogen have been described in a previous paper (7).

Freshly prepared solutions of cobaltous chloride were injected intraperitoneally on Mondays and Fridays in the appropriate groups. The schedule of injections started on Friday of week 1 of each trial, thereby allowing the mice to be exposed to two previous applications of the carcinogen.

The occurrence of fatalities was recorded throughout the experiment (Tables I and II). At the end of Trials 1 and 2, all tumors which measured at least one dimension (width *versus* height) of 1 mm. or greater were drawn and recorded on individual data sheets (7). The mice with tumors were chloroformed, and the growths were excised and immersed in 10% formalin in preparation for histopathological study.

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RESULTS

Table III shows the composite results obtained from the various doses of cobaltous chloride on the incidence of tumors induced by the MCD_{50} of methylcholanthrene.

Approximately 4 days after the initial application of methylcholanthrene, a progressive epilation was observed which persisted for 3 weeks. After this period, excessive hair growth was noticed in the interscapular region which exceeded that in other areas of the body. Seven to 10 days after the first application of the carcinogen, vasodilation of subcutaneous blood vessels was observed. During the last week of the experiment, in both Trials *1* and *2*, all groups showed epilation and a healthy appearance of the epidermis at the interscapular

TABLE I.—INCIDENCE OF DEATHS WITH 10 mg./Kg. COBALTOUS CHLORIDE

Day No.	Total No. of Injections	Total Dose, mg./Kg.	Hr. After Injection	Deaths, No.
16	4	40	72	1
65	18	180	48	1
72	20	200	24	1
			Total	3
Incidence of Deaths with 25 mg./Kg. Cobaltous Chloride				
13	3	75	48	2
24	6	150	72	1
27	7	175	48	1
35	10	250	24	1
42	12	300	24	1
54	15	375	48	1
72	20	500	24	1
			Total	8

			Injection	No.
3	1	50	Immediately	1
6	1	50	48	5
8	2	100	48	2
22	5	250	48	1
28	8	400		
30	8	400		
37	10	500		
48	12	600		
49	13	650	24	4
			Total	60

TABLE III.—EFFECT OF INTRAPERITONEAL INJECTIONS OF COBALTOUS CHLORIDE ON TUMOR INCIDENCE IN MICE RECEIVING THE MCD_{50} OF METHYLCHOLANTHRENE

Treatment and Group	Trial *1* Tumor Incidence	Trial *1* % Tumors	Trial *1* % Inhibition	Trial *2* Tumor Incidence	Trial *2* % Tumors	Trial *2* % Inhibition
Methylcholanthrene controls (A, E)	27/55	49	...	28/58	48	...
Cobaltous chloride, 10 mg./Kg. (B)	15/58	25	49[a]	...	..	...
Cobaltous chloride, 25 mg./Kg. (C, F)	6/54	11	77[a]	17/60	28	42[a]
Cobaltous chloride, 30 mg./Kg. (G)	...	..	...	11/55	20	58[a]
Cobaltous chloride, 40 mg./Kg. (H)	...	..	...	8/57	14	71[a]

[a] P value <0.005.

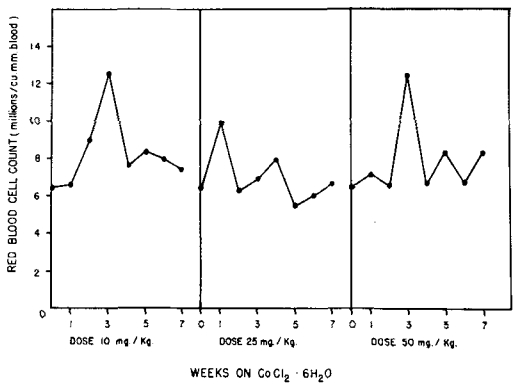

Fig. 1.—Results of blood tests during Trial *1*.

region, except for the control group, which showed marked degeneration of this area.

On each of the first seven Fridays of Trial *1*, an erythrocyte count was performed approximately 7–10 hr. after the injection of cobaltous chloride to determine whether polycythemia had occurred. Hermatocrit studies also were conducted on 42 mice apportioned as follows: a group of 12 animals, 9–10 weeks old were employed as controls, while additional groups of 15–20 mice were examined at each dosage level (Fig. 1 and Table IV).

DISCUSSION

As indicated in Table III, a significant reduction of the tumor incidence in response to the topical application of the MCD_{50} of methylcholanthrene occurred in the surviving groups (Trials *1* and *2*) which received intraperitoneal injections of cobaltous chloride. In Trial *1*, the administration of cobaltous chloride in doses of 10 and 25 mg./Kg. caused an inhibition of tumorigenesis by 49 and 77%, respectively; while in Trial *2*, cobaltous chloride in doses of 25, 30, and 40 mg./Kg. caused a 42, 58, and 71% inhibition. These results are in close agreement with the findings of Orzechowski *et al.* (1) that biweekly intraperitoneal administrations of either sodium cobaltinitrite or sodium nitrite significantly reduced the tumor incidence in response to the MCD_{50} of methylcholanthrene. Also, the *ad libitum* administration *per os* of sodium cobaltinitrite and sodium nitrite individually has been shown by Thompson *et al.* (8) to exert a similar inhibitory effect in mice.

It is well known that cobaltous chloride causes polycythemia, which inevitably leads to a state of hypoxia due to increased viscosity of the blood (9). Also, it has been suggested that cobaltous chloride may interfere with the cellular oxidative processes of the bone marrow, thereby producing a cytotoxic hypoxia which, in turn, leads to an increase in erythropoietic activity (9). Consequently, the polycythemia observed in this experiment (confirmed by blood studies) might lead to the conclusion that the resulting hypoxia arising from this condition was responsible for the retardation of tumorigenesis.

Liquier-Milward (10) has advanced a pertinent hypothesis which might explain the mechanism by which cobalt influences the growth of tumor tissue. Using radioactive cobalt-60 in tracer studies, he has demonstrated a high magnitude of cobalt incorporation in malignant cells and suggested that the embodiment of the trace metal is due to a chemical fixation and the formation of chelated compounds with newly formed proteins and nucleoproteins.

At the termination of this experiment, no noticeable change in the appearance of the skin was observed at the interscapular region of the mice treated with cobaltous chloride. Although epilation had occurred, little degeneration or inflammation of the epidermis was seen in the interscapular region compared to the controls. These findings agree with a recent report in which it was shown that the anti-inflammatory response caused by Δ-1 hydro-

TABLE IV.—BLOOD STUDY ON CF-1 MICE DURING TRIAL *1*

Group	Av. Hematocrit	Av. Red Blood Cell Count/cu. mm. (1000's)	Day No.
Cobaltous chloride			
A (controls)	21	6,500	
B (10 mg./Kg.)	21	6,500	
C (25 mg./Kg.)	48	10,060	3
D (50 mg./Kg.)	36	7,300	
B	42	9,080	
C	24	6,303	10
D	27	6,690	
B	46	12,600	
C	36	7,000	17
D	46	12,565	
B	39	7,560	
C	42	8,040	24
D	42	6,740	
B	38	8,500	
C	42	5,650	31
D	38	8,500	
B	37	8,010	
C	41	6,610	38
D	39	6,890	
B	40	7,500	
C	40	7,800	45
D	40	8,500	

cortisone was accentuated by the addition of the cobalt atom to the C-20 carbonyl function (11). The observation of Mancini *et al.* (12) that cortisone administration significantly delays the onset of carcinogenesis in response to the MCD_{50} of methylcholanthrene in mice, may further emphasize the importance of anti-inflammatory reactions in retarding the cancerization process.

SUMMARY

The biweekly intraperitoneal administration of cobaltous chloride in doses of 10 and 25 mg./Kg. in mice subjected to the MCD_{50} of methylcholanthrene resulted in a per cent tumor inhibition of 49 and 77, respectively. Cobaltous chloride, at a dose of 50 mg./Kg., employed over a 6-week period during Trial *1* (total dose 600 mg.), eventually killed 46 of the 60 animals. Toxic symptoms were observed in all groups in Trials *1* and *2*, except for the 10-mg./Kg. group and the controls.

A repetition of the experiment with cobaltous chloride, at dosage levels of 25, 30, and 40 mg./Kg., resulted in percentage tumor inhibitions of 42, 58, and 71, respectively.

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Thyroxinlike Activity of Several Thyroxin Analogs

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Five analogs of thyroxin were tested for thyroxinlike activity on the basis of the *Rana catesbiana* tail fin assay procedure. Two of them, 3,5-diiodo-4-(3′,5′-diiodo-4-hydroxyphenoxy)-phenylpropionic acid and 3,5-diiodo-4-(3′,5′-diiodo-4-hydroxyphenoxy)-phenylbutyric acid, were by this test many times more potent than L-thyroxin; one of them, 3,5-diiodo-4-(3′,5′-dimethyl-4-hydroxyphenyl)-phenylalanine, was slightly more potent than L-thyroxin.

IN AN EARLIER publication Bruice *et al.* (1) reported upon the thyroxinlike activity of a number of analogs of thyroxin when tested for their ability to influence the rate of metamorphosis of the bullfrog *Rana catesbiana*. This paper describes similar studies on additional analogs.

EXPERIMENTAL

The assay method employed was based upon the same principle as that of the earlier investigators, involving the measurement of the rate of tail resorption of the larva of *R. catesbiana*. The validity of the method, with ample literature citation, is discussed in detail in their paper, and will not be repeated here. However, for reasons to be outlined, some deviation from their protocol was introduced.

Assay Method.—Solutions of L-thyroxin and of the compounds to be tested were prepared, and the pH was adjusted to 8.0 to 8.5 by the addition of potassium carbonate and hydrochloric acid. Groups of tadpoles, whose tail widths ranged from 1.2 to 2.0 cm. at the widest point, were placed in tap water, fasted, and observed for 48 hr., at the end of which time tadpoles not completely healthy were discarded. Those selected for testing procedures were placed in individual shallow, transparent flat-bottomed vessels, each containing 200 ml. of the appropriate solution. Eight animals were used at each level of dosage, and a control group of eight others was employed in each determination. If, in any test series, a majority of animals failed to survive, the test was repeated to yield data from a total of at least eight animals. The animals were placed in a large air thermostat at a constant 30° and maintained in their respective solutions for 6 days. Zero time in the measurements was taken as the time at which the tadpoles were placed in test solution. Width of the tail fin at its widest point was measured every 24 hr. with a celluloid ruler held vertically at the side of the resting animal.

Treatment of Data.—Because the decrease in tail fin width of all control animals was reasonably constant, these were combined. For a total of 27 such animals, the average decrease after 6 days was 5.5 ± 0.94 mm. The average decrease for each group of test animals at each dosage level after 6 days' exposure was computed. The value for the control groups was subtracted in each case, yielding a corrected decrease in tail fin width that can be attributed to the influence of the test substance.

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