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Effect of Sodium Fluoride on Tumor Growth. (30150)

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Recently a report(1) from this laboratory indicated that NaBr in relatively low concentrations accelerated the growth of mouse and egg cultivated tumor tissue. This result occurred when the drug was introduced by way of the drinking water in mice, by injection over the embryonic membranes of eggs inoculated with tumor tissue, and when added to tumor tissue suspensions before implantation into eggs or mice. Preliminary tests with NaI and NaF gave evidence that low levels of these halides also accelerated tumor growth.

An extensive investigation of the effect of NaF on growth of mouse and egg cultivated tumor tissue has now been completed.

Materials and methods. Mice of the DBA strain implanted with RC mammary adenocarcinoma were used in the mouse experiments. Tumor suspensions for transplantation were made by adding 0.4 ml dispersed tumor tissue to 5.0 ml of 0.85% saline, and 0.2 ml of this suspension was injected subdermally in the right inguinal area. NaF was introduced into the mouse-tumor system in 3 different ways.

In one series of tests the compound was added to the tumor tissue suspension prior to inoculation into the mice. Concentrations of the drug were at the levels, 0.000,01-0.005 mg and 0.4-0.8 mg per mouse injection. The controls received the untreated tumor suspension. Control and experimental tumor suspensions were taken from a common pool of tumor-saline preparation.

In another series, NaF was added to the drinking water of the experimental animals immediately after they were inoculated with tumor tissue. Concentrations of the drug varied. In most of these tests, 1-5 mg of NaF per liter of distilled water was utilized. Controls were maintained on untreated distilled water.

A third series involved subdermal injections of NaF into mice bearing tumor transplants. In these tests, 0.25 mg of the compound dissolved in 0.25 ml of saline was injected subdermally on 4 consecutive days, beginning 4 days after tumor implantation. The tests were terminated one day after the final treatment.

In all the mouse experiments, the animals were sacrificed 8 to 10 days after tumor implantation, at which time the tumor tissue was removed and weighed. The results of a test were evaluated on the basis of the average weight of tumor tissue per mouse in the experimental as compared with the control animals.

A C3H mouse mammary tumor which grows readily in eggs by the yolk sac method (2) was utilized in the egg work. The techniques involved have been described(2,3). Tumor tissue suspensions were made up of 0.4 ml dispersed tissue in 5.0 ml of 0.85% saline, and 0.2 ml of this suspension was injected into the yolk sacs of 4-day incubated embryonated eggs.

Two types of tests were utilized in the experiments with eggs. In one series, NaF was added to tumor suspensions before inoculation into eggs. Concentrations of the drug were used at the levels, 0.000,01-0.000,5 mg and 0.1-0.8 mg per egg injection. The controls received the untreated tumor suspensions. Tumor preparations for control and

TABLE I. NaF Added to Tumor Suspensions Before Implantation and Growth of Tumor Transplants in Mice.

Exp	No. mice Control	No. mice Exp	Dosage, mg/.2 ml injection	Tumor wt, control = 100
1	9	7	.000,01	125
2	8	5	.000,01	139
3	9	8	.000,1	125
4	11	11	.000,1	106
5	9	6	.000,1	118
6	9	9	.000,1	140
7	6	5	.000,5	141
8	8	8	.000,5	163
9	7	8	.000,5	96
10	8	5	.000,5	113
11	10	9	.001	126
12	10	7	.001	103
13	10	10	.002,5	124
14	9	9	.005	144
15	9	8	.005	126
Total No. mice	132	115		

Control avg tumor wt (g) 1.29; S.D. ± .441.
Exp avg tumor wt (g) 1.63; S.D. ± .536.
Significance of difference in avg tumor wt, P = <.001.

experimental groups were taken from a common pool of tumor-saline suspension.

In another series, NaF was introduced into tumor-bearing eggs over the chick membranes. A level of 0.05-0.1 mg of the drug in 0.1 ml saline was injected into each egg. The controls were treated with saline. The membrane injection technique has been described(4).

The egg tests were terminated on the 13th day of incubation. The results were evaluated on the basis of the average weight of tumor tissue in experimental as compared with control eggs.

In all the experiments, both with mice and those involving embryonated eggs, every effort was made to randomize as much as possible all proceedings.

TABLE II. Subdermal Injections of .25 mg NaF Given on 4 Consecutive Days and Growth of Tumor Transplants in Mice.

Exp	No. mice Control	No. mice Exp	Tumor wt, control = 100
1	6	5	125
2	7	6	145
3	5	3	157
4	5	5	119
5	9	6	133
6	10	7	159
7	9	9	121
8	10	8	103
9	10	8	105
10	12	10	112
11	11	9	125
12	11	11	125
13	15	14	120
14	10	8	107
15	9	8	121
Total No. mice	139	117	

Control avg tumor wt (g) 1.20; S.D. ± .553.
Exp avg tumor wt (g) 1.45; S.D. ± .623.
Significance of difference in avg tumor wt, P = <.025.

TABLE III. NaF Added to Drinking Water and Growth of Tumor Transplants in Mice.

Exp	No. mice Control	No. mice Exp	Dosage, mg/l	Tumor wt, control = 100
1	17	14	1	117
2	12	11	1	113
3	10	7	2	129
4	20	19	2	113
5	8	8	2	161
6	9	9	2	113
7	8	6	2	103
8	9	7	2	161
9	10	10	2	132
10	7	6	2	117
11	10	10	2	118
12	8	6	5	101
13	5	7	5	106
14	18	17	20	113
15	10	8	20	136
16	16	13	20	89
17	9	10	44	100
18	7	7	55	114
19	10	9	55	123
Total No. mice	203	184		

Control avg tumor wt (g) .97; S.D. ± .360.
Exp avg tumor wt (g) 1.11; S.D. ± .387.
Significance of difference in avg tumor wt, P = <.013.

A total of 54 tests involving 991 animals was completed with mice bearing tumor transplants, and 58 tests including 1817 eggs were carried out with tumor-bearing eggs. Statistical significance, or P, was obtained by dividing the difference in tumor size for control and experimental tumors of a series by the standard error of the difference.

Results. The results of the various tests are summarized in Tables I-VII. Tumor growth was accelerated in association with NaF, at the concentrations and with the methods of introduction in tumor-bearing mice and eggs, as given in Tables I-V. The degree of growth stimulation was quite simi-

TABLE IV. NaF Added to Tumor Suspensions (.000,01–.000,5 mg per Injection) Before Inoculation and Growth of Tumor Tissue in Eggs.

Exp	No. eggs Control	No. eggs Exp	Tumor wt, control = 100
1	10	10	119
2	18	16	164
3	16	15	144
4	14	11	200
5	15	18	71
6	22	16	106
7	14	12	133
8	14	16	160
9	10	11	113
10	19	12	107
11	12	9	127
12	16	12	100
13	10	7	118
14	10	8	158
15	10	10	103
16	14	12	107
17	8	11	160
18	13	8	102
19	16	15	126
20	17	12	123
21	11	11	180
22	13	12	181
23	10	7	132
Total No. eggs	312	271	

Control avg tumor wt (g) .37; S.D. ± .186.
Exp avg tumor wt (g) .46; S.D. ± .211.
Significance of difference in avg tumor wt, P = <.001.

lar in tumors grown in mice as compared with tumors cultivated in eggs. In the 3 series of experiments with mice, accelerations of tumor growth averaged 126, 121 and 114% of the control, while in the 2 series of egg experiments, the per cent increase in tumor size was 124 and 121. The difference in average tumor size between control and experimental groups was statistically significant in each of these series of experiments, and in combination this significance is greatly increased.

When the drug was added to tumor suspensions before implantation in mice or eggs at the relatively high levels of 0.4-0.8 mg per mouse, or 0.1-0.8 mg per egg, tumor growth was markedly inhibited.

Discussion. The data appear to establish the association of NaF with acceleration of the growth of tumor transplants in mice and embryonated eggs, at concentrations and under the conditions described here. It has been reported(1) that NaBr likewise has a stimulating effect on tumor growth when administered to tumor-bearing mice or eggs, under conditions similar to the present tests. NaBr is effective at higher levels of concentration as compared with NaF. Concentrations of NaF, as low as 0.000,01 mg per mouse injection, when added to tumor suspensions before implantation in mice, accelerated tumor growth, while levels in the range 0.000,1-0.000,01 mg/injection of NaBr did not affect tumor growth.

In experiments where the compound was added to the drinking water, wide variations

TABLE V. NaF Given as a Single Injection of .05-.1 mg over Chick Membranes and Growth of Tumor Tissue in Eggs.

Exp	No. eggs Control	No. eggs Exp	Tumor wt, control = 100
1	28	23	130
2	12	10	103
3	26	27	143
4	19	15	147
5	24	16	148
6	16	14	146
7	25	23	119
8	23	23	143
9	17	24	144
10	22	19	113
11	24	24	164
12	20	20	106
13	39	39	114
14	20	23	110
15	24	16	123
16	21	19	108
17	21	15	143
18	12	10	136
Total No. eggs	393	360	

Control avg tumor wt (g) .23; S.D. ± .138.
Exp avg tumor wt (g) .28; S.D. ± .162.
Significance of difference in avg tumor wt, P = <.003.

TABLE VI. Tumor Inhibiting Effect of Relatively High Concentrations of NaF Added to Tumor Suspensions Before Implantation in Mice.

Exp	No. mice Control	No. mice Exp	Dosage, mg/.2 ml injection	Tumor wt, control = 100
1	8	8	.4	8
2	13	12	.5	18
3	11	12	.5	29
4	9	9	.8	2
5	9	10	.8	8
Total No. mice	50	51		

Control avg tumor wt (g) 1.99.
Exp avg tumor wt (g) .24.

TABLE VII. Tumor Inhibiting Effect of Relatively High Concentrations of NaF Added to Tumor Suspensions Before Implantation into Eggs.

No. of experiments	No. eggs Control	No. eggs Exp	Dosage, mg/.2 ml injection	Tumor wt, control = 100
4	63	58	.1	70
4	62	65	.2	51
3	37	35	.4	42
6	78	83	.8	6
Totals: 17	240	241		

Control avg tumor wt (g) .39.
Exp avg tumor wt (g) .14.

in dosage increased tumor growth in the instance of both NaF and NaBr. It was recently reported(5) that the plasma fluoride is regulated in rats. This probably accounts for the similarity of tumor growth acceleration when NaBr is given over a range of 1 to 1,000 mg per liter, and NaF in concentrations of 1 to 55 mg per liter.

Unpublished results of tests with NaI indicate that this halide also stimulates tumor growth when added to tumor suspensions before implantation in eggs or mice at low levels, 0.000,1-0.001 mg per mouse or egg.

Summary. In 54 tests involving 991 mice bearing transplanted tumors and 58 tests including 1817 tumor-bearing eggs, data were obtained which indicated a statistically significant acceleration of tumor tissue growth in association with comparatively low levels of NaF.

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Effect of Adrenalectomy on Hyperglycemic Responses of Fed and Fasted Rats to Catecholamines.* (30151)

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The hyperglycemic response to epinephrine has been difficult to fit into the classification of adrenergic receptors proposed by Ahlquist (1,2). The problem has been considerably resolved, at least in the case of the rat, by evidence that liver glycogenolysis is mediated by an α-receptor and that muscle glycogenolysis is mediated by a β-receptor(3-6). Fleming and Kenny(7) have demonstrated that in *fed* rats the hyperglycemic response has the characteristics of an α-response and in *fasted* rats of a β-response. These investigators pointed out that α-responses are best studied in the *fed* rat using norepinephrine and β-responses in the *fasted* rat using isoproterenol.

Adrenalectomy causes changes in the effects of epinephrine on carbohydrate metabolism (8,9). In view of the new evidence concerning the importance of the prandial state of rats and a greater understanding of the receptors involved in the hyperglycemic response, it was considered important to re-evaluate the effect of adrenalectomy using dose-response curves and both *fed* and *fasted* animals.

Materials and methods. Blood glucose experiments. Male Holtzman rats, from 6 to 8 weeks old, were maintained on a commercial laboratory diet throughout the study. When the rats were *fasted,* food was withheld during a period of from 16 to 20 hours before the beginning of the experiment. All rats, whether *fed* or *fasted,* were bled in the morn-

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