

vitro. In order to obtain only a slight effect of this enzyme in our experiments, we were forced to raise the salicylate concentration ten times higher than Fulton by using 10 mg of sodium salicylate per cc which is beyond the range of therapeutic dosage. In view of recent clinical investigations,(17) there is a possibility that the *modus operandi* of salicylates is a stimulation of the adrenal glands liberating a hormone which relieves the rheumatic manifestations. Sodium gentisate, which is a metabolic product of salicylate, is even weaker. In the study of 4-amino salicylic acid (PAS), the anti-hyaluronidase activity approaches that of ortho cresol with, however, a higher therapeutic index.

Of the utmost importance is the fact that

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neither benzoic acid nor para aminobenzoic acid inhibit hyaluronidase, whereas their hydroxyl derivatives do.

Summary. The anti-hyaluronidase activity of phenol substitution products increases markedly with the length of the carbonic side chain. Salts of aromatic acids, like sodium salicylate, have an activity of a lower order as compared with that of phenols with long carbonic side chain. A moderate increase in activity is also obtained by substituting chlorine, nitro or amino groups.

As the data indicate, there exists in general, a parallel course of the inhibitory power of the substances and their phenol coefficient.

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Synergistic Action of Estrogenic Hormone and X-rays in Inducing Thymic Lymphosarcoma of Mice.* (17522)

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Lymphocytic neoplastic disease was induced by estrogens(1) or x-rays(2) in mice of certain genetic constitution when these agents were given in appropriate doses. The carcinogenic hydrocarbons are another group of lymphoma-inducing agents.(3) Synergistic or additive lymphomogenic effects of x-rays and the carcinogenic hydrocarbon, methylcholanthrene, were demonstrable(4) providing

lymphoid tissue of the test animals was susceptible to the neoplasia-inciting action of each of the agents used independently.(5) In the present experiment the synergistic action of x-rays and estrogens was investigated. It had previously been demonstrated that the CBA and Bagg albino stocks of mice are susceptible to the induction of lymphomas by the action of either estrogens or x-rays.(6) A single dose of 200 r of x-rays is a minimum inducing dose; weekly injections of 5 micrograms of estradiol dipropionate for 14 weeks

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TABLE I.
Occurrence of Lymphomatosis in Bagb Albino Mice Receiving Threshold Doses of Lymphomogenic Agents Either Singly or in Combination.

Agent	Total mice	Mice over 250 days	Total lymphoma	Mediastinal lymphoma	Age lymphoma (in days)	Dead and negative	No. living*
200r†	105	70	3	1	365	10	92
EDP‡	59	30	2	2	350	7	50
200r + EDP	70	36	15	12	283	13	42
Controls	101	101	32	1	725	69	0
18MC§	108	76	1	0	490	23	84
200r + 18MC	109	75	3	2	365	18	89
EDP + 18MC	58	19	3	1	285	5	50

* The oldest EDP-treated test animals (alone or in combination) are 500 days of age. The oldest MC-treated mice are 700 days of age.

† 20 r of x-rays in one exposure.

‡ Estradiol dipropionate—5 μ g weekly for 14 weeks. This estrogen was supplied by Ciba Pharmaceutical Products through the courtesy of F. E. Houghton.

§ Methylcholanthrene—18 skin paintings with 0.25% solution in benzene.

TABLE II.
Occurrence of Lymphomatosis in CBA Mice Receiving Threshold Doses of Lymphomogenic Agents Either Singly or in Combination.

Agent	Total mice	Mice over 250 days	Total lymphoma	Mediastinal lymphoma	Age lymphoma (in days)	Dead and negative	No. living*
200r	148	83	6	1	410	8	134
EDP	57	42	2	1	294	2	53
200r + EDP	55	33	11	11	293	2	42
Controls	63	63	20	0	608	43	0
18MC	126	103	1	0	405	20	105
200r + 18MC	118	79	8	0	445	17	93
EDP + 18MC	54	16	0	0	—	6	45

* The oldest EDP-treated test animals (alone or in combination) are 500 days of age. The oldest MC-treated mice are 700 days of age.

represent a threshold dose for this agent. Two groups of mice, one in the Bagb albino and the other in the CBA strain, each received a single dose of 200 r of x-rays at ages ranging from one to 70 days. Two groups from the same stocks received 14 weekly injections of 5 μ g of estradiol dipropionate in peanut oil beginning at 42 days of age. In a third treatment series 2 groups received 200 r at 42 days followed by the 14 weekly injections.

Although 32% of untreated Bagb albino mice developed lymphomatous disease, the average age of appearance was 725 days, with the earliest spontaneous case being observed at 490 days. Only one of the 32 cases was a mediastinal (thymic) lymphosarcoma (Table I). In the CBA strain also (Table II) the average age of appearance of leukemia was late, 608 days, with only 2 of 20 spontaneous cases appearing before 515 days. None of these spontaneous cases was a mediastinal

lymphosarcoma. Thus, out of a total of 52 spontaneous cases of lymphomatosis in these 2 stocks of mice only one was a mediastinal lymphosarcoma. The low incidence of mediastinal lymphosarcoma can be correlated with a delayed appearance of systemic lymphomatosis (leukemia). Where spontaneous leukemia appears relatively early, as in the F strain, the incidence of mediastinal lymphosarcoma is high.(7) Microscopic study of early mediastinal lymphosarcoma indicates that in the mouse the site of origin is within the thymus itself.(8) Thymectomy has decreased the spontaneous incidence.(9)

When threshold leukemogenic doses of x-

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TABLE III.
Occurrence of Lymphomatosis in dba Mice Receiving Threshold Doses of Methylcholanthrene and X-rays
Either Singly or in Combination.

Agent	Total mice	Mice over 250 days	Total lymphoma	Mediastinal lymphoma	Age lymphoma (in days)	Dead and negative	No. living*
200r	78	38	0	0	—	13	69
18MC	105	94	21	2	325	47	37
200r + 18MC	97	78	29	14	320	20	48
Controls	117	117	19	3	350	95	0

* The oldest living mice are 700 days of age.

rays and estradiol dipropionate were given simultaneously to Bagb albino or CBA mice, synergistic effects were observed. Although a large per cent of the mice are still living (Tables I and II), of the 39 mice on combined treatment which have died 25 had lymphomatosis, and in 22 cases the disease was mediastinal lymphosarcoma.

Methylcholanthrene is not leukemogenic for these stocks.(6) When 18 skin paintings of 0.25% solution in benzene were combined with either 200 r of x-rays or 5 micrograms of estradiol dipropionate weekly for 14 weeks, the incidence of lymphomatosis was no greater than when either of the latter agents was administered alone (Tables I and II). In the dba stock, in which methylcholanthrene is leukemogenic,(3,6) the incidence of lymphomatosis was increased (Table III) when x-rays and the carcinogen were given simultaneously. The increased incidence can be accounted for solely by the greater number of mediastinal lymphosarcomas, the incidence of generalized lymphomatosis being the same whether methylcholanthrene was given alone or combined with x-radiation.

These experiments indicate that the thymus of young mice of certain strains contains lymphoid tissue which is very sensitive to the lymphomogenic action of certain agents. Al-

though systemic lymphomatosis (leukemia) developed at an advanced age in the Bagb albino and CBA stocks, spontaneous thymic lymphosarcoma appeared only once in a population of 164 controls. Thus lymphocytic neoplastic transformation within the thymus represents a disease which appeared spontaneously only occasionally although it could be readily induced.

A total of 70 μ g of estradiol dipropionate acted as a synergist in the induction of mediastinal lymphosarcoma. This amount of estrogen, when given from 42 to 140 days of age, was not sufficient to inhibit the ability of test animals to resume reproductive activity. In the development of spontaneous leukemia multiple factors may be operating synergistically so that significant causative ones may be obscured.

Summary. Synergistic lymphomogenic effects were obtained when estrogenic hormone and x-rays were administered simultaneously to mice of the Bagb albino and CBA stocks. Thymic lymphoid tissue appeared to be more sensitive than other lymphoid tissue. This was also true when x-rays and methylcholanthrene acted synergistically in the dba stock.

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