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On the Sensitivity of Different Tissues in Street Strain Mice to 9,10-Dimethyl-1,2-Benzanthracene

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It is apparent from recent investigations into the action of carcinogenic hydrocarbons on mice of inbred strains that new growths may be produced in other organs than the site of application. This holds, however, only for the kinds of tumor which occur spontaneously in the strain in question, these tumors appearing earlier and/or in increased numbers. Simultaneously in 1939 Bruus and Marble (3), Engelbreth-Holm (4), and Mider and Morton (17) were the first to call attention to this acceleration of the spontaneous development of tumors. Following the application of carcinogenic hydrocarbons they observed an acceleration of the development of leukemia in the strains used. These investigations have later been confirmed and elaborated by the same researchers as well as others: Morton and Mider (18), Law (11), Kirschbaum, Strong and Gardner (10), Kirschbaum and Strong (9), Kirschbaum and Kaplan (8), Engelbreth-Holm and Lefèvre (6), Engelbreth-Holm and Poulsen (7), and Lefèvre (12).

Acceleration has also been observed of mammary carcinomas occurring in certain strains of mice by Maisin and Coolen (14), Engelbreth-Holm (5), Engelbreth-Holm and Lefèvre (6), Engelbreth-Holm and Poulsen (7), Lefèvre (12) and of pulmonary adenomas by Andervont (1, 2), Lynch (13), and Lefèvre (12).

The most important feature about this process of acceleration is the fact that only tumors encountered spontaneously lend themselves to acceleration. This fact forms the basis of Lefèvre's view (12) that carcinogenic hydrocarbons are only capable of "inducing"

new growths in tissues where spontaneous tumors are met with, but not in others.

For the purpose of elucidating this question and, if possible, arriving at an explanation of the mechanism of the acceleration the writers of this paper started a series of experiments. One of these will be reported below.

In this series a certain carcinogenic hydrocarbon was applied in small doses directly to various tissues—tissues which develop tumors spontaneously as well as those which do not. The purpose of this procedure was to investigate whether the process of acceleration observed, e.g. following painting of the skin, is due simply to the fact that certain tissues are more susceptible to carcinogenic action (because of special inherited qualities) than others, and whether new growths can be produced in tissues which do not exhibit spontaneous malignant growths.

MATERIAL AND TECHNIC

The experiments were carried out on mice of the Street strain, aged 4 to 6 weeks. This strain has not been inbred by brother to sister mating for a sufficient number of generations to secure the complete genetic uniformity obtainable by a longer period of inbreeding. Genetically it is, however, so pure that the incidence of new growths in mice of this strain has been constant during latter years. Leukemia has been observed in about 1 to 2 per cent, mammary carcinoma in about 7 and 35 per cent in non-breeding and breeding mice respectively, and pulmonary tumors in about 3 per cent (12). Moreover, a few cases of tumors have been observed in other organs (carcinoma of the liver, hemangioma of the spleen, granulosa-cell tumor of the

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ovary, carcinoma of the small intestine, squamous-cell carcinoma of the skin).

The mice used for each experiment were litter mates, as far as possible an equal number of males and females. Half of each litter was left untreated, serving as controls.

In the experimental animals 0.02 mgm. of 9, 10-dimethyl-1, 2-benzanthracene dissolved in 0.01 cc. of paraffin was injected directly into one of the following tissues: thymus, lymph node, subcutaneous tissue, mammary gland, testis, lung, spleen, kidney, bone marrow.

The following technic was employed in injecting the hydrocarbon: Injection into the thymus was made by plunging the needle from the upper border of the manubrium sterni, about 2 to 3 mm. down, immediately behind the sternum, where the hydrocarbon was placed. Before injecting an inguinal lymph node the skin was cut about 1 cm. medial to the lymph node. The skin was pulled to one side and injection made directly into the lymph node. The site of injection into the subcutaneous tissue was in the left flank, approximately midway between the axilla and groin. Injection into the mammary tissue was made by placing the hydrocarbon subcutaneously below one of the lower nipples. Injection was made into the testis after the latter had been made to descend into the scrotum by means of light pressure. Injection into the lung was made by pushing the needle through the abdominal wall immediately below the right costal border, through the diaphragm and up through approximately two-thirds of the length of the thorax. Before injecting the spleen the skin was incised longitudinally on the abdomen, about 1 cm. lateral to the midline; the spleen was grasped with a forceps through the abdominal wall. This maneuver fixed the position of the spleen sufficiently for making the injection through the abdominal musculature. The needle was plunged from the lower pole of the spleen through about two-thirds of its length. Injection into the kidney was performed with the same technic with the only difference that the kidney was exposed by pushing the overlying organs to one side by a gentle pressure with a forceps. The needle was plunged through the lower pole of the kidney upwards through about two-thirds of its length. Injection into bone marrow was made by injecting the hydrocarbon into the medullary canal of the femur. For this end the knee was placed in extreme flexion, the bone between the two condyles pierced, and the needle plunged into the medullary canal of the femur.

EXPERIMENTS

Table I shows the number of mice treated in these experiments, the number of mice reaching an age greater than 3, 6, 9, and 12 months respectively, and the number of survivors. Fifty to 100 mice were used in each experiment and a varying number of mice (up to one-fourth) still survive 13 to 15 months after the start of the experiments.

The death of a comparatively large number of mice within the first months after the injections can hardly be ascribed to the treatment. The mortality was mainly due to an epidemic of enteritis which prevailed among the mice for some length of time. This appears also

	No. of exper. mice					No. of control mice						
	At start of exper. Aged 1 mo.	3 mos.	6 mos.	9 mos.	12 mos.	Surviving	At start of exper. Aged 1 mo.	3 mos.	6 mos.	9 mos.	12 mos.	Surviving
0.02 mgm. of 9,10-Dimethyl-1,2-benzanthracene injected into												
Thymus	103	68	29	25	13	12	103	78	44	33	17	17
Inguinal lymph node	64	63	27	24	24	23	68	53	31	29	27	25
Subcutaneous tissue	98	68	44	18	17	15	96	68	41	23	17	16
Mammary gland	101	62	29	17	13	12	99	72	39	30	17	15
Testis	96	63	32	23	14	13	102	69	37	25	19	14
Lung	106	82	44	36	33	29	101	76	40	27	23	21
Spleen	80	61	39	29	27	20	79	66	36	20	16	14
Kidney	79	64	29	17	9	6	79	59	33	30	27	26
Bone marrow	50	44	22	21	20	20	49	48	24	24	21	21

from the fact that the mortality was, practically speaking, the same among experimental and control mice (Table I).

Table II contains a survey of the tumors which so far have been observed among the experimental and control mice. In the fractions the numerator represents the number of tumors observed, and the denominator the number of survivors at the time of death of the first tumor-bearing mouse within the experiment in question. The adjacent columns give the survival time (in months) of the tumor-bearing mice.

	0.02 mgm. of 9, 10-Dimethyl-1,2-benzanthracene injected into		No. of exper. mice exhibiting distant tumors (thymus)		No. of controls exhibiting tumors	
	No. of exper. mice exhibiting local tumors	Survival time, mos.	No. of exper. mice exhibiting distant tumors (thymus)	Survival time, mos.	No. of controls exhibiting tumors	Survival time, mos.
Thymus	8/68	3, 3, 3, 4, 4, 5, 7, 8	—	—	—	—
Inguinal lymph node	—	—	2/54	4, 5	—	—
Subcutaneous tissue	—	—	—	—	—	—
Mammary gland	—	—	—	—	2/30	9, 11
Testis	—	—	—	—	—	—
Lung	1/33	12	2/52*	5, 7	—	—
Spleen	—	—	—	—	—	—
Kidney	—	—	1/19	7	—	—
Bone marrow	—	—	—	—	—	—

* See Discussion.

Experimental as well as control mice exhibited only those kinds of tumor which are characteristic of the Street strain, *i.e.* leukemia, mammary carcinoma, and pulmonary tumor.

So far the controls have developed only 2 cases of spontaneous tumors, both mammary carcinomas. The

absence of leukemia and lung tumors is no doubt due to the relatively brief lapse of time since the experiments were performed, *i.e.* 13 to 15 months. It appears from earlier statistics of spontaneous tumors in the Street strain (12) that mammary carcinoma was observed in non-breeding animals from the age of 12 months and in breeding mice from the age of 6 months. Leukemia, on the other hand, usually did not appear until the age of 14 to 15 months, although a few cases were observed at the age of 7 to 8 months and one in a mouse aged only 4 months. Pulmonary tumors were not met with until the age of 19 months. Hence, the absence of leukemia and pulmonary tumor among the controls of the present experiment is in accordance with earlier observations.

Another feature apparent from the same statistics of spontaneous tumors in the Street strain (12) is that leukemia was usually generalized, exhibiting more or less marked leukemic changes in lymph nodes, spleen, and liver, and most often also lymphosarcomatous enlargement of the thymus. Only the case just mentioned of leukemia encountered in a 4 months old mouse, appeared in the form of a tumor of the thymus without leukemic changes in other organs.

As apparent from Table II injection of 0.02 mgm. of 9, 10-dimethyl-1, 2-benzanthracene into the thymus of 68 mice was followed by leukemic thymic tumors in 8 cases. The age of these 8 animals varied from 3 to 8 months. The same dose of hydrocarbon injected into an inguinal lymph node resulted in 2 cases of thymic tumor at the age of 4 and 5 months respectively among 54 mice. Following injection of the hydrocarbon into the lung, 2 cases of thymic tumor were observed at the ages of 5 and 7 months among 52 mice. In addition, a pulmonary adenoma was observed in a 12 months old mouse among a number of 33. Injection into the kidney was followed by 1 thymic tumor in a mouse aged 7 months.

Injection of the hydrocarbon into the subcutaneous tissue, mammary gland, testis, spleen, and bone marrow has so far failed to cause any tumors.

In all cases microscopical examination was made of the thoracic organs, the liver and kidney. The lymph nodes were examined in only a few cases, apart from the experiments in which injection was made into the lymph nodes.

Microscopical examination of the thymic tumors encountered revealed a picture exactly like the one met with in spontaneous leukemia. Nearly all cases exhibited invasive growth into the heart and pulmonary tissue. Moreover, nearly all the mice affected with thymic tumor had perivascular and peribronchial infiltrates in the central area of the lungs. Liver, kidney, and spleen failed to exhibit leukemic changes in most of the animals. Of the 8 mice dying from thymic tumor fol-

lowing injection into the thymus, only 1 exhibited a moderate leukemic infiltration in the liver. Moreover, 2 additional mice in the same experiment revealed leukemic changes in the kidneys. The lymph nodes were found to be of normal size except in one of the 2 cases exhibiting thymic tumor following injection into an inguinal lymph node. In addition to the thymic tumor, this mouse revealed some perivascular and diffuse infiltration in the liver and beginning leukemic changes in the peripheral lymph nodes which were slightly enlarged. These changes were rather more marked in the right inguinal lymph node which was the site of the injection, than in the other lymph nodes, but it was not a case of local lymphosarcoma. The other mouse, which had received the injection into an inguinal lymph node, failed to exhibit a similar condition. All its peripheral lymph nodes were normal.

Neither gross nor microscopical changes could be observed at the site of injection in those animals that died from thymic tumor following injection of the hydrocarbon into the lung or kidney.

The pulmonary adenoma met with in one mouse, aged 12 months and not affected with thymic tumor, proved to be a typical solitary adenoma, about 1 mm. in diameter. Still, pulmonary adenomas may have occurred in other instances, as microscopical examination was made only in cases with grossly visible changes. Therefore, adenomas visible by microscopical examination only, may have been overlooked.

During the 13 to 15 months' experimental period none of the experimental animals exhibited mammary carcinoma.

DISCUSSION

It appears from the experiments described above that injection of 0.02 mgm. of 9, 10-dimethyl-1, 2-benzanthracene in paraffin into mice of Street strain was followed by the development of leukemic tumors of the thymus only, apart from a single case of pulmonary adenoma.

Among the total of 13 thymic tumors 8 occurred following injection of the hydrocarbon into the thymus gland. Probably the 2 cases encountered following injection of the hydrocarbon into the lung belong to this experimental group, as the hydrocarbon may have been placed in a site whence it has been capable of acting directly upon the thymic tissue. Following injection into the lung the hydrocarbon in the paraffin solution usually settles in the pleural cavity between the pulmonary lobes. In a few cases of deaths within a couple of months of the injection, the paraffin was found in the pleural cavity immediately adjacent to the mediastinum.

One case of thymic tumor, occurring after injection of the hydrocarbon into the kidney, developed so late

(in a 7 months old mouse) that it may have been spontaneous, considering that spontaneous leukemic tumors of the thymus in Street mice are observed in animals aged 7 to 8 months (12). The same may apply to one of the thymic tumors encountered following injection into the lung (in a mouse aged 7 months) and to 2 of the 8 cases (in mice aged 7 and 8 months) following injection into the thymus. On the other hand, it is extremely unlikely that the remaining 6 thymic tumors, occurring in 3 to 5 months old mice among this group of 68, should be spontaneous. The same applies to the two cases occurring in mice aged 4 and 5 months respectively following injection of the hydrocarbon into a lymph node among a group of 54 animals. On the whole it must be kept in mind that the controls exhibited no case of leukemia.

The experiments have shown that 0.02 mgm. of 9, 10-dimethyl-1, 2-benzanthracene is capable of producing new growth in thymus only. In other words the thymus is considerably more susceptible to carcinogenic action than the other tissues investigated.

This accords well with the observation reported by McEndy, Boon and Furth (16). They found the incidence of spontaneous leukemia in mice of the high leukemia stock Ak to fall after thymectomy, from 77 to 8 per cent in females and from 61 to 11 per cent in males. The interpretation of these results by the authors is that the effect of thymectomy is due either to "removal of site of potentially malignant lymphoid cells" or "inhibition of neoplastic growth in general" or possibly both. The experiments described in the present paper appear to lend support to the theory that the thymus is the site of "potentially malignant cells."

Injection of the hydrocarbon into a lymph node, performed in one of the experiments, seems to indicate that the lymphatic tissue also is more susceptible to the action of carcinogenic hydrocarbon than the other organs investigated, excepting the thymus. As yet, however, the figures are too small to form the basis of definite conclusions.

As stated above, spontaneous leukemia in Street mice is usually attended with major or minor generalized leukemic tissue changes, frequently also with lymphosarcomatous degeneration of the thymus. In the present experiments, on the other hand, the leukemia was characterized by the development of a thymic tumor, in nearly all cases without leukemic changes in other organs.

It has been shown experimentally that leukemic changes develop in the course of a brief space of time, before the leukemia becomes manifest, in cases of spontaneous leukemia (Saxton, Boon and Furth [19]) as well as of induced leukemia (McEndy, Boon and Furth [15]). Considering the usually more rapid

course of induced leukemia on the whole, it is not at all unlikely that the development of the thymic tumors occurring in the experiments described above was so quick that death ensued before major changes had time to manifest themselves. One cannot, however, rule out the possibility that the induced thymic tumors have had a stronger tendency to lymphosarcomatous growth without dissemination of the malignant cells than usually encountered in cases of spontaneous thymic tumors.

Injection of 0.02 mgm. of the hydrocarbon into the mammary tissue failed to result in the development of mammary carcinoma. One might be entitled to expect it, considering that painting with carcinogenic hydrocarbons (Engelbreth-Holm [4, 5], Engelbreth-Holm and Lefèvre [6], Lefèvre [12]) proved capable of producing an acceleration of spontaneous mammary carcinoma. In these cases it was, however, a question of experiments with other strains of mice, *i.e.* Aka and Little's dilute brown. Using Street mice Lefèvre (12) did not succeed in producing any acceleration of the development of mammary carcinoma by painting with 9, 10-dimethyl-1, 2-benzanthracene or with methylcholanthrene. Still, Engelbreth-Holm and Poulsen (7) have observed acceleration of mammary carcinomas in Street mice following ingestion of 9, 10-dimethyl-1, 2-benzanthracene. No explanation has as yet been found of this apparent disagreement. Thus, there seems to be a difference between the various strains exhibiting spontaneous mammary carcinoma as regards the acceleration of mammary growths. This difference is presumably of genetic origin or possibly due to a difference regarding the presence of the milk factor.

Lung tumors were not encountered in the present experiments, a surprising finding in view of the fact that Lefèvre (12), painting Street mice, found a marked acceleration of the development of lung tumors. Possibly the explanation is that the hydrocarbon has been used in too small doses or that the time of observation has been too short.

The experiments have clearly shown the *different susceptibility to a given dose of a given hydrocarbon on the part of the different tissues*. In the strain investigated, in which leukemia is the growth most easily accelerated, the thymus has proved to be far more susceptible than any other injected tissue, reacting by developing growths following action from 0.02 mgm. of hydrocarbon. This dose was unable to produce local growths in any other tissue in the course of the experimental period (about 1 year).

The tumors most commonly met with in the strain: mammary carcinoma and pulmonary adenoma, failed to appear within the period of observation following local injection of 0.02 mgm. of the hydrocarbon.

The great susceptibility of the thymus might indicate a reason for the accelerated development of leukemia following painting of the skin. Possibly, small quantities of hydrocarbon, absorbed from the skin and circulating in the organism, are sufficient to produce neoplastic growth in particularly susceptible tissues, but not in others.

SUMMARY AND CONCLUSION

9, 10-Dimethyl-1, 2-benzanthracene, 0.02 mgm., was injected into each of the following tissues: thymus, lymph node, subcutaneous tissue, mammary gland, testis, lung, spleen, kidney, bone marrow. Leukemia (lymphosarcomatous tumor of the thymus, in nearly all cases without other leukemic changes) was observed following injections, into the thymus, in 8 of 68 mice; into a lymph node, in 2 of 54 mice; into the lung, in 2 of 52 mice; and into the kidney, in 1 of 19 mice. Apart from a single pulmonary adenoma, no other growths were encountered. Injection of the hydrocarbon into the other organs mentioned failed to cause local tumor formation. The controls exhibited two cases of mammary carcinoma but no leukemia.

It is concluded that the thymus is more susceptible to the direct application of the hydrocarbon used than are the other tissues investigated.

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