Damage to the Thymus and Other Lymphoid Tissues from 3-Methylcholanthrene, and Subsequent Thymoma Production, in Mice*

KIMIO YASUHIRA†

(Dept. of Medical Microbiology and Dept. of Pediatrics, Stanford University, Palo Alto, Calif.)

SUMMARY

Serious damage in lymphoid tissues was seen on post-mortem examination of CF1 Swiss and C57BL mice subjected to intraperitoneal injection of 3-methylcholanthrene (MC) between 12 hours and 9 days after birth. In life such mice exhibited an acute or chronic wasting syndrome clinically and histologically similar to runting syndromes occurring after immunological procedures, but presumably different in origin.

Thymomas were produced in many of the CF1 Swiss mice surviving more than 2 months after MC injection, but not in any C57BL mice. Lung adenomas and some other tumors were produced in other CF1 mice between 5 and 8 months after treatment.

The thymus is a lympho-epithelial organ whose function other than the production of lymphocytes had not been established until several years ago, although it had been the subject of much experimentation. Recently, there has been increased impetus to study the role of the thymus in immunological processes. It is well known that animals in the neonatal period are deficient in responses to antigenic stimulation, although at that time the thymus is relatively predominant in its developmental phase. In spite of the presence of abundant lymphoid cells, "thymocytes," antibody production is negligible in thymus tissue (4, 8). Furthermore, it has been reported that thymectomy has no effect on the immune response in some experiments involving young adult animals (8, 15, 16). On the other hand, MacLean et al. (21, 22) suggested the possible participation of the thymus in the control of antibody production on the basis of the simultaneous occurrence of acquired agammaglobulinemia and benign thymoma in man; but the experimental data did not appear to prove this postulation.

In contrast, a striking effect of thymectomy on antibody production has recently been established in newborn animals. Miller (26), Martinez et al. (24), and Arnason et al. (2) have reported that homograft rejection or delayed hypersensitivity, as well as antibody responses to certain humoral antigens (3, 11), is markedly depressed in appropriately sensitized animals that have been thymectomized at birth. Similar results have been seen in experiments on chickens subjected to bursectomy. The bursa of Fabricius is a lympho-epithelial organ that occurs in birds, and it is regarded as being similar to the mammalian thymus anatomically (1) and histologically (10). It is of interest that Meyer et al. (25) have observed serious damage to the development of the bursa after treatment of chicken embryos with testosterone. According to Mueller et al. (27) and Papermaster et al. (28), bursectomy provides a striking effect in depressing the antibody response in young chickens, regardless of whether the procedure has been carried out surgically or chemically.

It is well known that the thymus may undergo marked atrophy as the result of accident, serious diseases, or intoxication. In the experiments reported here, striking effects of 3-methylcholanthrene on the thymus and other lymphoid tissue of newborn mice have become evident. This might be described as "chemical thymectomy" with methylcholanthrene, in analogy to chemical bursectomy with testosterone. As a result of the regression of lymphoid tissues, a considerable number of the animals died of a runt-producing disease during the first 2 months following treatment. In addition, thymomas and lung tumors were frequently induced in surviving animals. Thymomas were produced in albino Swiss mice, as shown by Pietra et al. (30, 31), but not in C57BL mice. These results are to be contrasted with those obtained by Kaplan (17), who used radiation to produce thymomas in C57BL mice. Strain specificity in thymoma production may be a subject of interest in considering the possible role of immunological responses in carcinogenesis.

MATERIALS AND METHODS

Methylcholanthrene (MC) solution.—A 0.3 per cent solution of MC in heavy mineral oil was prepared. The mix-
ture became clear on standing overnight after the addition of a small quantity of acetone. A 1 per cent mixture was also prepared in the same manner, although some of the MC remained undissolved.

**Animals.**—A stock strain of CF1 mice and an inbred strain (C57BL/Ka) were used for these experiments. The CF1 originated from an albino Swiss stock and has been bred in this laboratory for many years. The other strain, C57BL/Ka, was kindly supplied by Dr. Henry S. Kaplan of the Department of Radiology.

**Treatment of mice with MC.**—Mice of both strains were given a single intraperitoneal injection of one of the MC solutions at various times from 12 hours to 40 days after birth. All treated mice were inspected daily and were weighed every 10 days. Dead animals were subjected to thorough autopsy. In some litters all treated newborns were killed by the excited mothers immediately after treatment. These cases are omitted from the data.

**Autopsy examination.**—After gross examination suitable organs were removed for weighing and were fixed in formalin. The tissues were sectioned in paraffin blocks and were stained with hematoxylin and eosin.

**RESULTS**

**Development of organs with age in control animals.**—Fig. 1 shows relationships between increasing body weight and lymphoid organ weight in the two strains of mice used in these experiments. In CF1 mice, the increases in the weights of the spleen and lymph nodes are in good proportion to the increases in body weight during the first 3 months after birth. The thymus shows a characteristic involution after 1 month of increasing weight.

Development of organs with age is recognizable histologically during the 1st month after birth. The thymus, lymph nodes, spleen, kidneys, liver, thyroid gland, and other organs are especially immature at birth, and development after birth is significant in some of them. For example, the thyroid gland consists only of primitive cells, and the lymphoid tissues have only a small number of lymphocytes scattered in the fibrocytic network at birth; the thymus appears somewhat more developed, since it is differentiated into two parts, and the cortex consists of typical thymocytes.

**Toxicity of MC for C57BL mice.**—The toxicity of MC was tested in C57BL newborn and suckling mice. The details of the experiments and the results are indicated in Table 1, which shows the numbers of deaths and the varieties of toxic effects observed. A dose of 1 mg. of MC was highly toxic to the newborn, resulting in six deaths among 31 treated mice on the 1st or 2d day after treatment (Table 1, Exp. I, A). Nineteen additional animals died with signs of acute wasting disease within the 1st month, exhibiting diarrheæ, weight loss, and marked regression of lymphoid tissues at autopsy. The remaining six animals also died in the following 2 months, with signs of chronic wasting, which is characterized by marked retardation of growth.

In Experiment I, B and C, it was found that 0.3 mg. of MC was much less toxic than 1 mg. to newborn or 3- to 5-day-old mice, although the additional application of 1 mg. of MC 1 week after the first treatment resulted in lethal damage to these animals. In the next experiments (Exps. II, A to F) the toxic effects of smaller amounts of MC were determined in animals of various ages. It is clear from these trials that mice over 7 days of age are resistant to the dosage of MC used.

The growth curves of the mice in these experiments are shown in Chart 2. The depressive effect of MC on animal growth is evident in all groups of mice tested with the drug and is dependent on the dosage of the drug and the age of the animals.

**Anatomical evidence of MC toxicity in C57BL mice.**—All mice in Experiments I and II dying after more than 10 days were autopsied, and the tissues were studied histologically. In nineteen out of 58 cases accumulations of milky fluid were found in the pleural and peritoneal cavities. The cells in the fluids were lymphocytic and appeared to be of normal character. Some attempts to transplant the cells into newborn animals provided no evidence suggesting a possible leukemic nature.

A marked reduction in the lymphoid tissues was seen in the treated animals as contrasted with the nontreated animals of the same age (Table 2). This depression was most significant in the mesenteric lymph nodes, which were invisible after treatment in the majority of cases. Spleens were also markedly reduced in size and appeared fibrous and anemic. They sometimes weighed only 2 or 3 mg., even at ca. 1 month of age. Damage to the thymus was serious. In some cases the thymus was invisible, and in others it appeared one-tenth the size of the normal organ or even smaller.

In contrast, no reduction in lymphoid tissue was observable in animals sacrificed at ages of more than 100 days (Table 2). Such animals were all in the groups of Experiments II, B, C, E, and F which were treated with rather small amounts of MC or at rather advanced ages. Early changes in the lymphoid tissues of these animals subsequent to treatment have not been checked periodically, but no significant damage has been demonstrated in some animals killed at ages of 1–3 months.

**Microscopic findings in C57BL mice treated with MC.**—There were marked microscopic changes in the lymphoid

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tbody>
<tr>
<td><strong>TOXIC EFFECTS OF MC IN C57BL MICE</strong></td>
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<tr>
<td><strong>EXPERIMENTAL CATEGORY</strong></td>
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<tr>
<td><strong>AGE (days)</strong></td>
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<tr>
<td>I, B</td>
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<td>I, C</td>
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<td>II, A</td>
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<td>II, E</td>
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<td>II, F</td>
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</tbody>
</table>

* Deaths after 30 days of age.
† Deaths after 1 month of age.
tissues of mice treated with MC at birth or a few days after dying at 10-20 days of age. The thymus showed massive degeneration in the cortex. Thymocytes were sensitive to MC and were completely destroyed after treatment. Extensive pyknosis and karyorrhexis were evident, together with leukocytic infiltration in all cases.

After resolution of necrotic substances, the cortex revealed a new cell sequence completely different from the ordinary one. Instead of normal thymocytes, the sequence consisted of large cells similar to fibrocytes—i.e., they had abundant protoplasm, and the nuclei were less chromatophilic. In the medulla lymphoblasts were also sensitive to MC, like thymocytes; and, hence, fibrocytes became predominant, only a small number of small lymphocytes resistant to the drug being scattered through the fibrous tissues. The appearance resembled that of a scar and was similar in nature to that of lymphoid damage in runting animals caused by homotransplantation technic (7) in this laboratory. This characteristic cell appearance was designated "fibrolymphocytic" degeneration in this paper.

After treatment lymphoid cells were reduced in the spleen and in the lymph nodes, much as in the thymus. Germinal centers in the secondary nodules disappeared, lymphoblasts in the white pulp were destroyed, and small lymphocytes were scattered in the fibrocytic scar. The appearance of the cells in these tissues was also of the type referred to here as "fibrolymphocytic." Proliferation of giant cells was frequently seen in the spleen as a sign of fibro-lymphocytic degeneration. In some cases, the spleen was infiltrated with copious myeloblasts. The proliferation of these myeloblasts seemed to originate in situ, was limited to the spleen, and had no tendency toward malignant degeneration as yet. This proliferation was due to regeneration of the damaged tissues, whereas myeloblasts generally appear at birth. Lymphocytes also increased in number in the lymphoid tissues of animals surviving more than 20 days after treatment.

A conspicuous degeneration of young cells was also observed in the bone marrow. In particular, degeneration and reduction of myeloblasts were marked in the marrow, being associated with significant hemorrhage. Atrophy of the tissue was evident and seemed to be lethal to the animals. In some cases small configurations of myeloblasts appeared in parts of the atrophied marrow of mice more than 25 days after treatment. The proliferation of myelo-
blasts gradually spread through the whole marrow, inducing a tendency toward leukemoid reaction in the animals.

The thyroid gland is another organ that shows marked retardation of development after treatment with MC at birth. In many of the treated animals showing acute and chronic runting syndrome, the thyroid gland appeared as only a configuration of immature primitive cells. No tubular structures or accumulations of colloidal substances were seen. The disturbance in the development of the thyroid was especially significant in animals with the chronic runting syndrome, and the cell configuration seemed to be similar to that in some cases of thyroid tumors.

In many treated animals lymphocytic infiltration, although visible in the liver, was insignificant. Hydroceles in the kidney were characteristic, indicating the toxicity of MC to newborn mice, and they appeared, without any signs of nephrosis, only in mice treated with a large amount of the drug at birth.

Wasting disease, thymoma, and lung tumor in CF1 mice.—To test the toxicity of MC in CF1 mice, 0.3 or 1.0 mg. of the drug was injected into newborn or suckling mice of this strain. Although these dosages were highly toxic for these mice, the results obtained were relatively different from those seen in C57BL mice with respect to the occurrence of thymomas and lung tumors. The details of the experiments and results are indicated in Table 3.

1. Wasting disease: A single injection of 0.3 mg. of MC was so toxic to newborn CF1 mice that half of them died with the acute or chronic wasting disease (Exp. III, A). A depression in body weight was evident in this group: the average weight of 5.5 gm. at the age of 1 month was in significant contrast to the 12 gm. of controls. The same amount of the drug did not affect suckling or young adult mice, with the exception of one litter (Exp. III, B and C). Suckling mice more than 5 days of age were also resistant to a dosage of 1 mg. (Exp. IV, C), although newborn and 3-day-old mice were susceptible (Exp. IV, A and B). Doses of 0.3 mg. at birth and 1.0 mg. on the 7th day were
lymphoid tissue in the acute wasting disease. Consider animals some days before death. Atrophy of the lymphocytic degeneration appeared in the atrophied tissues was also evident on autopsy. Table 4 shows the weights of lymphoid tissues of animals dying of the disease in contrast to normal mice of the same strain. Histological findings in these runted animals were similar to those in C57BL mice previously described. "Fibrolymphocytic" degeneration appeared in the atrophied lymphoid tissue in the acute wasting disease. Considerable hemorrhage also occurred in the thymus, bone marrow, and other tissues. Marked disturbance in the postnatal development of tissues coexisted in the thyroid gland during the chronic wasting.

2. Incidence of thymomas: After recovering from the initial disturbances, the surviving mice were observed to gain weight again and to become as large as controls, except for some mice with the chronic wasting. In the 3d to 5th month of treatment some of the surviving mice died suddenly, without any evident signs of illness. Autopsy revealed enlarged thymuses compressing the lungs and sometimes weighing over 1,000 mg. The average weight of the tumors was 590 mg. (range, 155-1270 mg.) in the fifteen cases listed in Table 3. Lymph nodes associated with the thymus were also enlarged, but no enlargement was seen in remote nodes; for example, the mesenteric nodes were sometimes invisible in the adipose mesenterium, and the average weight was 57 mg. (range, 0-95 mg.), with the exception of one enlarged mesenteric node weighing 335 mg. There was no evident hepatosplenomegaly; the average weight of the spleen in fifteen thymoma-bearing mice was 164 mg. (range, 50-432 mg.). The weights of the spleens and mesenteric lymph nodes exhibited no significant differences between animals dying with and without thymoma (excepting runts), but these organs appeared rather regressive as compared with those in control animals. A small number of scattered lesions were observed in the lungs of about two-thirds of the thymoma-bearing mice. They were small in size and semitransparent in appearance. There were no visible lesions in other organs.

Histological examinations have led to the recognition of the tumors of cell lines in thymic tumors: lymphocytic and stem-cell type (9, 30). In lymphocytic tumors, medium-sized lymphocytes were predominant, mature and immature lymphocytes of various sizes being scattered. Stem-cell tumors consisted of so-called "undifferentiated reticular cells" with cytoplasms which were inconspicuous; and nuclei, showing distinct nuclear membranes, appeared to be relatively large. The nuclear chromatin was fine and distributed in many, and some or no nuclei were recognizable. Some of the cells were rather developed, resembling immature or mature lymphocytes. All development phases from the undifferentiated reticular cells to the lymphocytes could be pointed out in the tumors. Such thymomas appeared in five out of fifteen cases. It was clear, in some cases of thymomas in an early stage (though they are not counted as thymomas in the table), that the tumor cells surrounded venules in portal canals in the liver and in the intralobular connective tissues of the lungs, where adenomas coexisted with tumor infiltration. These

<table>
<thead>
<tr>
<th>AGE (days)</th>
<th>THYMUS (mg.)</th>
<th>SPLEEN (mg.)</th>
<th>MENT. LYMPH NODE (mg.)</th>
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<td></td>
<td>Normal*</td>
<td>Treated</td>
<td>Normal*</td>
<td>Treated</td>
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<tr>
<td>0-5</td>
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<td>—</td>
<td>20</td>
<td>—</td>
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<td>6-10</td>
<td>34</td>
<td>—</td>
<td>35</td>
<td>—</td>
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<td>11-15</td>
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<td>5.5 (0-17)</td>
<td>50</td>
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<td>9.1 (0-30)</td>
<td>55</td>
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<td>105</td>
<td>8.0 (1-15)</td>
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<td>10.5 (7-15)</td>
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<td>36-40</td>
<td>103</td>
<td>7.5 (3-10)</td>
<td>70</td>
<td>12.3 (7-25)</td>
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<td>93</td>
<td>0, 5</td>
<td>90</td>
<td>30, 20</td>
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<td>61-80</td>
<td>65</td>
<td>0, 0, 15</td>
<td>105</td>
<td>70, 15, 25</td>
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<td>55</td>
<td>0</td>
<td>125</td>
<td>15</td>
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<tr>
<td>130-190</td>
<td>90 (60-130)</td>
<td>125 (110-150)</td>
<td>95 (90-100)</td>
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<tr>
<td>135-155</td>
<td>85 (30-115)</td>
<td>135 (90-170)</td>
<td>55 (35-75)</td>
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<td>155-165</td>
<td>90 (30-140)</td>
<td>115 (90-170)</td>
<td>60 (30-90)</td>
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<td>105</td>
<td>80 (50-115)</td>
<td>140 (120-155)</td>
<td>45 (30-60)</td>
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</table>

* Average weights of five control mice.
### TABLE 3

**EFFECTS OF MC DOSAGE ON CF1 MICE**

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<tr>
<th>Experimental Category</th>
<th>Treatment</th>
<th>No. Letters</th>
<th>No. Mice Treated</th>
<th>Category of Disease</th>
<th>No. Deaths</th>
<th>Age at death (months)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5 6 7 8 9 KILLED†</td>
</tr>
<tr>
<td>III, A</td>
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<td>4</td>
<td>26</td>
<td>Wasting</td>
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<td></td>
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<td>Lung tumor</td>
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<td></td>
<td></td>
<td></td>
<td>Others†</td>
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<td>0.3</td>
<td>3</td>
<td>18</td>
<td>Wasting</td>
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<td>Lung tumor</td>
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<td>Others</td>
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<td>12</td>
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<td>Others</td>
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<td>IV, A</td>
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<td>1.0</td>
<td>4</td>
<td>30</td>
<td>Wasting</td>
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<td>Others</td>
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<tr>
<td>IV, B</td>
<td>3</td>
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<td>2</td>
<td>17</td>
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<td>V, A</td>
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<td>31</td>
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<td></td>
<td></td>
<td></td>
<td>Others‡</td>
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*Surviving at least 10 months.
† The death at 6 months was due to leukemia.
‡ Leukemia.
§ Cause of death undetermined.

adenomas were similar in nature to those about to be described.

3. Occurrence of lung tumors: After surviving 4 months some of the treated mice gradually lost weight and died with severe emaciation. Scattered lesions, exclusively adenomas, were evident in the lungs of all these mice. The lesions were the same in nature as those observed in the lungs of mice with thymoma. They were transparent or translucent and 2 mm. or less in diameter. There was no dissemination of tumors from the lungs to other organs. A majority of the tumors were benign, but others tended to be malignant. The cells of the malignant tumors resembled those of adenocarcinoma or so-called "alveolar cell carcinoma." Deaths from lung tumors occurred in the period from 5 to 8 months of age. Many of the mice surviving more than 5 months died with such tumors.

4. Myeloid leukemia: Two cases of leukemoid reaction have been observed in CF1 mice with the acute wasting disease. One occurred in a mouse that died on the 23rd day after 1 mg. of MC and the other on the 27th day after 0.3 mg. of the drug at birth. Leukopoiesis was evident in the spleen, but there was no hepato-splenomegaly, or any leukocytosis in the peripheral blood. The appearance was the same as has already been described for C57BL mice.

In contrast to these indistinct responses in young mice, two definite cases of leukemia have been induced in adult mice. One mouse died on the 128th day after 1 mg. of MC and the other on the 156th day after 0.3 mg. The spleen weighed 550 mg. in the former case and 190 mg. in the latter. The thymus and the lymph nodes were markedly enlarged. Leukemic infiltration was histologically evident in the liver and all lymphoid tissues. The leu-
within 1 month after the treatment. The surviving ani-
to lymphoid tissues, with subsequent atrophy. These
are one of these mice.

As postulated by Billingham (6). In contrast, direct
responses of the host to previously introduced cellular anti-
gens, the wasting disease is the same as homologous disease
similar to those which were seen in homologous diseases.

CLINICAL DETAILS

IMMUNOLOGICAL PROCEDURES

Toxicity of MC is an important possibility in MC-induced
wasting, and this is the case even if the carcinogen is shown
not to play any role as an antigen in the immunological
production of the runts after combining with tissue com-
ponents in the animals. Therefore, the wasting disease
in these experiments should also be considered at present
to be different from the “homologous wasting disease” in-
duced in X-radiated hybrid mice with parental cells (18).

Haddow and his associates (12, 14) called attention to
the toxicity of carcinogenic hydrocarbons and described the
association between the carcinogenicity and the growth-inhibiting effects of the drugs (13). In some other studies
these observations were confirmed. According to Picard and Laduron (29), prolonged administration of 3,4-
benzpyrene resulted in atrophy of lymphoid tissues.

Thus, the wasting syndrome described in this paper may
be attributed to the direct action of MC in inhibiting the
growth of mice, especially in acute wasting, though Kelly and O’Gara (19) did not recognize such acute toxicity to
newborn mice of the drug at the small dose used. It
should be noted, however, that cases of chronic wasting
showed, at autopsy, disturbances in the development of
the thyroid gland. In such animals, the hypophyseal
gland was not investigated, and hence the indirect action of
MC through hormonal disturbances cannot be ruled out.

The likelihood of damage to corresponding tissues prior
to tumor induction has sometimes been noted, and such
damage has actually occurred to some extent in the present
study—i.e., thymomas have been produced in CF
mice after striking reduction in the thymus following ad-
ministration of MC at birth or some days later. Pietra
et al. (30, 31) reported a significant effect of carcinogenic
hydrocarbons in producing thymomas in newborn Swiss
mice. According to a recent paper by Rappaport and
Baroni (33), these thymomas were induced in the animals
by the carcinogens, although there was no definite correla-
tion between the origin of necrotic damage in the cortex of
the thymus and tumor production. In addition, attention
should be called to the fact that no thymomas have ever
been induced in C57BL mice in the experiments presented
in this paper, no matter how serious the damage done to
the thymus in this strain of mice by the application of MC
immediately after birth. That being the case, factors
other than direct damage should be considered in the case
of tumor production.

In this respect, anatomical evidence of retarded regen-
eration of damaged lymphoid tissues in the treated CF1,
but not C57BL, mice could be pointed out. This observation
suggests the importance of retardation of cell matura-
tion in tumor production, as postulated for skin papillomas
by Berenblum (5). Additionally, the retarded regenera-

**TABLE 4**

**WEIGHT OF LYMPHOID TISSUES FROM CF1 MICE Dying of the
RUNTING SYNDROME (Listed in Table 3 as Wasting Disease)**

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>THYMUS (mg.)</th>
<th>SPLEEN (mg.)</th>
<th>MESENTH. LYMPH NODES (mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal*</td>
<td>Treated</td>
<td>Normal*</td>
</tr>
<tr>
<td>0-5</td>
<td>9</td>
<td>—</td>
<td>15</td>
</tr>
<tr>
<td>6-10</td>
<td>37</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>11-15</td>
<td>67</td>
<td>17</td>
<td>50</td>
</tr>
<tr>
<td>16-20</td>
<td>98</td>
<td>3.5</td>
<td>55</td>
</tr>
<tr>
<td>21-25</td>
<td>115</td>
<td>5.3</td>
<td>80</td>
</tr>
<tr>
<td>26-30</td>
<td>135</td>
<td>4.4</td>
<td>85</td>
</tr>
<tr>
<td>31-35</td>
<td>160</td>
<td>12.0</td>
<td>105</td>
</tr>
<tr>
<td>36-40</td>
<td>145</td>
<td>19.0</td>
<td>110</td>
</tr>
<tr>
<td>41-45</td>
<td>130</td>
<td>10, 5</td>
<td>142</td>
</tr>
<tr>
<td>46-50</td>
<td>127</td>
<td>3, 10</td>
<td>100</td>
</tr>
<tr>
<td>51-55</td>
<td>103</td>
<td>30</td>
<td>190</td>
</tr>
<tr>
<td>56-60</td>
<td>90</td>
<td>—</td>
<td>245</td>
</tr>
</tbody>
</table>

* Average weights of tissues from five control animals.

kemic cells were myeloid in nature and showed all se-
quences of cell development from immature to mature
forms. Leukocytosis was evident in the peripheral blood of
one of these mice.

**DISCUSSION**

After a preliminary sketch (7) on running disease,
Billingham (6) reported that the constant and diagnostic
syndrome of the disease consists of retardation of growth
and involution of lymphoid tissues. On the basis of the
present study it is evident that the treatment of newborn
mice with MC provides a significant number of instances
of the running syndrome, similar in appearance to those
following immunological procedures. MC-induced runts
also had marked reduction of growth and serious damage
to lymphoid tissues, with subsequent atrophy. These
disturbances resulted in many deaths among the mice
within 1 month after the treatment. The surviving ani-
mal sometimes weighed only half or a third as much as
normal animals at the same ages. In treated mice lymph-
oid tissues were negligible or extremely reduced at autopsy
and revealed histologically a specific appearance which has
been called "fibro-lymphocytic." These effects were
similar to those which were seen in homologous diseases.

It is difficult at present to decide whether the MC-in-
duced wasting disease is the same as homologous disease
in pathogenesis. The latter is due to immunological re-
sones of the host to previously introduced cellular anti-
gens, as postulated by Billingham (6). In contrast, direct

FIG. 1.—Normal thymus of a 3-day-old C57BL mouse. ×150.

FIG. 2.—Thymus of a CF1 Swiss mouse that died on the 12th
day after the injection of 1 mg. of MC at birth. "Lympho-
epithelial" degeneration is seen. ×150.

FIG. 3.—"Lympho-epithelial degeneration" in the spleen of a
CF1 mouse which died on the 17th day after the same treatment
as was described above. ×150.

FIG. 4.—Thyroid gland showing marked retardation in develop-
ment in a CF1 mouse that died 23 days after neonatal injection
of MC. ×150.
Fig. 5.—Leukemoid proliferation of myelocytes in the spleen of a CF1 mouse which died 27 days after the injection of 0.3 mg. of MC at birth. ×300.

Fig. 6.—Large magnification of myelocytic infiltration in the spleen. ×1200.

Fig. 7.—Stem-cell type of thymoma in a CF1 mouse which died 5 months after injection of 0.3 mg. of MC at birth. ×300.

Fig. 8.—Large magnification of the stem-cell thymoma. ×1200.
tion may lead the animals to immunological unresponsiveness which may take part in chemical carcinogenesis, as was postulated by Prehn and Main (32) or in spontaneous occurrence of thymomas, as was suggested by McKean et al. (21). In fact, suppressed antibody responses were observed rather more extensively in MC-pretreated CF1 mice than in control mice after a test injection of a soluble protein; data confirming these findings, which previously appeared in other papers (20, 23, 34), will be reported elsewhere, though genetic susceptibility essential for thymoma production may not refer only to these phenomena.

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Kimio Yasuhira

Cancer Res 1964;24:558-569.