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Influence of Adrenalectomy on Homologous Disease.* (31064)

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It is well established that the syndrome known as "homologous disease," "runt disease" or "secondary disease" is the result of an immune reaction elicited by the introduction of immunologically competent lymphoid cells into susceptible recipients incapable of rejecting the donor cells. This graft-*versus*-host reaction has been induced in fetal(1) newborn(2) and adult animals previously made tolerant of the homologous cells(3) and in adult animals pretreated with a lethal dose of whole body X-irradiation(4). Homologous disease can also be produced by injection of parental strain lymphoid cells into genetically tolerant F1 hybrid mice(5). Animals with the disease develop anemia, lethargy, and a hunched posture, and they frequently succumb as a result of the process.

We became interested in the influence of the adrenal glands on homologous disease because we had observed that animals suffering from homologous disease frequently had enlarged adrenal glands. In addition, it had been reported by Kaplan and Rosston(6) that adrenalectomy ameliorates homologous disease. We therefore considered the possibility that homologous disease might be in part the result of adrenal hyperactivity. Furthermore, there is good evidence that the adrenal steroid hormones exert an effect upon lymphoid tissue and upon immunological responsiveness. The injection of certain adrenal corticosteroids causes involution of lymphoid organs (7) and decreases immunological responsiveness to antigenic stimulation(8,9). Conversely, adrenalectomized animals frequently develop higher titers of circulating antibody than normal animals(10).

In the experiments to be reported here, we have studied the development and course of homologous disease produced by injection of spleen cells from parental strain mice into their F1 hybrids previously subjected to bilateral adrenalectomy. These experiments demonstrate that not only does removal of the adrenal gland fail to avert homologous disease produced in this way, but such manipulation increases both the incidence and the severity of this process. They also demonstrate that these effects of adrenalectomy can be prevented by administration of cortisone acetate.

Materials and methods. Inbred mice of the A and C3H strains and F1 hybrid mice resulting from the cross between A and C57-Bl/1 and between C3H and C57Bl/1 strains were used for these experiments. The A, C3H and C57Bl/1 strains of mice have been maintained in our colony by strict brother-sister mating since 1956, and are directly descended from the inbred colony of the late Dr. J. J. Bittner. All F1 hybrid mice were between 1½ and 3½ months of age at the time they received the spleen cell injection. Spleen cell suspensions were prepared by slicing spleens into several pieces and then expressing the splenic pulp gently from the capsule in a loose fitting Potter-Elvehjem glass homogenizer. All suspensions were prepared in lactate-Ringer's solution and were injected within one hour of preparation. The dosage of spleen cells injected in all experiments was 150 million per mouse, administered intravenously in a volume of approximately 0.25 cc into one of the lateral veins

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TABLE I. Effect of Adrenalectomy on Homologous Disease Induced in (A × C57Bl/1)F1 Hybrid Mice.

Treatment before cell injection	Cell donor strain	No. cells inj (millions)	No. died/No. in group*	Mean survival time (days ± S.E.)†
Bilateral adrenalectomy	A	150	36/36	9.6 ± .3
Sham-adrenalectomy	"	"	15/16	22.9 ± 1.7
Non-operated	"	"	6/8	24.5 ± 3.8
Bilateral adrenalectomy	(A × C57Bl/1)F1	"	0/8	—

* Deaths occurring during 40 day period after cell injection.
† Mean survival time of mice dying during 40 day period after cell injection.

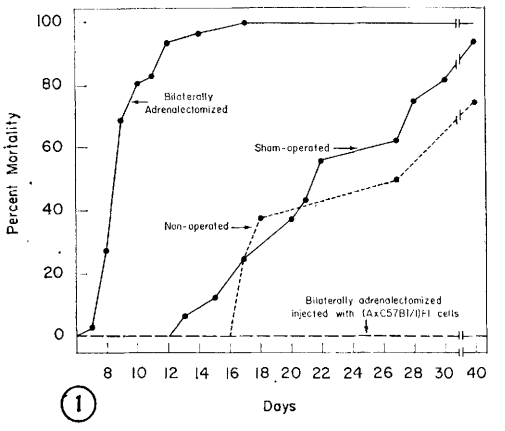
of the tail. Adrenalectomies and sham-adrenalectomies were performed 24 hours prior to spleen cell injection. All bilaterally adrenalectomized mice were maintained on 0.9% saline drinking water and Purina laboratory chow.

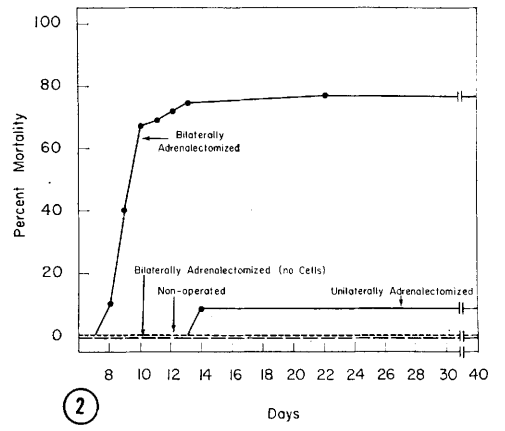
Three experiments were performed. In the first, (A × C57Bl/1)F1 hybrid mice were bilaterally adrenalectomized, sham-adrenalectomized, or non-operated prior to the injection of spleen cells from adult A strain donors. A control group of bilaterally adrenalectomized (A × C57Bl/1)F1 hybrid mice was injected with spleen cells from syngeneic (A × C57Bl/1)F1 donors. In the second experiment, (C3H × C57Bl/1)F1 hybrid mice were bilaterally adrenalectomized, unilaterally adrenalectomized or non-operated prior to the injection of spleen cells from adult C3H strain donors. A control group of bilaterally adrenalectomized (C3H × C57Bl/1)F1 hybrid mice was not injected with spleen cells. Finally, in the third experiment, 2 groups of bilaterally adrenalectomized (C3H × C57-Bl/1)F1 hybrid mice were injected with spleen cells from adult C3H donors. Mice in one of these groups received a daily subcutaneous injection of 0.25 mg cortisone acetate† for 10 days, beginning the fourth day after the cell injection, and mice of the other group did not receive cortisone acetate and served as controls. Mice in all experiments were observed and weighed daily, and at death representative mice were autopsied and tissue sections obtained for histological studies.

Results. The results of the first experiment are shown in Table I and Fig. 1. The adrenalectomized (A × C57Bl/1)F1 hybrid mice injected with spleen cells from A strain donors began to die 7 days after cell injection, and all mice in this group had died by the 17th day after injection. By contrast, the sham-operated and non-operated F1 hybrid mice similarly injected did not begin to die until 13 days following injection. By 40 days after injection 94% of the sham-operated mice and 75% of the non-operated mice had died. The mean survival times of the mice succumbing to homologous disease in these groups were 9.6 ± 0.3 days for the bilaterally adrenalectomized mice, 22.9 ± 1.7 days for the sham-operated mice, and 24.5 ± 3.8 days for the non-operated mice. There were no deaths in the group of bilaterally adrenalectomized hybrid mice injected with syngeneic hybrid spleen cells demonstrating that injection of spleen cells without immunological activity against the host does not produce death in bilaterally adrenalectomized recipients.

The results of the second experiment are shown in Table II and Fig. 2. Bilaterally adrenalectomized (C3H × C57Bl/1)F1 hybrid recipients of spleen cells from C3H strain donors began to die 8 days following cell injection, and by 13 days after injection 75% of these mice had died. Less than 10% of the unilaterally adrenalectomized mice and none of the non-operated mice had died by 40 days after cell injection. No deaths occurred in a control group of similar bilaterally adrenalectomized F1 hybrid mice which were not injected with spleen cells. The mean survival time of the bilaterally adrenalectomized mice that succumbed to homologous disease was 10.4 ± 0.5 days. Only one of the unilaterally adrenalectomized mice died 14 days after cell injection.

† Upjohn Co., Kalamazoo, Mich.





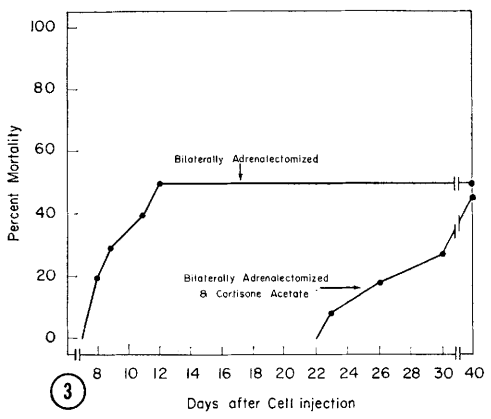


FIG. 1. Cumulative mortaility of operated and non-operated (A x C57Bl/1)F1 hybrid mice injected I. V. with 150 million A strain spleen cells. Abscissa represents days after cell injection.

FIG. 2. Cumulative mortality of operated and non-operated (C3H x C57Bl/1)F1 hybrid mice injected I. V. with 150 million C3H strain spleen cells. Abscissa represents days after cell injection.

FIG. 3. Cumulative mortality of bilaterally adrenalectomized (C3H x C57Bl/1)F1 hybrid mice injected I. V. with 150 million C3H strain spleen cells. One group received additional treatment with 0.25 mg cortisone acetate subcutaneously daily for 10 days beginning 4 days after cell injection.

The results of the third experiment are shown in Table III and Fig. 3. Two groups of (C3H $\times$ C57Bl/1)F1 hybrid mice were bilaterally adrenalectomized and injected with spleen cells from C3H strain donors. One of these groups was given no further treatment; mice of the other group were injected daily with 0.25 mg of cortisone acetate for 10 days beginning on the fourth day after cell injection. Deaths occurred in mice in the group given no further treatment after cell injection 8 days following injection. By 12 days post-injection 50% of these mice had died. In the group of mice treated with cortisone acetate the first deaths occurred 23 days following cell injection and by 40 days after cell injection 45% of these mice had died. The mean survival times for these groups were 9.6 $\pm$ 1.8 days for the untreated group and 29.4 $\pm$ 2.7 days for the group of mice treated with cortisone acetate.

Discussion. The results of the first 2 experiments demonstrate that bilateral adrenalectomy of F1 hybrid mice 24 hours before injection of parental strain spleen cells leads to early death of the adrenalectomized mice as compared to unilaterally adrenalectomized, sham-operated or non-operated F1 hybrid mice similarly injected with parental strain spleen cells. The fact that bilaterally adrenalectomized hybrid mice injected with hybrid spleen cells did not die indicates that cell injection alone, in the absence of immunological attack by the injected cells, is not a factor contributing to the early death of the adrenalectomized mice injected with parental strain spleen cells. It is of interest that in the strain combination in which very few unilaterally adrenalectomized and none of the non-operated mice succumbed to homologous disease, that is (C3H $\times$ C57Bl/1)F1 hybrids injected with C3H strain spleen cells, bilateral adrenalectomy resulted in early death of the hybrids and in a marked increase in the incidence of death from the disease. The in-

TABLE II. Effect of Adrenalectomy on Homologous Disease Induced in (C3H × C57Bl/1)F1 Hybrid Mice.

Treatment before cell injection	Cell donor strain	No. cells inj (millions)	No. died/No. in group*	Mean survival time (days ± S.E.)†
Bilateral adrenalectomy	C3H	150	30/39	10.4 ± .5
Unilateral adrenalectomy	"	"	1/11‡	—
Non-operated	"	"	0/6	—
Bilateral adrenalectomy	—	—	0/16	—

* Deaths occurring during 40 day period after cell injection.
† Mean survival time of mice dying during 40 day period after injection.
‡ One animal died 14 days after cell injection.

TABLE III. Effect of Cortisone Treatment on Homologous Disease Induced in Bilaterally Adrenalectomized (C3H × C57Bl/1)F1 Hybrid Mice.

Treatment after cell injection	Cell donor strain	No. cells inj (millions)	No. died/No. in group*	Mean survival time (days ± S.E.)†
Cortisone acetate	C3H	150	4/11	29.4 ± 2.7
None	"	"	5/10	9.6 ± 1.8

* Deaths occurring during 40 day period after cell injection.
† Mean survival time of mice dying during 40 day period after injection.
‡ Given daily, subcutaneously, 0.25 mg, the 4th through 13th day after cell injection.

creased incidence and severity of homologous disease in adrenalectomized mice are undoubtedly related to adrenal cortical secretion deprivation, since treatment with cortisone prevents the early death of the hybrids.

The results of the experiments described herein indicate that the pathogenesis of homologous disease is not due to hyperfunction of the adrenal glands. Two hypotheses can be put forward to explain our experimental findings. First, lymphoid cells injected into adrenalectomized recipients may exhibit an increased capacity to respond immunologically against the host antigens. This hypothesis would be in keeping with the findings of Char and Kelley(10) that adrenalectomized animals can show increased immunological responsiveness. Furthermore, Santisteban and Dougherty(7) have shown that within 5 days after adrenalectomy, mice exhibit an increase in total lymphoid mass. Thus, in the adrenalectomized host, injected lymphoid cells may have an increased capacity to proliferate and to function as immunologically competent cells. The F1 hybrid recipient in our experiments would not benefit from increased immunological responsiveness, since these hybrids are known to be genetically incapable of rejecting parental strain tissue. An alternative hypothesis is that, because of their decreased ability to withstand stress, the adrenalectomized mice are unable to cope with the stresses which may be an early component of the graft-*versus*-host reaction.

Our findings are apparently contradictory to those of Kaplan and Rosston(6) who reported amelioration of homologous disease in mice by adrenalectomy. These investigators, however, gave several doses of X-irradiation to their recipients, performed adrenalectomy 3 to 6 weeks prior to injection of parental strain lymphoid cells, and used thymus cells rather than spleen cells to induce homologous disease. Their experimental design was so different from ours that direct comparison of the results is impossible. Nonetheless, we would conclude from our observations that adrenal hyperactivity is not an important basic pathogenic mechanism of homologous disease and that adrenalectomy under the conditions described herein enhances rather than ameliorates homologous disease.

Summary. Adrenalectomy performed 24 hours before the induction of homologous disease, by the injection of parental strain spleen cells, resulted in early death in both (A × C57Bl/1)F1 recipients of A spleen cells, and in (C3H × C57Bl/1)F1 recipients

of C3H spleen cells. In addition there was an increased incidence of death in the latter strain combination. The early death of adrenalectomized (C3H × C57Bl/1)F1 recipients of C3H parental strain spleen cells was prevented by the administration of cortisone acetate.

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Carcinogenic Effect of N-Hydroxy-N-2-Fluorenylacetamide, 2′,4′-Dimethylacetanilide, and 2′,4′,6′-Trimethylacetanilide on Liver in Suckling Mice. (31065)

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Adult mice are resistant to liver tumorigenesis when exposed to carcinogenic hydrocarbons or urethan despite the fact that treatment with these agents will produce or enhance tumorigenesis in a variety of other organs(1,2). On the other hand, the liver is highly susceptible to the development of hepatomas when these agents or N-2-fluorenylacetamide (FAA, 2-acetylaminofluorene) are administered orally during the suckling period (3-6). In contrast, adult mice are much less prone to hepatotumorigenesis with FAA(7,8). In the present experiment, advantage was taken of the sensitivity of suckling mice to liver tumorigenesis by testing the aromatic compounds 2′,4′-dimethylacetanilide, and 2′,4′,6′-trimethylacetanilide. These chemicals are suspected of possessing weak tumorigenic activity(9,10). In addition, the well known liver carcinogen N-hydroxy-N-2-fluorenylacetamide (N-OH-FAA)(11), was included as a positive control. This compound is considered to be a proximate carcinogen derived from FAA(12). It already had been demonstrated that as little as 7 mg of FAA was sufficient to induce a high yield of hepatomas when administered orally to suckling mice(4). Since FAA is less potent than N-OH-FAA in liver tumorigenesis(12), it seemed of interest to determine the relative activity of the latter carcinogen in suckling mice when a moderate or low dose was employed.

Materials and methods. C57BL6/A_JF_1 hybrid mice of both sexes were employed. Litters born in this laboratory were divided at random among the different groups. The infants were 1 week old at the start of treatment and were weaned and separated by sex at 4-5 weeks. Animals were housed in stainless steel cages, in air-conditioned quarters, and were fed a diet of Wayne Lab Blox Chow and water *ad libitum*. The mice were examined daily and those found dead autopsied as quickly as possible. Cases of advanced postmortem changes or cannibalism were discarded and do not appear in the data. Approximately the same number of mice (Table I) were lost in each group. Surviving mice were killed at the end of the experiment and grossly visible tumors or lesions recorded and then excised for tissue processing. Tumor diagnoses were confirmed by histologic examination. The data include only those surface hepatomas that measured at least 1 mm in diameter.

All mice were treated by stomach tube, receiving 0.05 ml of inoculum per dose. The tube was introduced directly into the esopha-