The Effect of Adrenalectomy on the Susceptibility of Rats to a Transplantable Leukemia*

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Transplantable tumors, as a rule, develop more readily in young animals. A transplantable lymphatic leukemia of the rat, which has been under investigation in this laboratory (12), resembles the tumors in this respect. Animals from a susceptible strain, if inoculated under 5 months of age, prove susceptible in something over 95 per cent, while rats between 12 and 15 months of age develop the disease in less than 50 per cent. It would appear, therefore, that the lowered receptivity becomes evident even in the early middle age period, and is certainly acquired before there are definite senile changes. The parent strain (Wistar) from which our rats are derived has an estimated average life span of 33 months, with the menopause occurring between 15 and 18 months of age (4).

The regression of the thymus is the most obvious change taking place with the aging process. According to Donaldson (4) the thymus reaches its largest size in the rat at about 2½ months of age, which corresponds to the beginning of puberty. Thereafter the gland regresses, till at 1 year of age only a small fragment remains. The possibility that the underlying conditions responsible for the thymus regression may be of significance in determining susceptibility to inoculated malignant cells has not received experimental consideration.

Jaffe (10) demonstrated some years ago that removal of the adrenals was followed by regeneration of the thymus in older rats and resulted in a definite stimulation of growth of the gland in young ones. A similar stimulation of the thymus reduced in size by x-ray has been noted by Grégoire (8). In the present study advantage has been taken of this method of thymus stimulation and accompanying reactions following the removal of the adrenals on the natural resistance of old rats and induced resistance of young rats to transplantable lymphatic leukemia.

METHODS

The rats used throughout the following experiments were from a substrain of Wistar rats from which our original leukemia case was derived. Both adrenals were removed with a generous margin of surrounding fat through a dorsal incision. Immediately following the operation each animal was given 2.5 cc. of 1.25 per cent saline solution intraperitoneally and this was repeated daily for 2 to 3 weeks. Throughout the experimental period the drinking fluid provided was Ringer’s solution containing 1 per cent lactose and 1 per cent dextrose. Otherwise the diet was the standard one used in this laboratory, consisting of bread soaked in milk with oats and fox chow once a week. This postoperative procedure is a modification of that recommended by other investigators (1, 3, 7, 16).

During the first few days following operation the rats were kept under even temperature conditions. The death rate in the older animals was approximately 16 per cent and no deaths attributable to the adrenalectomy occurred later than 9 days. With the younger group (3 to 6 months) the rate was 20 per cent between the second and 13th days, with no later fatalities due to the operation occurring during the experimental period.

EXPERIMENTS

Group I.—In the four individual experiments making up this group identical procedures were carried out, and the results were so nearly the same that they may be presented together. From 60 rats surviving the immediate effects of adrenalectomy, 39 were inoculated intraperitoneally with 0.2 cc. of leukemic cells 14 to 16 days after the operation. The remaining 21 adrenalectomized animals were not inoculated, and served as survival controls. As control of the activity of the inoculum, 39 intact rats of the same age period were inoculated at the same time as the test rats with 0.2 cc. of leukemic cells. All the rats used were from the same strain, ranged in weight from 250 to 349 grams, and were estimated to be over 15 months of age.

The results of the foregoing experiments are given in Table I and the first three experiments shown graphically in Fig. 1. Of the 39 inoculated adrenalectomized animals, 38 died. Of the 21 controls, 20 were still living 90 days after the inoculation. The death rate in the test group was 97.5 per cent, and in the control group 95 per cent. Notwithstanding the acceleration of the adrenalectomized animals there was no significant difference in the rate of development of the leukemia when the inoculum was examined.
ectomized rats 89.7 per cent developed the characteristic disease picture of leukemia, manifested by extreme enlargement of the thymus and superficial and mesenteric lymph nodes, increase in circulating lymphocytes, and often involvement of the spleen. The average survival time for the diseased animals was 6.2 days, with the majority dying between the fifth and sixth day and only 1 living as long as 9 days. Of the 39 inoculated intact rats only 43.5 per cent developed leukemia, and these had an average survival time of 9.7 days. Only 1 died as early as 7 days and the majority lived for 11 days. There were no deaths among the uninoculated adrenalectomized rats for the duration of the experiment or for weeks afterwards. The extent of regeneration of the thymus may be judged by comparing the size of the gland in a normal rat in Fig. 2 with that of an adrenalectomized rat of the same age in Fig. 3.

Group II.—The Rockefeller Institute strain of hooded rats, which normally have a high degree of
resistance to our transplantable leukemia, was used in the next experiment. The adrenals were removed from 11 rats and 2 weeks later they, with 12 intact rats of the same strain, were inoculated intraperitoneally with leukemic cells. All the 11 adrenalectomized rats developed the disease promptly, with an average survival time of only 6.5 days. The control intact rats showed no evidence of the disease.

The foregoing experiments demonstrated clearly that removal of the adrenals reduces the natural resistance of older rats to inoculated lymphatic leukemia. Furthermore, resistance to the disease in at least one refractory strain of rats is completely obliterated by different intervals before and after the immunizing treatment. These results were compared with the degree of susceptibility of immunized intact rats, adrenalectomized nonimmunized rats, and untreated controls. The figures for the various groups, with records of time intervals between the different procedures, are given in Table II. All the rats used in the test were from the same strain, of about the same weight, and were approximately 3 months old. The immunizing dose of 0.5 cc. of defibrinated rat blood from another strain of rats was given intraperitoneally 14 days before the leukemia inoculation. With the exception of one small group, there was an interval of adrenalectomy. The next experiments were designed to test the effect of adrenalectomy on induced resistance in young rats, a state that develops after an injection of homologous living normal cells some days prior to inoculation. Such a procedure has long been known to induce a degree of resistance to many of the transplantable malignant tumors of animals. Sturm (15) has reported recently that susceptibility to inoculation of our strain of lymphatic leukemia may be also materially reduced by the same method.

**Group III.**—There were 5 individual experiments in this group, which include observations on a total of 253 young rats. Tests were made of the effect of adrenalectomy on induced resistance to inoculated leukemic cells when the operation was performed at different intervals before and after the immunizing treatment. As noted above, the deaths directly attributable to the adrenalectomy in our control groups occurred before the 14th day. All adrenalectomized animals received the same sustaining postoperative treatment as that used in the first experiment.

The results show clearly that removal of the adrenals definitely interferes with the development of a resistant state, which in intact animals follows the injection of homologous living normal cells. When the glands are removed before the immunizing dose the reduction in potential resistance is perhaps somewhat less than that observed when the immunizing treatment precedes the adrenalectomy. As in the first group, the average survival time of the adrenalectom-
TABLE II: Effect of Adrenalectomy on Induced Resistance to Transplanted Leukemia

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Interval</th>
<th>Procedure</th>
<th>Number</th>
<th>Leukemic survival time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenalectomy</td>
<td>24-48 hrs.</td>
<td>Immunization</td>
<td>34</td>
<td>76.5% 6.3 days</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td></td>
<td>9</td>
<td>88.8 7.1 days</td>
</tr>
<tr>
<td>Immunization</td>
<td>24 hrs.</td>
<td>Adrenalectomy</td>
<td>32</td>
<td>90.6 6.6 days</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td></td>
<td>10</td>
<td>100.0 6.1 days</td>
</tr>
<tr>
<td>Immunization</td>
<td>14 days</td>
<td>Inoculation</td>
<td>59</td>
<td>33.9 9.7 days</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>2 days</td>
<td>Inoculation</td>
<td>10</td>
<td>100.0 6.4 days</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td></td>
<td>23</td>
<td>100.0 6.5 days</td>
</tr>
<tr>
<td></td>
<td>26 days</td>
<td></td>
<td>9</td>
<td>100.0 6.1 days</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>No inoculation</td>
<td></td>
<td>10</td>
<td>All survived</td>
</tr>
<tr>
<td>Controls</td>
<td>Inoculation</td>
<td></td>
<td>57</td>
<td>96.5 8.6 days</td>
</tr>
</tbody>
</table>

ized animals that developed leukemia was significantly shorter than that of the susceptible animals of the other groups.

DISCUSSION

There has accumulated in recent years considerable experimental evidence that hormones play a role in the development and growth of certain types of malignant neoplastic disease. Estrogens have been shown to influence the development of mammary, uterine, and testicular tumors in animals (2, 6, 14) and the male sex hormone appears to stimulate the growth of prostatic cancer in man (9). In some strains cancer of the adrenals frequently develops in mice that have been castrated early in life (18). It is now demonstrated that removal of the adrenals renders a rat susceptible, or more susceptible, to a transplanted lymphatic leukemia. This gives still another indication that interference with the endocrine balance may have an influence on the development of a malignant condition.

It does not necessarily follow that because an induced condition affects susceptibility to transplants of neoplastic cells, such an induced condition may play any part in the origin of a malignant tumor. Yet there is at least one example where this is true. Estrogens may play a role in the experimental production of interstitial cell tumors of the testicle, and in several instances such tumors will grow on transplantation only in mice receiving estrogens (6, 14). While it is not definitely established that the male sex hormone is involved in the origin of prostatic cancer, there is evidence that testosterone stimulates the growth of this type of tumor and that elimination of the male sex hormone by castration, or its neutralization by estrogen, is often followed by notable diminution of growth, particularly of metastases from prostatic cancer in man (9). Considering the fact that the functional activities of certain organs are so completely under the control of the endocrines, it may well be that certain hormones may play some part in the origin and growth of tumors arising in such organs. The indication that lymphoid tissue is at least to a certain extent influenced by the endocrines (5, 13), taken with the present findings that elimination of one of the endocrine glands influences resistance to malignant lymphoid cells, is sufficiently suggestive of a possible hormonal involvement in leukemia to warrant further investigation.

There are a number of effects from removal of the adrenals besides that on the thymus, which must be considered in an analysis of our results. The fact that rejuvenation of the thymus is a prominent feature in the older rats rendered susceptible to leukemia by adrenalectomy cannot be taken as indicative of a relationship between the two conditions. The mere presence of an active thymus in young rats of a resistant strain does not affect their susceptibility, but removal of the adrenals renders these animals 100 per cent susceptible to the disease. This finding does not completely eliminate the thymus changes from consideration, for even in young animals adrenalectomy causes a definite stimulation of the still active gland. It is considered more likely that adrenalectomy results in a condition that stimulates the lymphoid tissue or releases it from control, and that these factors are equally effective in stimulating the development of the inoculated malignant lymphoid cells. If this be the true explanation, the increased susceptibility to leukemia and the stimulation of the thymus would be the result of the action of a common factor with no causal interrelationship.

There is as yet no convincing evidence that the adrenals play any significant role in cancer (11, 17).

SUMMARY

In the experiments reported removal of the adrenals reduced the natural resistance of old rats and the induced resistance of young rats to a transplantable lymphatic leukemia. Inoculation of intact, middle-aged animals of a special strain resulted in 43.5 per cent mortality, while 89.7 per cent of adrenalectom-
ized rats of the same strain and age developed the disease. Young rats with induced resistance gave 33.9 per cent takes following inoculation. Animals in which the adrenals were removed after the resistance-inducing treatment were over 90 per cent susceptible while in another group, adrenalectomized before the immunizing treatment, 78.8 per cent died of leukemia. A different strain of rats, highly resistant to the transplanted leukemia used in the tests, became 100 per cent susceptible following removal of the adrenals.

A prominent feature in the adrenalectomized rats is the regeneration of the retrogressed thymus in old animals and an active stimulation of this gland in young ones. It is suggested that the greater receptivity of adrenalectomized rats to transplanted leukemia is the result of action of the same stimulating factors on the malignant lymphoid cells.

REFERENCES

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