INFLUENCE OF LIGHT ON THE GROWTH AND MALIG-
NANCY OF A TRANSPLANTABLE NEOPLASM
OF THE RABBIT.

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One of the striking features of the disease induced by a certain transplantable neoplasm of the rabbit is the variability of its course and of the general character of its manifestations (1). Not only do individual animals differ in respect to the plane or level of malignancy but well defined group variations are also observed between series of rabbits inoculated at one time and those inoculated at another. On the whole, this variability is seasonal in character in that the disease is most severe in the spring and fall and is usually milder in the summer than in the winter months (2). The influence of the seasonal factor has been interpreted as operating upon or affecting general animal economy and susceptibility or resistance to disease is considered as a functional activity of the animal organism.

Although many external and internal factors undoubtedly participate in the seasonal variability of this malignant disease, there appears to be a correlation between sunshine and the manifestations and outcome of the condition. That is, the actual hours of sunshine, together with the rate, extent and persistence of change in the curve of sunshine over a given period, can be related to the observed general level of malignancy. The experiments upon which this relationship has been based were carried out with rabbits kept in rooms which receive practically all light diffused through ordinary window glass so that the effects observed cannot be wholly attributed to the shorter ultra-violet rays.

The idea of a correlation between the external factor of light on the one hand, and the physical state and functional activities of the animal organism on the other, has been tested by a series of experiments in which conditions of light could be controlled. The particular
points studied in beginning the work were the effects produced by a continuous illumination of maximum intensity practicable with simple equipment and by an absence of light, first upon the physical state of normal rabbits as shown by the weights of organs and second, upon the course and character of the malignant disease. The results of the first tumor experiment which were summarized in a preliminary note published soon after the conclusion of the work are here reported in full.

*Methods and Material.*

The experiment was begun on January 16 and concluded on April 15, 1925. Three animal rooms, with similar environmental conditions other than those of lighting, were employed. These rooms will be referred to as the light, the dark and the control or unaltered room, respectively.

A constant source of light was furnished by twelve 1000 watt Mazda lamps and three Cooper Hewitt 50 inch low pressure mercury arcs, Type P, in crown glass, arranged on a rectangular frame in the center of a room from which all other light was excluded. The lamps and arcs were distributed in three horizontal rows 2½ feet apart and each row contained four lamps and one arc. The animal cages were placed parallel with and on either side of the light source at a distance of 3½ feet.

The intensity of light reaching the cages was not entirely uniform, but on the average was 425 foot candles. The spectrogram of the mercury arc furnished by the Cooper Hewitt Company, shows that the crown glass absorbs all light below a wave-length of 3022-28 Ångström units while that of the Mazda lamps is cut off at about 3100 Ångström units. Our primary object in so far as the quality of the light was concerned, was the absence of rays which are absorbed by ordinary glass, as in the case of the diffused sunlight of the laboratory.

A second room was arranged so that all light was constantly excluded except during the time the animals were fed or examined, when a single 30 watt Mazda lamp was employed. This light was not used for more than 1 hour per day.

An ordinary animal room which has a southern exposure was used as the control room. Practically all the light of this room was diffused sunshine which passed through the glass of two large windows.

The temperature of the three rooms was maintained at 70° to 75°F. by automatic regulation of the common heating and ventilating systems. In the case of the light room, this was supplemented by an electric fan intake of outside air which also served as an aid to satisfactory ventilation. With this equipment, it was not possible to keep the temperature of the light room within the above limits during the warmer months, so that in subsequent experiments to be reported later, the
type of illumination was modified. The humidity of the three rooms varied with
that of the outside air.

The rabbits employed were representative of the usual breeds and types com-
monly used in this laboratory. They were adult male animals, approximately
1 year old, and were assembled from a carefully selected stock 1 week prior to the
experiment. During the entire period of observation, each rabbit was separately
caged and fed the standard diet of oats, hay and cabbage.

On January 16, 1925, 10 rabbits were placed in each of the three rooms where
they remained for the duration of the experiment. On February 16, 1 month after
they had been living in an environment of constant light or of constant darkness
or in the variable diffused sunlight of the laboratory, each rabbit was inoculated
in the right testicle with 0.3 cc. of a tumor emulsion prepared from an actively
growing primary tumor. This neoplasm is considered to be of epithelial origin (1).
The experiment was terminated on April 15, 2 months after inoculation, at which
time all surviving animals were killed by an injection of air into the marginal ear
vein. This period was selected upon the basis of previous experience as being suffi-
ciently long to include the majority of deaths due to tumor involvement or to
permit recovery to take place.

Detailed records including body weight determinations were kept covering the
clinical course of the disease and each animal received a careful postmortem exami-
nation with special reference to the presence or absence of metastases, the number
and distribution of metastases, the degree of involvement of different organs and
the state of the growth. The distribution of metastatic foci in particular has been
used as a basis for estimating the general character of the disease in those cases in
which secondary growths were found. The method used involves the calculation
of percentage values of the number of tumor sites or foci in terms of the number
of theoretical foci as shown by the actual location of metastases in the first twenty
generations of tumor animals (5). There are certain obvious objections to this
method. For instance, such organs as the liver or kidneys may be markedly in-
volved with numerous tumors or by only a few which destroy little tissue. How-
ever, a comparative classification of the disease, whether of high, moderate or low
malignancy, may be brought out in percentage terms by an arrangement of meta-
static distribution according to the following divisions:

| I. Skin and subcutaneous tissue, superficial lymph nodes, muscles, heart and pericardium, bones and bone marrow, glands of internal secretion with the exception of the suprarenals, the spleen and the central nervous system | 30 possible foci |
| II. Lungs and pleura, liver, kidneys and pancreas | 5 |
| III. Extensions and implantations to the retroperitoneal and mediastinal tissues, omentum, mesentery and parietal peritoneum | 19 |
| IV. Suprarenals and eyes | 4 |
This system of grouping metastatic foci was selected for these reasons: It has been found, from the study of several hundred rabbits inoculated in the testicle with the tumor, that there is usually a widespread distribution of metastases, extensions and implantations in those animals in which the most malignant disease develops and in which death occurs within 3 to 5 weeks after inoculation. In these instances, tumors may be found in many organs and tissues as in the skin, the superficial lymph nodes, the muscles, the bones and bone marrow, the heart, the spleen, and the endocrine glands as well as in the liver, the kidneys, the lungs, the retroperitoneal and mediastinal tissues and the serous membranes of the abdominal and pleural cavities. In instances of a somewhat less malignant disease, the most conspicuous and frequent metastases are found in the liver, kidneys, lungs and pancreas. A level of still lower malignancy is chiefly characterized by the predominance of extensions of tumor from the primary growth in the testicle to the retroperitoneal and mediastinal tissues and by implantations upon the omentum, mesentery and parietal peritoneum. If death occurs in these animals during the first 2 months after inoculation, the extensions and implantations referred to are found to be of an extreme grade, or, more frequently, some organ such as the kidneys or hypophysis is also involved. Finally, in those animals in which the disease is very mild, metastases may be found only in such sites as the eyes or suprarenal glands which of course are also involved in instances of high malignancy. On the other hand, metastases to the skin, muscles, bones and endocrine gland group practically never occur in cases of low malignancy.

Results.

The results of this experiment consist, first, of the clinical observations in which the general physical condition of the animals, the character of the primary growth and the development of metastases recognizable during life, are of especial interest; second, the mortality rate including the time of death with reference to the time of inoculation; and third, the postmortem observations, particularly as regards the condition of the primary tumor, the animal incidence of metastases and the number, distribution and state of these growths.

As far as could be determined by observation and by body weight, the health of the rabbits kept under the conditions of constant illumination was excellent, and while no outspoken deleterious effects were observed in the animals kept in the dark, there were indications furnished by body weight determinations to the effect that this environment was a less favorable one (Table 1). Only the weights of rabbits surviving the experiment have been used in this connection since a loss of weight together with other manifestations of cachexia
frequently occur in association with a widespread tumor growth in animals which succumb to the disease. Reference to the table shows that the groups of rabbits kept in the environment of constant light and of the variable sunlight conditions of an ordinary animal room for 3 months showed a well marked increase in body weight while the group in constant darkness gained comparatively little. Reckoning from the mean weight on admission the controls gained more than the light group due, probably, to the lower initial figure of the controls. However, the final mean weights of both groups were the same. The net gains over the first observations available after the rabbits from the light and dark rooms had been placed in these rooms were practically the same for the light group and the controls, but the dark room group gained only one-third as much as the others.

### TABLE I.

*Changes in Mean Body Weight.*

<table>
<thead>
<tr>
<th>Group</th>
<th>On admission (gm.)</th>
<th>1 wk. (gm.)</th>
<th>4 wks.</th>
<th>12 wks.</th>
<th>Over admission weight gain</th>
<th>Over first experimental weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>2120</td>
<td>2350</td>
<td>2465</td>
<td>2660</td>
<td>25.5</td>
<td>13.2</td>
</tr>
<tr>
<td>Lights</td>
<td>2264</td>
<td>2342</td>
<td>2428</td>
<td>2675</td>
<td>18.2</td>
<td>14.2</td>
</tr>
<tr>
<td>Darks</td>
<td>2283</td>
<td>2238</td>
<td>2288</td>
<td>2346</td>
<td>2.7</td>
<td>4.8</td>
</tr>
</tbody>
</table>

A primary tumor developed in each rabbit of the experiment. In the control group, there was a rapid and extensive primary growth for the first 3 weeks, after which time regression took place in the 5 rabbits which survived the experimental period of 2 months, and in 3 of these complete absorption with healing occurred. In the 4 rabbits dying from tumor, there was relatively little necrosis of the primary growth. In the rabbits from the dark room the primary growth was very rapid in 3 animals which early succumbed to the disease, while in 7 rabbits it grew more slowly and ultimately regressed and at the end of the experiment it was entirely necrotic in 2 and

1 One of the control rabbits died of an intercurrent infection so that this group consists of 9 animals.
healed in 5 instances. In the group exposed to constant illumination, the initial growth of the tumor in the majority of the animals was unusually rapid and extensive but it eventually became entirely necrotic in 5 and completely healed in 4 rabbits. In the remaining animal, which finally succumbed to the disease, approximately one-half of the primary tumor was living.

**TABLE II.**

*Analysis of Results.*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rabbits</th>
<th>Animal incidence</th>
<th>Mortality</th>
<th>Postmortem observations of metastatic foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls...</td>
<td>9* 44.4</td>
<td>10</td>
<td>4</td>
<td>44.4 4, 4, 4.5, 4.5, 6.0</td>
</tr>
<tr>
<td>Darks......</td>
<td>10 30.0</td>
<td>7</td>
<td>3</td>
<td>30.0 3, 3.5, 4</td>
</tr>
<tr>
<td>Lights....</td>
<td>10 20.0</td>
<td>6**</td>
<td>1</td>
<td>10.0 6.5</td>
</tr>
</tbody>
</table>

* 1 rabbit of the original 10 died of an intercurrent bacterial infection during the experiment.

** Two of these metastases eventually became completely healed.

† Three of these metastases occurring in 2 rabbits were small and entirely necrotic.

Metastases in superficial parts of the body which were recognized during life developed in several rabbits (Table II). In the control group there were ten such secondary growths in the eyes, superficial lymph nodes, subcutaneous tissue and bones distributed among 4 rabbits—an animal incidence of 44.4 per cent. In the group kept under conditions of constant darkness, the animal incidence of metas-
tases detected clinically was 30.0 per cent; seven tumors were found in the eyes, lymph nodes, skin and subcutaneous tissue. On the other hand, in only 2 rabbits of the light group—20.0 per cent—were such growths recognized. In 1 rabbit the unusual occurrence of regression and ultimate healing of eye metastases was observed while in another, four tumors were found in the muscles, bones, subcutaneous tissues and superficial lymph nodes.

The mortality rates of the three groups were—controls 44.4 per cent; darks 30.0 per cent; lights 10.0 per cent (Table II). Among the controls, 4 rabbits were killed, 4, 4½ and 5½ weeks after inoculation because of the development of paralysis or other symptoms indicative of impending death. A similar procedure was necessary in three instances in the dark room group, 3½ and 4 weeks after inoculation. Among the rabbits in the light room, on the other hand, there was but one such instance. The symptoms which necessitated the sacrificing of this animal 6½ weeks after inoculation, developed much later and more slowly than those of similar cases in either the control or dark room groups.

At postmortem examination, it was found that the incidence of metastases was approximately the same in all three groups, but there were distinct group differences in the number of foci² (Table II). Among the 9 control rabbits there were 81 foci of metastatic growths, or 9 per animal, 69 foci in the dark group, or 6.9 per animal, and only 27 foci in the light group, or 2.7 per animal. A comparison of actual metastatic rates in which only rabbits with secondary growths are considered, shows that there was little difference between the control and dark groups, but in the case of the light group the rate was approximately one-third those of the others.

The number of metastatic foci in individual rabbits is given in Table II. In the fatal cases, the largest number occurred in a con-

² It should be pointed out that the figures refer to the number of organs or tissues involved, and not to the numbers of secondary growths, and consequently the expressions “foci of metastases,” “distribution of metastases,” or “metastatic rate” are used rather than “number of metastases.” On the other hand, the figures for clinical metastases detected during the life of the animal refer to the actual number of individual secondary tumors found. The present basis of counting foci of metastases is slightly different from that employed in the preliminary note (4).
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Control animal in which 30 foci were counted and in the others, there were 15 to 26. But in so far as the severity of the disease may be

judged from the mean number of metastatic foci, there was practically no difference in the three groups—(controls 19.25, darks 22.7,
lights 22.0). Among the rabbits which survived the experimental period of 2 months, postmortem examination revealed 4 metastatic loci in one control, a single focus in a dark room rabbit and 5 foci distributed among 4 light room animals. In 2 of the rabbits from the light room, the three secondary growths found were small and entirely necrotic. It is probable that none of the 6 surviving rabbits with metastatic foci would have died from the effects of tumor growth.

The character of the disease which developed in the three groups may be compared upon still another, and in many respects a more impressive basis, namely, the distribution of metastatic foci, as is illustrated by Text-fig. 1. In this graph, each rabbit is represented by a horizontal column, divided into four sections corresponding to the four general divisions of secondary growths previously described, while the shading of the sections represents the metastatic rate in percentage terms of the possible number of foci.

This diagrammatic representation illustrates in a striking manner the low plane of malignancy of the disease in the light room group. The metastatic rate in all four divisions is seen to be much lower than that of either the controls or the dark room groups. As far as the dark room series is concerned, the metastatic rate is, on the whole, somewhat lower than that of the controls if one takes into consideration the number of animals with secondary growths and the degree of involvement of the two divisions of foci which are predominately affected in instances of severe or well marked malignancy, namely, the division which includes the skin, muscles, bones and glands of internal secretion, and that of the lungs, liver and kidneys. In the case of the light room group, there was but 1 rabbit in which a disease characterized by a widespread distribution of metastatic growths developed while there were 3 such instances among the dark room and 4 among the control animals. In a 5th control rabbit, there was a wider distribution of tumor than in a 4th dark or in 4 other light room rabbits in which metastases were found and in 2 of these last animals, it will be recalled that the secondary growths were entirely necrotic. Finally, there were 4 controls as contrasted with 6 dark and 5 light room animals in which no metastases were found.
DISCUSSION AND CONCLUSIONS.

This experiment was undertaken for the purpose of obtaining information on the influence of light on the character of disease in rabbits induced by a transplantable malignant neoplasm. To this end, the attempt was made to insure conditions which would be common to all animals of the experiment with the exception of the light environment. Comparable conditions of temperature and humidity prevailed in the three rooms in which the experiment was carried out, all the rabbits were fed the same diet, and the animals themselves, assembled at the laboratory at the same time, were well matched as to breed and age. Individual animal variation could not, of course, be wholly eliminated, but this factor was controlled by the selection and number of rabbits employed in each group.

The lighting of the three rooms was the variable factor of the experiment. One group of rabbits, kept in a well lighted animal room was exposed to the daily fluctuations of sunshine diffused through ordinary window glass. Another group was placed in a room which was continuously and constantly lighted by Mazda lamps and mercury arcs in crown glass. The third group was kept in a room from which all light was constantly excluded except for very brief daily periods.

The clinical and postmortem observations of the three groups of rabbits inoculated with the malignant tumor and kept in these different surroundings bring out distinct variations in the course and character of the tumor process. The most highly malignant disease occurred in the control group living under ordinary indoor daylight conditions. The mortality rate was the highest, the incidence of secondary growths detected during life or found at postmortem examination was also the highest and there was the widest distribution of metastatic foci. In contrast to these effects was the extremely mild disease which developed in the rabbits kept under conditions of constant illumination. The mortality rate of this group was only one-fourth that of the controls and the duration of life of the single rabbit which succumbed to the disease was considerably longer than that of comparable controls. In addition, there was a lower incidence and a smaller number of metastases found during life together with a smaller number of metastatic foci observed at autopsy.
The disease which developed in the group living in practically constant darkness was also not as severe as that of the controls but was considerably more malignant than that of the light room group. As compared with the controls, the mortality rate was slightly lower, the incidence and number of clinical metastases were somewhat less, and there were fewer sites of secondary growths as revealed by postmortem examination. In addition, a higher percentage of rabbits either recovered wholly or was in process of recovery. Although the effect of constant light exclusion was much less pronounced than that of constant and continuous illumination, it appeared to be in the same direction of diminished malignancy.

There were certain special features of the disease that developed in the rabbits kept in the light and dark rooms which have a direct bearing on the influence associated with these environments. The course and character of the primary tumor in the control and dark room groups were in general accord with what is usually observed in the majority of normal rabbits. That is, the tumor underwent a rapid growth during the first 2 weeks and, in animals succumbing to the disease 3 to 5 weeks after inoculation, showed little tendency toward regression while in most rabbits surviving as long as 2 months, it was found to be wholly or largely necrotic or completely healed. In contrast with the control and dark room groups, the initial growth of the primary tumor in the majority of the light room rabbits was unusually rapid and extensive so that the first impression of the disease was one of heightened severity. The subsequent course of the primary growth, however, was in agreement with the low level of malignancy shown by the disease of this group, for it became entirely necrotic or became completely healed in all but 1 rabbit.

Although there was practically no difference in the incidence of metastases in the control and light room groups, the greatly lessened number and the markedly restricted distribution of the growths in the rabbits in the light room, indicate that these animals were able to restrain or inhibit the development of the majority of metastatic foci. This finding is further supported first, by the unusual occurrence in 1 rabbit of the complete healing of the metastatic growths in the iris of both eyes; second, by the postmortem finding in 2 other rabbits of but 3 small and entirely necrotic metastatic tumors and
finally by the comparative lateness with which the terminal stages developed in the 1 rabbit of the group which eventually succumbed to the disease.

The incidence of metastases in the dark room group was slightly lower than in the others. The secondary growths were confined almost wholly to 3 rabbits in which the disease exhibited well marked malignant characteristics, while in the other animals it was extremely mild. These results indicate first, that constant darkness has a slight but definite effect upon the course and character of the disease and second, that this effect differs in some respects from that observed under conditions of constant illumination. It would appear that under the influence of the conditions obtaining in the dark room, those rabbits which possessed a high resistance to tumor growth, either natural or acquired, were able to exercise this power more efficiently but that the influence was not sufficient to enable less resistant animals to deal successfully with the disease. On the other hand, the restraining or inhibitive effect associated with constant illumination was, to a greater or less degree, seen in all rabbits of the group.

The results of this experiment are interpreted as furnishing experimental evidence in support of the idea referred to in the beginning of this paper, that there is a relation between the factor of light and the manifestations and outcome of the malignant disease with which we have been working. It will be recalled that, in general, the periods of maximum and minimum sunlight, corresponding with summer and winter were associated with relatively low levels of malignancy while the periods of greatest malignancy occurred at times of abrupt and rapid changes in the hours of sunshine, coinciding roughly with the spring and autumn months.

In the experiment here reported, the most malignant disease developed in the group of rabbits living in an ordinary room, exposed to the influence of the variations in sunlight, passing through ordinary glass, that prevailed during these months (February, March, April). In contrast with these results, a disease of low malignancy occurred in the group kept in an environment of constant and intense illumination. Furthermore, the plane of malignancy of the disease in the rabbits living in a room from which almost all light was excluded was...
found to be somewhat lower than that of the controls although it did not approach the low level of the group exposed to constant illumination. It would appear, therefore, that the conditions of light in the experiment reported were associated with variations in the course and character of the malignant disease comparable with those of a seasonal nature which normally occur throughout the year.

The mechanism by which these effects are induced is not known. We have considered the influence of the seasonal factor in disease as operating upon or affecting general animal economy and susceptibility or resistance as a functional activity of the animal organism. Hence it appears that resistance to tumor growth as displayed by rabbits living under conditions of constant intense illumination and, to a less extent, in almost constant darkness is more potent or efficacious than it is in the case of rabbits exposed to the daily fluctuations of diffused sunlight.

Finally, it should be stated that no attempt was made in this experiment to produce ideal conditions for diminishing or enhancing tumor malignancy. The purpose was to determine whether variations in the course and character of the malignant disease took place, first, under conditions of constant and continuous illumination in which none of the shorter ultra-violet rays participated and, second, under conditions of constant darkness.

**SUMMARY.**

An experiment is reported in which an environment of constant and continuous light excluding the shorter ultra-violet rays, and one of constant darkness, have influenced the course and character of a malignant disease of rabbits induced by a transplantable neoplasm.

Under the influence of constant light the level of malignancy was observed to be low; under the influence of constant darkness the level of malignancy was somewhat lower than in the control animals living under ordinary indoor light conditions, but the level was not as low as among the animals constantly illuminated.

These observations furnish experimental evidence in support of the idea that there is a correlation between the external factor of light on the one hand and the manifestations of an experimental malignant disease on the other.
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BIBLIOGRAPHY.


