EFFECTS OF LIGHT ON NORMAL RABBITS, WITH ESPECIAL REFERENCE TO THE ORGANIC REACTION.

I. Clinical and Postmortem Observations.

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For several years a study of the animal organism in these laboratories has included an investigation of the mass relationships of various organs with especial reference to the subjects of variation, the effect of environmental influences, and the relation of these conditions to phenomena of animal resistance as indicated by the occurrence and general course of disease.

A striking feature both of experimental syphilis and of a transplantable neoplasm of the rabbit is the variability of the disease manifestations, and observations over a period of several years have led us to the opinion that a relationship exists between the season of the year and the general character of the disease. It was further found that the weights of many organs not directly involved by the disease process were affected and that these alterations could be correlated with the character and clinical course of the disease. In addition, variations of a rhythmic character which appeared to have a seasonal significance could be demonstrated in the organ weights of normal rabbits, and the organs in which these changes were especially marked were those in which the more pronounced weight alterations were observed in association with syphilis and the malignant tumor.

The influence of the seasonal factor has been interpreted as affecting animal economy, while susceptibility or resistance to disease has been considered as a function of the animal organism. Among the several factors contributing to weather conditions during the time of our experiments, sunshine appeared to be most closely correlated with variations in disease manifestations. That is, the actual hours of sun-

shine together with the rate, extent, and persistence of change in the curve of sunshine over a given period could be related to the character of the experimental diseases. Furthermore, when the curve of sunshine was compared with the organ weights of a sufficiently large number of normal rabbits examined during the time of our experiment, it was found that the fluctuations of the curve corresponded with variations in the mass relationships of practically all the organs, and perhaps most strikingly so in the case of certain of the ductless glands and representative lymph nodes. Since our experiments were in each instance carried out with rabbits kept in rooms which received practically all sunlight only as filtered through ordinary window glass, the effects observed cannot be attributed wholly to the short or so called therapeutic ultra-violet rays.

The observations led to the conception of a relationship between the nature or character of light, on the one hand, and the physical state and functional activities of the animal organism, on the other. In order to test this conception experimentally, an investigation was undertaken in which conditions of light could be controlled. Since the significant features of the factor of sunlight appeared to be its total amount together with the change in actual hours of sunshine, our initial experiments were carried out with a constant illumination of the maximum intensity that was practicable with a simple equipment. Its effect was studied, first, upon the normal rabbit with especial reference to organ weights, and second, upon the course of the malignant tumor and of experimental syphilis. A summary of the first experiment dealing with the results of tumor inoculation has already appeared (1), and those in which *T. pallidum* was employed will be published shortly.

The results of the experiment with normal rabbits are reported in the present series of papers. The methods employed, the clinical observations, and the postmortem findings are described in Paper I. In Paper II, the actual organ weights and the weights of organs per kilo of net body weight are presented in tabular form. An analysis of these statistical data, including a consideration of their reliability as an index of existing conditions, is discussed in Paper III.

Methods and Materials.

The rooms in which this experiment was carried out were three in number. One, the so called "light" room, measuring 19 × 9.5 × 10.25 feet or including 1850 cubic feet, was kept constantly and solely illuminated by 1000 watt Mazda lamps and Cooper Hewitt 50 inch low pressure mercury arcs, Type P, in crown glass, arranged in three superimposed horizontal rows on the long axis of the room. Each row contained four Mazda lamps and one mercury arc. The animal cages were placed in racks along the sides of the room parallel with and equidistant from the source of lighting. The intensity of the light reaching the cages averaged 425 foot candles; these determinations were made by the Electrical Testing Laboratories to whom our appreciative thanks are due. The spectrogram supplied by the Cooper Hewitt Company shows that the crown glass of the mercury arc does not transmit light below wave-lengths of 3022–28 Ångström units, while a spectrogram of the Mazda lamps which was kindly made for us by Dr. Frederick L. Gates shows that their light is cut off at about 3100 Ångström units. For the purpose of the present experiment there was no need to analyze further the nature or quality of the light.

In the "dark" room all light was constantly excluded except for the time, approximately 1 hour per day, when the animals were fed or examined. On these occasions a single 30 watt Mazda lamp was used. Satisfactory ventilation and temperature regulations of both the light and dark rooms were obtained by a system of fans and ventilating vents.

The "control" room was an unaltered animal room; it has a southern exposure and is lighted by two large windows. During the time of the experiment practically all the sunlight in this room diffused through the ordinary lead glass of these windows.

The experiment was conducted as follows: 150 male rabbits were assembled from November 14 to December 15, 1924, separately caged, and kept in ordinary animal rooms. The rabbits were chosen from carefully selected stocks of well nourished animals free from any external manifestations of disease. They were obtained chiefly from breeders or dealers in eastern Pennsylvania and included all the ordinary breeds of rabbits that are commonly used in our laboratory. The various breeds were proportionately distributed in the major divisions and subgroups of the experiment. The exact age of the animals was unknown but the great majority of them were between 8 months and 1 year old. The diet of all was the same throughout the experiment and consisted of hay, oats, and fresh cabbage or carrots.

On December 31, 1924 and January 2, 1925, 50 rabbits were placed in the light room, 50 in the dark room, while the third lot of 50 animals remained in the control or unaltered animal room. Groups of 10 rabbits from each room were killed and examined 2, 4, 6, 8, and 12 weeks later.[1] In addition, other groups of normal

[1] Data for a 10 week period were obtained from additional groups of rabbits placed under the same conditions on January 29 and killed on April 8,

rabbits designated as "outside" controls were killed and examined at the same time.[2] These animals had been in the laboratory for only a few days and represent a stock which had been living under unknown but presumably different conditions from those of the control rabbits in the experiment.

Upon the day the rabbits were killed the feeding was omitted; they were weighed and then killed by air embolism, a method quicker and less disturbing than etherization. Each animal was at once bled from the inferior vena cava and the autopsy performed immediately. The details of our method of post-mortem examination are elsewhere described (2). The organs weighed were: the gastrointestinal mass, the heart, the liver, the kidneys, the spleen, the brain, the thyroid, the parathyroids, the hypophysis, the suprarenals, the pineal, the thymus, the testicles, the posterior axillary and popliteal lymph nodes, the deep cervical lymph nodes, and the main mass of mesenteric nodes. In order to avoid drying after removal from the body, the small organs were placed in Petri dishes and kept moist with physiological salt solution. Before weighing the excess fluid was removed by absorption on filter paper.

The records kept included the source of each rabbit, the date of receipt, an age grouping, a breed or type classification, a weight chart, and a record of the general physical condition of the animal during the period of observation.

The statistical analysis of the organ weights has included both actual and relative weights, that is, the weight of organs per kilo of net body weight.

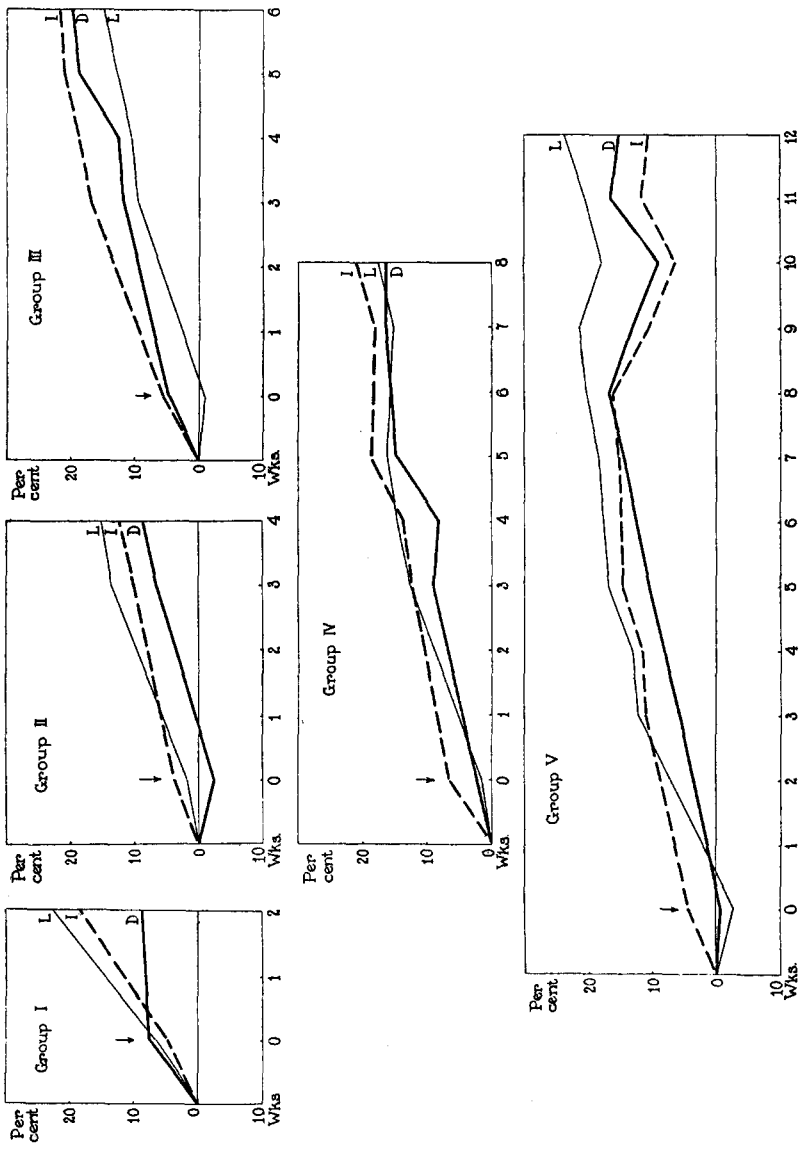
No animal has been omitted because of any abnormality detected either during life or at autopsy, and the figures, as given, include all data irrespective of whether an organ was normal or pathological. Certain groups contain one or more goiters and in these instances additional figures for the thyroid are given from which these weights have been omitted. Similar supplementary figures are given for the deep cervical lymph nodes in those cases in which very large nodes were associated with a purulent infection of the middle ear or cranial sinuses.

RESULTS.

The clinical observations on the 150 rabbits comprising the experiment were made over a period of 6 to 16 weeks, reckoning from the time the animals were first brought to the laboratory; while the dura-

1925. These observations are not included in the present paper because of the introduction of a number of factors not uniform with those reported. Much of the data from this additional experiment, however, is in general conformity with that of the first.

[2] No outside control rabbits are available for the last group of experimental animals. We have, however, included the results obtained from 20 normal rabbits killed and examined on March 12, midway between the time when the fourth and fifth experimental groups were examined. These animals had been caged indoors for 1 to 4 weeks.



TEXT-FIG. 1. Percentage deviations of mean body weights from the mean weights on admission. The number of weeks refers to the time the groups of rabbits had been under conditions of constant light or of constant darkness.

Light groups ——
Dark groups ——
Control groups — —

tion of the experiment, that is, of exposure to constant illumination or to constant exclusion of light, was 2 to 12 weeks. The physical state of all rabbits at the beginning of the experiment was excellent and it continued to be so in the case of the animals in the light room and those in the unaltered or control room. Although the same was generally true for the rabbits caged under conditions of constant darkness there were some indications that this environment was less favorable.

Evidence for the satisfactory condition of the rabbits is furnished by body weight curves (Text-fig. 1) in which the average or mean value for each group of 10 animals is used. The changes in weight are expressed in percentage deviations from the average weight of the group on admission. The curves show that, on the whole, each group of rabbits gained in weight steadily and fairly uniformly. In Groups I and II (2 and 4 weeks exposure) the light room animals increased in weight more than the "inside controls," while those in the dark room gained the least. In Group III (6 weeks exposure) the inside controls and the dark room animals gained more than the light room rabbits. In Group IV (8 weeks exposure) the gain of all three sets approximated each other with a tendency of those in the dark room to gain the least and the inside controls to gain the most; the light room rabbits of this group showed a greater increase in weight during the first weeks than those in the dark room but toward the end of the experiment the two curves are practically identical. In the last group (12 weeks exposure) the greatest increase in body weight was made by the group of light room rabbits, while the inside controls gained the least. The dark room animals showed the smallest gains for the first 7 weeks, but during the last month they slightly exceeded in this particular the inside controls. It should be mentioned that this group of inside controls was not entirely comparable to the others in that it contained three older and larger rabbits. During the first 2 months, these animals gained slightly in weight but in the subsequent month lost weight, which largely contributed to the disproportionate drop in the average body weight of the group during the latter part of the experiment.

Reference to the curves brings out the fact that, except with Group I, the mean body weights of the various groups at the time the rabbits were placed in the light and dark rooms were not uniform. That is,

from the time of admission to the beginning of the experiment, some groups gained less weight than others, while some actually lost weight. A more accurate idea of the effect of the experimental environments upon general physical conditions as indicated by the criterion of body weight is obtained by comparing the final weight values with those at the beginning of the experimental period (Table I). All the control groups showed an increase in weight; the gain of the fifth group was much smaller than the others owing, as has been mentioned above, to the irregularity of the group in respect to the age and size of the animals. The greatest gains in weight were made by the rabbits

TABLE I.

Increase in Mean Body Weight during Experimental Period.

	Initial weights			Final weights			Actual and percentage gain					
	Light room	Dark room	Controls	Light room	Dark room	Controls	Light room		Dark room		Controls	
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	per cent	gm.	per cent	gm.	per cent
Group I	2200	2125	2198	2528	2190	2433	328	14.9	65	3.1	235	10.7
Group II	2178	2200	2095	2460	2450	2350	282	13.0	250	11.4	255	12.2
Group III	1995	2125	2225	2310	2415	2570	315	15.8	290	13.6	345	15.5
Group IV	2100	2225	2333	2433	2530	2653	333	15.9	305	13.7	320	13.7
Group V	2045	2035	2380	2603	2370	2530	558	27.3	335	16.5	150	6.3
Mean	2104	2142	2246	2467	2391	2507	363	17.3	249	11.6	261	11.6

kept in the light room, and this was true of all five groups. The dark room groups gained less than those in the light room and in the case of the first three groups, less than the inside controls. The percentage increase of the fourth groups of dark room and inside control animals was the same. The fifth group in the dark room showed a much greater increase than the inside controls due largely to the disproportionately high value of the latter group at the beginning of the experiment.

It should be mentioned, in connection with the body weight observations, that each rabbit was given all the food he would consume with the idea of supplying optimum conditions for the maintenance of a

good state of nutrition. The appetite of the rabbits in the light room was noticeably increased, especially during the first 6 weeks of the experiment, and it was found that they ate more food than the controls. The appetite of the dark room rabbits, on the other hand, showed no apparent deviation from the normal.

It is also of interest to note that the general behavior of the rabbits was not apparently disturbed by living in an environment of constant light. They did not try to avoid looking at the arcs and lamps and, as far as could be determined, slept as usual. During the first weeks of the experiment the animals seemed more alert and lively but this condition did not persist. On the other hand, there was nothing in the behavior of the rabbits in the dark room that was peculiar to this group of animals.

The health of the animals was, in general, excellent. Few instances of spontaneous disease developed during the period of observation that were recognizable clinically.

There were 2 cases of ear canker, 1 each among the light room and inside control rabbits; 1 instance of a small, encapsulated, subcutaneous abscess in an inside control animal, and 3 cases of clinical snuffles, all among the inside controls.

It was found by postmortem examination, however, that 59.3 per cent of the rabbits in these groups (light room 60 per cent, dark room 58 per cent, inside controls 60 per cent) showed gross lesions of some kind but in only a few cases were these more than slight or moderate in degree.

The great majority of the lesions, which were distributed fairly uniformly among the subgroups, were scarring of the kidneys and liver, but there was 1 example of a marked chronic coccidiosis of the liver with recent lesions, and 20 instances of a purulent infection of the cranial sinuses, middle ear, or mastoids. Other conditions noted included slight degenerations of the aorta, verminous infections of the intestinal tract, coccidial cysts of the abdominal cavity not associated with visible lesions of the liver, 1 case of a healed pulmonary abscess, 1 instance of a healing abscess of the myocardium and another of hypertrophy of the right ventricle of undetermined origin, 1 case of marked malformation of the kidneys, 1 of a parathyroid cyst, 2 axillary lymph node cysts, and 2 instances of hydrocephalus.

As a further indication of the health of the animal the condition of body fat was noted at autopsy in such general terms as excessive,

abundant, moderate, scant, or none. The body fat was either scanty or practically absent in only 13 rabbits (8.06 per cent) distributed as follows: 3 in the light, 7 in the dark, and 3 in the inside control groups respectively.

A number of gross lesions were found at autopsy in all save 2 of these 13 rabbits.

Of the 3 light room rabbits, there were 2 with active lesions of the kidneys, 1 with a purulent inflammation of both mastoids, and all 3 showed healed or inactive coccidiosis of the liver of slight degree. Among the 7 dark room rabbits, there were 5 with kidney lesions, 1 of which was active and 4 inactive with varying degrees of scarring; there was 1 instance of an active and 1 of a slight and inactive coccidiosis, and 1 of a purulent exudate in the nasal passages and mastoids. Among the 3 inside control rabbits, 2 showed kidney lesions, of which 1 was active and the other inactive with marked scarring; 1 animal had a slight active and 1 a slight inactive coccidiosis, and another had a purulent inflammation of the nasal passages and cranial sinuses. There was some degree of thymic mass depletion in 9 and an enlargement of the spleen in 10 of these 13 rabbits.

The 40 rabbits comprising the four groups of outside controls (recently acquired animals) were clinically free from any evidence of disease but there was practically the same incidence of visible lesions at autopsy as in the other groups, that is, 57.5 per cent.

In the case of 20 normal rabbits killed on March 12, 1925, midway between the fourth and fifth experimental groups, the incidence of postmortem lesions was 60 per cent. The types of lesion did not differ from those found in the light, dark, and inside control rabbits, the majority being slight or moderate and principally affecting the same organs. Among the outside controls there were 2 cases of a marked active coccidiosis of the liver and 5 instances of a moderate or marked scarring of the kidneys. In the March group, the incidence of active coccidiosis of considerable extent was higher—5 of the 20 rabbits showing this condition; in 2 animals there was marked scarring of the kidneys. In none of the outside controls was there any purulent inflammation about the head, while 2 of the March normals showed this condition. The body fat of 3 outside control rabbits was scanty. In each animal the thymic mass was small and the spleen enlarged, but only 1 showed gross lesions of probable significance, namely, a possibly active lesion of the kidneys and an active coccidiosis of the liver. A similar state of body fat occurred in 6 of the 20 March normals, 4 of which showed a small thymus and 3 of these a slightly enlarged spleen. There was 1 instance of an active and another of an inactive coccidiosis, 1 of a purulent inflammation of the mastoids, while in 3 rabbits no gross lesions were found. The occurrence of relatively little

fat in rabbits which presumably have not been living a sedentary life and have not necessarily been constantly provided with abundant food is obviously of less significance as an index of physical condition than it is in rabbits living a caged existence with an ample food supply.

DISCUSSION.

The health of the animals employed in an experiment of this type is of importance first, from the point of view of the general reaction to artificial environments, and second, because of the influence of physical condition upon the weights of organs. The rabbits comprising this experiment were all in apparent good health throughout the period of observation, such spontaneous disease as could be recognized clinically appearing unimportant.

Body weight observations have been used as an index to the effects of constant illumination or exclusion of light upon the general physical condition. There were five groups of 10 rabbits kept under each of these environmental conditions for 2, 4, 6, 8, and 12 weeks respectively. All groups gained in weight during these periods, but the greatest gains were made by the rabbits exposed to constant light. While there was also an increase in body weight of the groups kept in constant darkness it was less pronounced and more irregular than that of the groups in constant light and, on the whole, than that of the control rabbits kept in an ordinary animal room, a fact which suggests that the exclusion of light was relatively unfavorable.

At the end of the experimental period, all the rabbits appeared to be in good condition, but postmortem examination showed that the body fat of several was relatively scanty. The distribution of these animals, 3 in the light, 3 in the control, and 7 in the dark room groups, suggests that some impairment of nutrition may be related to prolonged caging in an absence of light. This is further suggested by the fact that 4 of the 7 dark room rabbits were of the group which had been kept in this environment for 12 weeks. Gross lesions of some kind were found in 11 of the 13 rabbits with scant body fat, those of the kidneys appearing most significant. The kidneys were affected in 9 instances and in 4 of these the lesions appeared active, the organs being enlarged, soft, opaque, and either markedly congested or yellow. To what extent inactive lesions of the kidneys, with moderate or

marked scarring, are associated with scant body fat is not known, but such was the condition in 5 rabbits. On the other hand, moderate or marked renal scarring is not necessarily accompanied by scant body fat, for there were 19 other rabbits among the 150 experimental animals with such lesions but with no evident fat reduction.

The thymic mass in the rabbit has been found to be a fairly delicate indicator of general physical condition in that its size, consistency, and the amount of associated fat are roughly proportional to the state of nutrition of the animal. Among the 13 rabbits of the experimental series which showed a scant amount of body fat, the thymic mass was considered to be depleted to a greater or less extent in 9 animals, 5 of which were in the dark room groups.

Enlargement of the spleen was frequently seen in the entire series of rabbits and no relationship could be made out between its occurrence and the presence or character of gross lesions, or the condition of the body fat.

At postmortem examination it was found that visible lesions of some kind were present in 59.3 per cent of the 150 rabbits caged under conditions of constant illumination, of constant darkness, and of an ordinary animal room. Lesions of a similar type and distribution were found in 57.5 per cent of one group of 40, and in 60 per cent of a group of 20 rabbits recently brought to the laboratory. These figures are higher that those of two groups of 350 and 295 normal rabbits examined during $3\frac{1}{2}$ years, namely, 40.9 and 53.2 per cent (3). While the larger number of animals in these two series suggests a possible explanation for these differences, the uniform incidence of lesions among the four major groups of this experiment indicates a higher proportion of pathological change in the rabbit stock at this particular time. The state of the lesions found in the experimental animals did not suggest that the conditions of constant light or of constant darkness were associated with heightened activity of the pathological process.

It has been found from the observations of the two large series of normal rabbits referred to, that as long as the animals remain in good health the values obtained for organ weights of animals with lesions do not differ materially from those of animals that are entirely free from lesions (3, 4). There is a slight tendency toward an increase

in organ weight as one passes from rabbits with slight or moderate lesions to those with marked lesions, particularly in relation to the activity of such conditions. The number of marked or active lesions was extremely few in this series of rabbits, and it has been found by actual determination that these conditions have not essentially affected the mean organ values employed in analyzing the experimental results obtained, except in certain particular instances, as in the case of the deep cervical lymph nodes, a marked enlargement of which is associated with a purulent infection of the cranial sinuses. The effect of an impaired state of health upon organ weight does not have to be considered in the present connection as all the rabbits of the experiment were in good physical condition during the period of observation.

SUMMARY AND CONCLUSIONS.

A group of 50 normal male rabbits kept under conditions of constant light that had none of the shorter ultra-violet rays and another group kept in constant darkness for 2 to 12 weeks were observed clinically and subjected to postmortem examination for the purpose of determining the effect of these environmental conditions upon general body health and the weights of organs. A similar group of 50 rabbits caged in an ordinary animal room for the same period, and two groups of 40 and 20 rabbits respectively, which had recently been brought into the laboratory, served as controls.

It was found that the general health of the rabbits was not impaired by the artificial light or the exclusion of light. The gain in body weight which occurred in all groups was especially marked in the case of those kept under conditions of constant light.

The incidence of spontaneous disease recognizable clinically during the experiment was extremely low and of a mild character and did not obviously disturb the health of the animal. It was found at postmortem examination, on the other hand, that 59.3 per cent of the rabbits caged indoors, that is in the light, dark, or unaltered rooms, and 58.3 per cent of those recently brought to the laboratory had visible lesions of some kind. The great majority of these lesions, however, were of a slight grade, and none appeared to have any deleterious effects upon the general physical state of the animals.

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