# Light-Dark-Shift Stress, With Special Reference to Spontaneous Tumor Incidence in Female BN Rats [1,2]

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ABSTRACT—As a way to induce mild chronic stress, light-dark (L-D)-shift stress was applied to inbred BN virgin female rats during their whole life-span (group I, 100 animals); the incidences of spontaneous tumor and nontumor processes were recorded. A group of rats (group II, 100 animals) exposed to a standard lighting system served as the control group. Total tumors of 128 in group I and of 154 in group II were found in 74 and 86 animals, respectively. Neither were these differences nor was the pattern of spontaneous tumors statistically significant. Although in earlier studies L-D-shift stress had proved to be effective, especially with regard to its capacity to induce a substantial decrease in cellular immune response, apparently such alterations did not unfavorably affect longevity of BN female rats. Although as a side issue of this study, a strong predisposition for tumor incidence appeared to exist, in particular for the incidence of Langerhans' islet tumors, in fat animals at weaning. JNCI 1986; 76:439-446.

Promotional effects of environmental conditions on tumor incidence have been a subject of numerous studies (*1-3*). In a report on the contribution of the environment to cancer incidence by Wynder and Gori (*4*), they postulate that 80% of the observed cancer incidence can be considered "preventive potential." This preventive potential mainly involves defined environmental factors, such as diet, tobacco, alcohol, and occupation but also cultural and behavioral patterns, psychological factors, or stress (*3, 5*). Prospective studies by Grossarth-Maticek et al. (*6*) on the mechanism of psychosomatic factors in the process of cancerogenesis showed that traumatic life events or personality traits could lead to stress, eventually inducing neuroendocrinologic factors, and to enhanced cancer. In the last decade, studies on the influence of stress on illness or more specifically on carcinogenesis originated from this hypothesis or related hypotheses, leading to numerous comprehensive studies (*5-8*).

In epidemiologic studies of the influence of stress on tumor incidence, it is always very difficult to discriminate between stress and other factors that may cause increased tumor incidence. In animal studies in which the conditions can be controlled generally more easily than those in studies of humans, substantial evidence existed for a correlation between stress and increased tumor growth; furthermore, no decreased tumor incidence was noted (*9-11*). The mechanism that is thought to be, as a rule, responsible for the observed promotion is the decrease of the immune response caused by stress, for which there is much evidence from experimental as well as clinical studies (*12, 13*). However, although there is a strong correlation between stress and immunosuppression, there is not much proof that the temporarily or more permanently induced immunosuppression will result in an enhanced tumor incidence. Certainly, in humans, in which most tumors are nonimmunogenic, stress-linked immunosuppression causing tumors would have been surprising (*14*).

When a possible effect of stress on tumor promotion is studied, one has to consider essential differences found between acute and chronic stresses. Whereas acute stress immediately resulted in elevated levels of serum corticosterone and other endocrine functions, with prolonged exposure to stress the reverse effect was seen: Serum adrenal steroid concentrations became subnormal (*15, 16*). Although certain stressful events, such as bereavement and hospitalization, may be considered acute stress, most stressful conditions in the modern life-style are usually regarded as chronic stress. Therefore, in animal studies preferably a mild form of chronic stress should be given, for proper study comparisons to effects of human stress.

Prolonged exposure of hostile and adverse stress to the animal will lead to the General Adaptation Syndrome (GAS) as described by Selye (*17*). Less hostile inducement of chronic stress often led to habituation or compensation by other means than the above-mentioned adaptation, such as partial loss of hearing as seen in an experiment in which lifelong exposure to noise was used to induce chronic stress (*18*). Other ways to obtain mild chronic stress, such as isolation stress or prolonged sequential bleeding (*19, 20*), will encounter logistic problems when used in large groups of animals and are not very workable. In earlier studies by these authors,

ABBREVIATIONS USED: L-D-shift=light-dark-shift; MST=median survival time.

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L-D-shift stress was used on rats for 35 weeks. This stress procedure significantly decreased cellular immune response and body weight (*16*). This finding implicates that this procedure does not seem to lead to adaptation and is potentially useful in a mild chronic stress study.

Spontaneous tumor incidence of BN female animals was recorded earlier by our group (*21, 22*) and by others (*23*); these studies proved that, by establishing spontaneous tumor incidence, the influence of, for instance, dietary factors on carcinogenesis could be investigated (*22*). Expanding on this model we investigated the influence of mild chronic stress on tumor incidence. Apart from weighing the animals every 2 weeks and apart from recording the data gathered by autopsies, no other determinations were made so as not to induce potentially stressful procedures other than the given L-D-shift stress (*24*).

## MATERIALS AND METHODS

*Experimental design.*—This experiment was part of a larger study on the effect of environmental conditions on cancer incidence, all having the same protocol and, apart from specific environmental conditions studied, having identical conditions (*21, 22*). The same protocol for the experimental design, strain of animals, and husbandry was followed as that in (*22*).

We used 2 groups of 100 animals each, equally divided into group I, L-D-shift stress, and group II, untreated controls. From weaning (3 wk) until termination of the experiment (150 wk) L-D-shift stress was used, while body weights and the conditions of the animals were recorded. Furthermore, at least twice daily the animals were carefully checked for sickness and death, without handling them. Those animals suspected to be sick were removed and housed individually; when moribund they were sacrificed. Animals with substantial tumor growth, which impeded their eating and drinking habits, were also killed.

The protocol for the sick and dead was the same as that described elsewhere (*21*). Body weight, which was determined every 2 weeks, was used as a criterium for the condition of the animals and for establishment of possible predispositions for survival or tumor incidence.

*Experimental animals.*—The BN female rats used (exclusively virgins) were at the time of the experiment at their 17th generation of inbreeding. Immediately after weaning (3 wk), the animals were weighed and equally divided into 2 groups: an experimental group and a control group. A total number of 70 litters, from which exactly 50% came from a second and 50% from a third litter, were necessary to complete the 2 groups. The time difference between the first and the last animal entering the study was 9 weeks. The animals were housed in Makrolon cages (41 cm long×25 cm wide×15 cm high) in groups of 5 in air-conditioned animal rooms with a controlled day-night rhythm. To prevent possible effects of the dominating animals, for instance, in food consumption or uncontrolled stress, we regrouped the animals every 2 weeks, keeping the numbers of rats at 5 per group. The location of the cage in the animal room was changed every 2 months. The animals received a commercial diet ad libitum (Hope Farms AM II, Woerden, The Netherlands).

*L-D-shift stress.*—Mild chronic stress was given by alternating the light-dark rhythm of the animals undergoing the L-D-shift stress. Every Friday the automatic timer controlling the light for these animals was changed half a cycle; i.e., from the standard lighting conditions (7 a.m.-7 p.m. light and 7 p.m.-7 a.m. dark) to dark during the day and to light during the night; the next week standard lighting conditions again were used. In earlier studies, this procedure had proved to be stressful, which could be established by decreased immune response, by a slightly inhibited increase in body weight, and by a decreased weight of the adrenal glands (*16*). Control animals were housed under a standard lighting regimen: 7 a.m.-7 p.m. light and 7 p.m.-7 a.m. dark. Both animal rooms were adjacent and were identical in temperature, humidity, air-conditioning, and level of background noise. Handling of the animals was limited to cleaning (twice/week), which was done during daytime. In the dark phase for the animals in group I, an infrared light was used to perform the necessary handling.

*Autopsy.*—Autopsies were conducted according to the protocol described in one of our previous longevity studies (*21*). Each organ system was examined, and the macroscopic findings were recorded. Thymus, adrenal, and spleen weights, as a measure of immune capacity, were determined. Routine microscopic examinations were performed on samples of liver, spleen, mesenteric lymph nodes, peripheral lymph nodes, kidneys, adrenal glands, stomach, duodenum, ileum, cecum, colon, uterus-cervix-vagina, pancreas, pituitary gland, thyroid, thymus, heart, lungs, aorta, muscle, ovaries, mammary gland, and bone marrow. Other organs were only examined when suspected of neoplasms. Tissues were fixed in 4% buffered formaldehyde and, after being processed in an Autotechnicon, were embedded in paraffin. Sections at least 5 $\mu$m thick were stained with hematoxylin and eosin. Other stains were used when required by our pathologist (P. Z.). All materials were screened at least twice by the pathologist; in numerous cases additional expertise was requested from other pathologists.[5] Cardiac ischemia was considered to be present when areas of "waviness," contraction bands, nuclear pyknosis, and deep eosinophilia of the cytoplasm were visible in the heart muscle.

*Statistical evaluation.*—A chi-square analysis, adapted to evaluate cumulative incidence data, according to Peto's incidental analysis (*25*), was used. In the results, the calculated values were given as $G$-values with their corresponding $P$-values. The correlations between body weight and tumor incidence were calculated with the use of the Fisher's exact probability test ($G$-values).

Because the experimental data consisted of rats found dead or killed when moribund (with animals surviving up to 150 wk) and rats sacrificed at 150 weeks, the evaluation of the differences in the ages of the animals

had to be adjusted to this terminal sacrifice. By use of the Fisher's exact probability test, the number of tumor-bearing animals (for any tumor site) killed or found dead before 150 weeks was compared with the number of animals sacrificed at 150 weeks. When no significant difference could be shown, a Mann-Whitney analysis was used to compare the differences for the tumor site by age of group I and group II animals living less than 150 weeks. Differences were statistically significant at $P<.05$.

## RESULTS

From the 200 animals in the study, 2 rats in group I could not be further evaluated, due to cannibalism and autolysis; from some other animals, limited numbers of organs were missing for the same reasons. However, the Peto analysis (*25*) used in determining the differences in incidence was adapted for these missing organs. In the results given in text-figures 1 and 2 and in tables 1-6, the above-mentioned 2 animals from group I were excluded.

All the animals ($n=198$), independent of the experimental treatment, litter size, age of the mother at litter, week of birth, mortality in the litter, and parity (second or third litter), all of which might influence life-span, were analyzed for possible relation to survival and tumor incidence. However, no relation between one of these husbandry variables and survival or tumor incidence was present. However, correlations between body weight of all animals at a certain age to survival in general or to occurrence of tumors at some specific tumor sites were positively present (table 1); e.g., a strong correlation could be determined between the body weight at weaning (3 wk of age) and tumor incidence and also between body weight at weaning and the incidence of Langerhans' islet tumors. When the animals were 10 weeks of age, no such correlation between body weight and tumor incidence could be detected anymore. However, at 80 weeks the same predisposition for fat animals for eventually developing a tumor, as was detected earlier, was found again. Because, at weaning, fat and thin animals were equally divided in both experimental groups, the observed predisposition of the body weight at this age to forthcoming tumor processes could not have led to a possible misconception of the results of the tumor incidence in any of the experimental groups.

Very soon after the start of the experiment, the mean body weight of the experimental group began to diverge from that of the control group. In the first part of the experiment, the mean body weight (text-fig. 1) in group I was slightly less to that in group II; although this difference was significant ($P<.01$), most of the time the difference did not exceed 10 g. A maximum of 21 g in body weight difference was reached in the animals at 128 weeks; however, this relatively large difference in body weight between group I and group II was due to the fact that a selection of fat animals in group I died in this period.

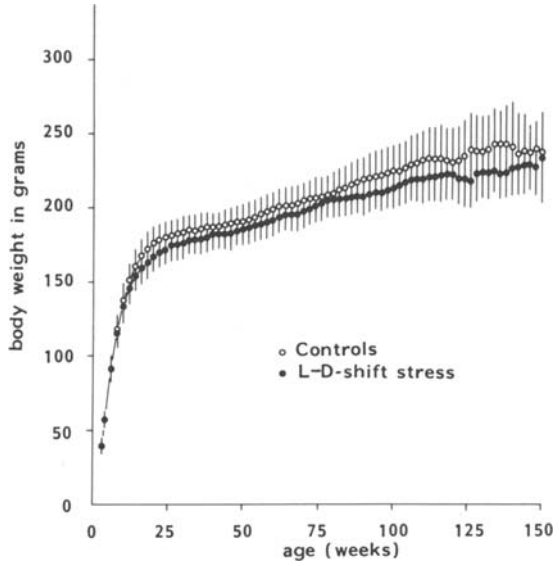
At autopsy the mean weights of thymus, spleen, and adrenal glands were not statistically significantly different. Although small differences existed in the weights of the thymus and adrenal glands (mean ± SE for thymus wt, 62.5±3.2 and 65.9±2.7, and for adrenal wt, 73.6±1.8 and 78.0±2.6, groups I and II, respectively), when these organ weights were corrected for the differences in body weight at termination they were almost equal.

TABLE 1.—*Body weight in relation to survival, occurrence of tumors (general), occurrence of mammary tumors, and Langerhans' islet tumors of groups I and II together*[a]

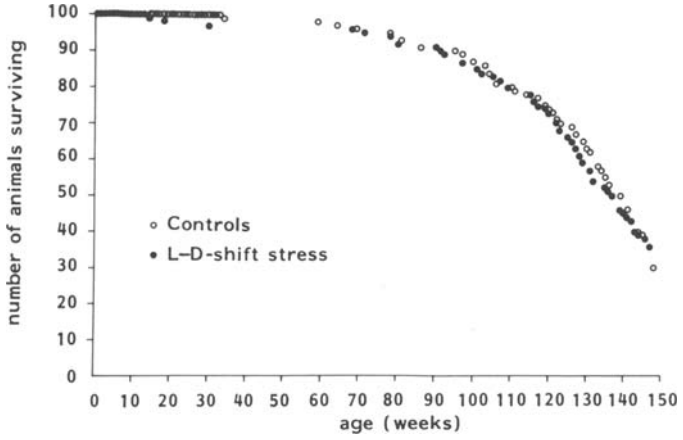
| Body wt, g/rat | Wk No. | No. of rats | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Surviving at wk: | | Having tumors | | Having mammary tumors | | Having Langerhans' islet tumors | | Total rats |
| | | ≤120 | >120 | Yes | No | Yes | No | Yes | No | |
| ≤40 | 3 | 59 | 43 | 73 | 29 | 14 | 88 | 17 | 85 | 102 |
| >40 | | 43 | 53 | 86 | 10 | 20 | 76 | 29 | 76 | 96 |
| | | [3.37] | | [10.15][b] | | [1.76] | | [5.08][b] | | |
| ≤136 | 10 | 53 | 49 | 79 | 23 | 18 | 84 | 21 | 18 | 102 |
| >136 | | 49 | 47 | 80 | 16 | 16 | 80 | 25 | 71 | 96 |
| | | [0.02] | | [1.08] | | [0.03] | | [0.82] | | |
| ≤171 | 20 | 55 | 49 | 79 | 25 | 17 | 87 | 21 | 83 | 104 |
| >171 | | 45 | 47 | 80 | 12 | 17 | 75 | 25 | 67 | 92 |
| | | [0.31] | | [3.85][b] | | [0.15] | | [1.32] | | |
| ≤185 | 40 | 51 | 55 | 85 | 21 | 16 | 90 | 24 | 82 | 106 |
| >185 | | 47 | 41 | 74 | 14 | 18 | 70 | 22 | 66 | 88 |
| | | [0.54] | | [0.50] | | [0.96] | | [0.15] | | |
| ≤207 | 80 | 44 | 50 | 71 | 23 | 12 | 82 | 17 | 77 | 94 |
| >207 | | 48 | 46 | 84 | 10 | 20 | 74 | 29 | 65 | 94 |
| | | [0.34] | | [6.21][b] | | [2.41] | | [4.14][b] | | |

[a] Group I: L-D-shift stress; group II: untreated controls. Body wt and survival parameters are divided into two categories, with the use of the median as point of division. *No. in brackets, G*-value (Fisher's exact probability test).

[b] Animals classified as fat at 3 wk (at weaning) and at 80 wk eventually developed significantly ($P<.05$) more often a tumor or in particularly a Langerhans' islet tumor than did the thin animals.




TEXT-FIGURE 1.—Comparison of body weight increase in groups I and II. *Vertical bars* represent the SD. Differences in body weight were statistically significant after 20 wk.




TEXT-FIGURE 2.—Comparison of the actuarial survival of groups I and II. Each group originally consisted of 100 animals; L-D-shift stress was given during the rat's whole life-span.

From all animals entering the study, 36 and 30 in groups I and II, respectively, survived the 150-week observation period and were sacrificed at that age while in good condition. From these 36 and 30 animals, respectively, 9 and 3 animals had, apart from some minor nontumor processes, no pathologic conditions. Difference in tumor incidence at 150 weeks ($G$-value, 2.48; $P<.10$) was probably one of the most convincing proofs in this experiment for the relative harmless effect exhibited by lifelong exposure to chronic stress.

In the actuarial survival curve (text-fig. 2) the congruent shape of both survival patterns can be seen. The MST of all animals was 138.5 and 140.5 weeks, a difference which was not statistically significant in the Mann-Whitney analysis ($Z$-value$=-0.05$, $P=.95$).

The animals dying in the course of the experiment from nontumor processes died mostly with signs of cardiac ischemia. In group I 4 of 12 and in group II 9 of 12 animals died from heart failure due to cardiac ischemia (the animals sacrificed at termination were not included in this comparison). All other animals, apart from the animals who some days before death had difficulties in breathing, died without any clinical symptoms or without a detectable loss of body weight, which could have predicted the oncoming death of the animal. From some of these rats, due to cannibalism, numerous organs were missing. For more details of all the important nontumor processes, *see* table 2. It has to be stressed that the pathologies given in this table were mostly not life threatening. The recorded nontumor processes were almost equally divided for both groups; no statistical significant differences in incidence or in mean age were present.

Totals of 128 and 154 tumors were found in 74 and 85 animals in groups I and II, respectively. The mean numbers of tumors per rat were 1.7 and 1.8, respectively. The pattern of the spontaneous tumor incidence is seen in table 3. In table 4 the numbers of tumors in tumor-bearing rats are given.

The differences in tumor incidence were not statistically significant for tumor-bearing rats in general or for the number of tumor-bearing rats for a particular tumor site. The fact that in group I fewer tumor-bearing rats (11) were present had, as a matter of course, also its influence on the difference in the total number of tumors found. Furthermore, it was striking that almost twice as many tumors of the cervix-uterus appeared in group II; this otherwise nonsignificant difference was caused by a high number of leiomyomas found in the

TABLE 2.—*Incidence of nontumor processes and mean ages of rats*[a]

| Nontumor processes | Group I | | Group II | | Peto analysis (*25*); Nontumor incidence | |
|---|---|---|---|---|---|---|
| | No. of rats | MST, wk | No. of rats | MST, wk | *G*-value | *P*-value |
| Uterine infection | 8 | 147 | 10 | 148 | 0.10 | 0.75 |
| Hydronephrosis | 52 | 144 | 49 | 148 | 0.37 | 0.55 |
| Biliary cysts | 35 | 150 | 49 | 148 | 1.48 | 0.22 |
| Pancreatic atrophy | 14 | 150 | 22 | 145 | 0.00 | 1.00 |
| Mammary hyperplasia | 49 | 144 | 53 | 148 | 1.26 | 0.27 |
| Cardiac ischemia | 14 | 140 | 22 | 138 | 1.14 | 0.29 |

[a] Group I: L-D-shift stress; group II: untreated controls.

TABLE 3.—*Incidence pattern of spontaneously occurring tumors*[a]

| Anatomic site and tumor type | Group I | | Group II | |
|---|---|---|---|---|
| | *n* | MST, wk | *n* | MST, wk |
| Pituitary gland | | | | |
| Carcinoma | 1 | 124 | 1 | 150 |
| Adenoma | 31 | 145 | 28 | 147 |
| Adrenal gland | | | | |
| Cortical carcinoma | 9 | 144 | 9 | 134 |
| Cortical adenoma | 2 | 145 | 6 | 146 |
| Medullary pheochromocytoma | 6 | 147 | 8[b] | 147 |
| Pancreas endocrine | | | | |
| Islet cell carcinoma | 2 | 150 | 1 | 150 |
| Islet cell adenoma | 23 | 150 | 20[b] | 150 |
| Thyroid | | | | |
| Medullary carcinoma | 2 | 150 | 4 | 132 |
| Mammary gland | | | | |
| Adenocarcinoma | 6 | 147 | 4 | 150 |
| Adenofibroma | 9 | 124 | 16[b] | 118 |
| Brain | | | | |
| Meningioma | 1 | 132 | — | |
| Granular cell myoblastoma | 3 | 144 | 3 | 148 |
| Cervix-uterus | | | | |
| Squamous cell carcinoma | — | | 1 | 148 |
| Leiomyosarcoma | 4 | 120 | 5 | 134 |
| Leiomyoma | 5 | 140 | 11 | 148 |
| Liver | | | | |
| Hemangioendothelioma | 1 | 150 | 4 | 130 |
| Gastrointestinal tract | | | | |
| Leiomyosarcoma | — | | 1 | 87 |
| Leiomyoma | — | | 1 | 137 |
| Carcinosarcoma | — | | 1 | 150 |
| Fibroma | 1 | 117 | — | |
| Papilloma | 1 | 150 | 5 | 150 |
| Skin and subcutaneous tissue | | | | |
| Osteosarcoma | 1 | 141 | — | |
| Melanoma | 3 | 124 | — | |
| Fibrosarcoma | — | | 1 | 150 |
| Squamous cell carcinoma | 1 | 145 | — | |
| Squamous cell papilloma | 1 | 140 | | |
| Basal cell carcinoma | — | | 1 | 140 |
| Angiosarcoma | — | | 1 | 143 |
| Salivary gland | | | | |
| Adenocarcinoma | 1 | 137 | — | |
| Lungs | | | | |
| Adenoma | — | | 1 | 135 |
| Kidney and bladder | | | | |
| Transitional cell carcinoma | 1 | 150 | 3 | 150 |
| Adenoma | — | | 2 | 134 |
| Lymphoreticular tumor | | | | |
| Histiocytic sarcoma | 5 | 150 | 1 | 79 |
| Myelomonocytic leukemia | 5 | 120 | 8 | 129 |
| Lymphoblastoma | 3 | 140 | 2 | 126 |

[a] Group I: L-D-shift stress; group II: untreated controls. *n* = number of animals with a tumor of the specified type. — = no tumor present.

[b] Includes No. of animals in which >1 tumor was found; *see* also table 5.

untreated control group. For the rest, the similarity in groups I and II for tumor incidences and age of tumor-bearing animals was evident. In both groups a gradual increase in the number of tumors was noted, eventually resulting in greater than 2 tumors per rat in the last 10-week period.

Table 5 shows the difference in tumor multiplicity (>1 tumor at the same anatomic site) between both groups. Whereas in group I, no tumor multiplicity was to be seen, in group II tumor multiplicity was shown seven times, at the indicated tumor sites.

In animals bearing mammary tumor, often certain endocrine tumors were found as well (table 6). Such conjunctions were seen in the same frequency in both groups of animals. The incidence of mammary tumors together with pituitary gland tumors or together with Langerhans' islet tumors was most pronounced.

The number of animals in which a tumor was found with distant metastases is given in table 7. Uniformly, in this rat strain only a limited number of tumors metastasize. In the results of this study, no exception to this finding was seen. The adrenalcortical carcinoma, the only tumor with a high metastatic rate, was found five times in group I and three times in group II, with metastases to lungs and/or liver. Also, in this respect, no significant difference between both groups was found.

## DISCUSSION

In the study of the effect of chronic stress on tumor incidence or on longevity, numerous factors may influence the results even more than in the study of acute stress. In particular, the effect of uncontrolled stress due to handling and to housing conditions, such as background noise, pheromones, and infections preexisting or entering during the study, may result in unwanted variation of the results. The elimination of these unwanted factors will make an experiment on chronic stress often a cumbersome and certainly a risky venture. Although we cannot be completely sure that all factors leading to uncontrolled stress were eliminated, we are almost certain that the conditions were near optimal and, what is even more important, that background stress was identical in both experimental groups.

With regard to the chosen way to induce the stress, one may wonder if the quality and quantity of L-D-shift stress can be compared with certain types of chronic stress in humans. Although caution must be exercised when rodent stress is compared to human stress, the comparison between L-D-shift stress and the stress that, for instance, shift workers undergo seems not to be too far-fetched. Rotation shift has been a subject in several studies in which the noxious effect of this social and physiologic stress on the organism has been investigated (*26, 27*). Without much explanation, to humans the social disrupture attended by rotation shift stress is an important part of the stress whereas to rats L-D-shift stress is accomplished exclusively by means of the physiologic impairment of endocrine circadian rhythm (*28, 29*). Either as a result of this disturbance of endocrine organ function or by other mechanisms, L-D-shift stress, like most other stresses, resulted in a decreased immune capacity (*16, 27*).

In the present study, the immune capacity was not measured, because of the earlier mentioned possibility to induce unwanted stress by this procedure. However, we had every reason to believe that, like in earlier studies, which were carried out under exactly the same condi-

TABLE 4.—*Number of tumors in tumor-bearing rats*[a]

| Age, wk | No. of rats dying | | No. of rats with the following No. of tumors | | | | | Total No. of tumors | Mean[b] |
|---|---|---|---|---|---|---|---|---|---|
| | Total | With tumor | 0 | 1 | 2 | 3 | 4 | | |
| **Group I with L-D-shift stress** | | | | | | | | | |
| 0-10 | 0 | 0 | — | — | — | — | — | — | — |
| 11-20 | 2 | 0 | 2 | — | — | — | — | — | — |
| 21-30 | 0 | 0 | — | — | — | — | — | — | — |
| 31-40 | 1 | 0 | 1 | — | — | — | — | — | — |
| 41-50 | 0 | 0 | — | — | — | — | — | — | — |
| 51-60 | 0 | 0 | — | — | — | — | — | — | — |
| 61-70 | 0 | 0 | — | — | — | — | — | — | — |
| 71-80 | 2 | 0 | 2 | — | — | — | — | — | — |
| 81-90 | 2 | 2 | — | 2 | — | — | — | 2 | 1.0 |
| 91-100 | 5 | 3 | 2 | 2 | 1 | — | — | 4 | 1.3 |
| 101-110 | 7 | 5 | 2 | 5 | — | — | — | 5 | 1.0 |
| 111-120 | 6 | 6 | — | 4 | 2 | — | — | 8 | 1.3 |
| 121-130 | 15 | 13 | 2 | 10 | 2 | 1 | — | 17 | 1.3 |
| 131-140 | 12 | 11 | 1 | 5 | 3 | 3 | — | 20 | 1.8 |
| 141-150 | 46 | 34 | 12 | 8 | 17 | 6 | 3 | 72 | 2.1 |
| Total | 98[c] | 74 | 24 | 36 | 25 | 10 | 3 | 128 | 1.7 |
| **Group II, untreated controls** | | | | | | | | | |
| 0-10 | 0 | 0 | — | — | — | — | — | — | — |
| 11-20 | 0 | 0 | — | — | — | — | — | — | — |
| 21-30 | 0 | 0 | — | — | — | — | — | — | — |
| 31-40 | 1 | 0 | 1 | — | — | — | — | — | — |
| 41-50 | 0 | 0 | — | — | — | — | — | — | — |
| 51-60 | 1 | 1 | — | 1 | — | — | — | 1 | 1.0 |
| 61-70 | 2 | 2 | — | 1 | 1 | — | — | 3 | 1.5 |
| 71-80 | 2 | 1 | 1 | 1 | — | — | — | 1 | 1.0 |
| 81-90 | 3 | 3 | — | 2 | 1 | — | — | 4 | 1.3 |
| 91-100 | 2 | 2 | — | 2 | — | — | — | 2 | 1.0 |
| 101-110 | 8 | 7 | 1 | 6 | 1 | — | — | 8 | 1.3 |
| 111-120 | 6 | 5 | 1 | 1 | 2 | 2 | — | 11 | 2.2 |
| 121-130 | 10 | 6 | 4 | 6 | — | — | — | 6 | 1.0 |
| 131-140 | 15 | 14 | 1 | 9 | 3 | 2 | — | 21 | 1.5 |
| 141-150 | 50 | 44 | 6 | 13 | 16 | 11 | 2 | 97 | 2.2 |
| Total | 100 | 85 | 15 | 42 | 24 | 15 | 2 | 154 | 1.8 |

[a] — = no tumor present.
[b] Mean = mean value calculated by dividing the No. of tumors by the No. of tumor-bearing rats.
[c] Two animals (dying at 69 and 133 wk) that could not be examined histologically were excluded.

tions, during a long period of time the immune capacity should be depressed (*16*). As others reported, for most tumor types this immunosuppression does not have to implicate that a higher incidence of neoplastic pathologies has to be observed (*30*). Inasmuch as most spontaneous tumors are not immunogenic or are very weakly

TABLE 5.—*Number of rats with >1 tumor at the same anatomic site*[a]

| Anatomic site | No. of rats in group I with No. of tumors: | | | No. of rats in group II with No. of tumors: | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | Total | 1 | 2 | Total |
| Mammary glands | 15 | 0 | 15 | 17 | 2 | 21 |
| Langerhans' islets | 25 | 0 | 25 | 19 | 2 | 23 |
| Adrenal gland (cortex) | 11 | 0 | 11 | 13 | 1 | 15 |
| Adrenal gland (medulla) | 6 | 0 | 6 | 6 | 2 | 10 |

[a] Group I: L-D-shift stress; group II: untreated controls.

TABLE 6.—*Number of rats with a mammary tumor together with a tumor at the following anatomic site*[a]

| Anatomic site | Group I | | Group II | |
|---|---|---|---|---|
| | No. of rats | Percent of mammary tumors[b] | No. of rats | Percent of mammary tumors[b] |
| Mammary gland + pituitary gland | 6 | 40.0 | 5 | 23.8 |
| Mammary gland + Langerhans' islets | 4 | 26.7 | 4 | 19.1 |
| Mammary gland + pancreatic gland + pituitary gland | 2 | 13.3 | 3 | 14.3 |
| Mammary gland + adrenal gland | 1 | 6.7 | 3 | 14.3 |
| Mammary gland + thyroid | 1 | 6.7 | 2 | 9.5 |

[a] Group I: L-D-shift stress; group II: untreated controls.
[b] Percentage is calculated by dividing the No. of rats with the specified combination of tumors by the total No. of mammary tumor-bearing rats (group I = 15; group II = 21)×100.

TABLE 7.—*Number of tumors found with distant metastases*[a]

| Original anatomic tumor site, tumor type | Total No. of tumors/ No. of tumors with metastases | |
|---|---|---|
| | Group I | Group II |
| Adrenal gland, cortical carcinoma | 9/5 | 9/3 |
| Adrenal gland, medullary pheochromocytoma | 6/0 | 8/1 |
| Pancreas, Langerhans' islet carcinoma | 2/1 | 1/0 |
| Bone, osteosarcoma | 1/1 | — |

[a] Group I: L-D-shift stress; group II: untreated controls. Tumors were metastasized to lungs (from adrenal gland, cortical carcinomas; from adrenal gland, medullary pheochromocytoma), to liver (from adrenal gland, cortical carcinomas), to brains (from adrenal gland, cortical carcinomas), and to pleura (Langerhans' islet carcinoma). — = no tumor present.

immunogenic (*14*), a very limited role of stress-induced immunosuppression on the incidence of spontaneous tumors would be expected. However, other mechanisms might be involved that are apart from a pathway via immunosuppression. Certainly, when one chooses L-D-shift stress, which not only disturbs the circadian rhythm of a number of hormones, such as insulin and corticosteroids, but may decrease or increase the basal hormone levels, a direct interaction between this altered hormone function and tumor incidence may become possible (*31*). In this respect, mention has been made of the stress-increased prolactin levels on mammary tumor incidence for example (*32*). Furthermore, endogenous opioids have been mentioned (*33*).

In the results of the present study, the small but significant retardation in body weight of the animals under L-D-shift stress shows that a certain disturbance of the normal environmental conditions of the animals is present. This decrease in body weight may have had a direct influence on tumor incidence. In the results, a strong correlation between thin animals and nontumor occurrence was found. Therefore, the L-D-shift stress may have had, by the effect observed on the body weight, a positive influence on survival. It will be difficult to assess the contribution of this difference in body weight to tumor incidence. Furthermore, because the tumor incidence from Langerhans' islet tumors, which was the tumor with the most obvious correlation between body weight and incidence, was distributed equally in both groups, a relation between an L-D-shift stress-linked decrease in body weight and tumor incidence seems very unlikely. The finding that weight at a certain age had consequences on survival or tumor incidence was seen in earlier studies by our group and by others (*21, 34*). Apart from this indirectly mentioned (via a decrease in body weight) effect on tumor incidence and longevity, other factors must have been involved. An effect from L-D-shift stress more directly focused on the organism must be assumed to be responsible for the generally favorable outcome of chronic stress in regard to tumor incidence and longevity. This finding seems to receive little support from the data obtained by others; however, we must realize that most evidence for a positive correlation between stress and cancer came from studies on acute stress and cancer (*8*). In studies in which chronic or repeated acute stress was investigated, no hazardous effects were found; and in some other studies even a beneficial effect on longevity or of less chance for tumor incidence was found (*8, 35*).

Organ weights not significantly different between group I and untreated controls suggest that the chosen way of inducing stress was not effective, for stress is correlated with an increased adrenal gland weight and decreased thymus and spleen weight. With chronic stress this finding is not true: At the most no change in adrenal gland weight was seen; however, slightly decreased weights were found (*15, 16*). Furthermore, it is important to realize that all organ weights were established at termination, which means that they represent an end-point situation.

Distribution according to tumor site and tumor incidence as was found in the present experiment is not essentially different from the results that Burek (*23*) found in his study in BN female rats under conditions comparable with those for untreated controls. In this study (*23*) also a relative high incidence of cervix-uterus tumors was noted. In 19% of the animals, such a tumor was found, at an incidence in agreement with the frequency of the tumors as were detected in the untreated animals in our study (17%). Therefore, the low cervix-uterus tumor incidence in the animals in group I reveals the positive contribution of chronic stress in lessening the potential for tumor incidence at this anatomic site. So far we do not have a reasonable explanation for this phenomenon.

We conclude that chronic L-D-shift stress certainly does not contribute to increase of tumor incidence or lessen the mean age of the animals. Furthermore, the group receiving chronic stress showed favorable results in regard to the total number of tumors, the number of tumor-bearing animals, the incidence of cardiac lesions, and the incidence of tumors on the cervix-uterus. As to how far this finding may be of some comfort to those persons living under a strain comparable to the investigated L-D-shift stress is hard to assess; but for now there is not much evidence, epidemiologically as well as experimentally, that such stress is a high risk for cancer or decreased longevity.

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