Insect Tumors Induced by Nerve Severance: Incidence and Mortality*

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Among the causative and contributory factors in abnormal growth, the nervous system deserves attention—even though, to date, very little experimental evidence is available to suggest a relationship between nervous disturbance and tumor development. The insect, *Leucophaea maderae*, is one example which illustrates such a relationship. In this species, tumors[1] can be induced experimentally by severance of the recurrent nerve. Branches of this autonomic nerve innervate the organs affected, i.e., the anterior portion of the alimentary canal (foregut and anterior midgut, or stomach) and the salivary organs (salivary glands and reservoir). This observation has been reported in preliminary form (1–3). A series of studies is intended to deal with certain aspects of this problem in somewhat more detail.

A large proportion of animals in which the recurrent nerve has been cut succumb to these tumors after varying periods of time. It had been noted early in this work that males and females show differences in their response to the development of tumors (4). The nature of these differences was analyzed in experiments to be reported here.

MATERIALS AND METHODS

Leucophaea, the insect used in this work, is a large, ovoviviparous roach belonging to the subfamily Panchlorinae. Of South American origin, the animal has been bred in the laboratory for over 10 years. Stock colonies are kept in large earthenware crocks; for experimental animals, pint-size glass jars are suitable. The food consists of apples, carrots, dog chow, and occasionally hard-boiled eggs. This insect offers many advantages as an experimental animal. Neither anesthesia nor sterile precautions are necessary for the operations performed. Postoperative losses are small. Since the normal life span of the species is long (2–3 years), there is ample time for tumors to develop.

In the experimental series on which this report is based, conditions were kept as constant as possible. The animals used for operations were "dated," i.e., the time of their emergence from nymphs into adults was recorded. All animals were young adults (not exceeding 48 days of "adult age") at the time of the nerve operation. As many suitable specimens as became available were subjected to nerve severance within approximately the same period of time. For reasons to be discussed later, the gonads were removed from about one-half of the group several weeks prior to nerve severance.

Thus, four groups of animals were available for comparison:

a) Females with nerve severance
b) Males with nerve severance
c) Castrate females with nerve severance
d) Castrate males with nerve severance

The animals were regularly checked. When a specimen had died or had to be sacrificed because it seemed close to death, it was autopsied, and the presence or absence of tumors was recorded. As "positive" were marked those animals in which at least one of the organs normally innervated by the recurrent nerve showed a tumor. There are no certain criteria by which the presence of tumors can be ascertained in living, unopened animals. In cases where the response to the nerve injury is slow, death may occur before tumor growth has proceeded to the point where it can be identified with certainty at autopsy. Even with daily checks for deaths in the tumor colonies, a small number of animals are unsuitable for histological examination, because the organs involved may show post mortem decay. Another small proportion of animals is lost in each experimental series for various reasons, such as escape, occasional cannibalism,[2] death from postoperative or unidentified causes.

RESULTS

Tumor Incidence

A precise determination of the incidence of insect tumors due to nerve severance meets with difficulties. For one, the interval between operation and death from the tumors varies widely—from a few days to several months. The longer the animals survive, the greater is their chance to be lost for reasons mentioned in the preceding chapter. Among the lost animals may be potential positive tumor cases. Since only those specimens in which

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[1] The term "tumor" as used here does not imply that the growths under investigation are in every respect of the same nature as neoplasms in vertebrates. Histopathological and histophysiological data have been accumulated and will be reported in subsequent papers in which these growths will be characterized in detail and in which their differentiation from lesions such as injury reactions (6) will be discussed.

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[2] This difficulty could be avoided by placing experimental animals in individual containers. However, it has been found advantageous to keep two to three specimens together in order to promote activity, feeding, etc.

tumors could actually be identified are listed as positive in our experiments, the figures on tumor incidence obtained represent conservative values.

Of the 150 specimens in which the recurrent nerve had been cut, 95 were classified as positive, 39 negative, and 16 inconclusive—i.e., they were difficult to diagnose on account of post mortem changes, etc. The tumor incidence in this group was therefore 71 per cent (63 per cent for the males, 76 per cent for the females). Among the 189 gonadectomized specimens with nerve severance, 117 were positive, 39 negative, and 30 inconclusive. The tumor incidence for this group as a whole was 75 per cent (83 per cent for the males, 67 per cent for the females).

These values are in line with those of earlier experimental series in which the estimated incidence ranged from about 70 to 80 per cent. It can thus be stated that in the insect, *Leucophaea*, the incidence of tumors developing due to nerve severance, is high, i.e., roughly 75 per cent. Castration preceding nerve operation does not seem to influence the tumor incidence appreciably. Although it would appear that more castrate males develop tumors than castrate females and noncastrate males, these differences should not be considered significant unless they can be further substantiated. In part, they can be explained on the basis of differences in mortality rates, which will be discussed in the following section.

It has been mentioned that tumors develop in several locations below the level of nerve transection. Post mortem examination of a given specimen may show the presence of tumors either in one or more of the organs in question. The relative frequency with which each of these sites is involved differs markedly. These differences seem to depend, at least in part, on varying speeds of tumor growth, according to the organ affected, and on differences in the lethal properties of the various tumor types.

The site which was found most frequently affected was the midgut (stomach). Of 212 positive cases (with one or more tumor sites) in the present series of experiments, 160 had tumors in the midgut, 88 in the salivary organs, and ten in the foregut.

Mortality of Tumor-bearing Insects

It has been stated before that the time elapsing between nerve severance and death due to tumors varies considerably. Since the onset of tumor growth cannot be determined without sacrificing the animal, no precise statement can be made as to when tumors start to grow and how long a tumor-bearing animal can survive. It may well be that first signs of tumorous change appear soon after nerve severance in all specimens concerned. This process may lead to rapid tumor growth, in some cases causing early death, and may proceed so slowly in others that it does not become fatal before death from other causes intervenes.

For the duration of the survival period also the site of tumor formation is important. Tumors in the midgut tend to appear soon after operation and often develop so rapidly that death occurs before tumors in the salivary organs have a chance to develop. With increasing survival rates, therefore, the incidence of tumors in the salivary organs, particularly in the salivary reservoir, increases. Tumors in the salivary organs alone may or may not be lethal to the host. At any rate, in the experiments reported here midgut tumors must be considered the prime cause of death.

In an evaluation of the tumor mortality in *Leucophaea*, it is necessary, therefore, to focus attention on those tumor deaths that occur relatively soon after nerve severance. Consequently, it was decided to consider only those animals that died of tumors within 200 days after the nerve operation. Tumor-bearing animals that survived longer were not included in the analysis. Their tumors, being located for the most part in the salivary reservoirs, could not be considered responsible with reasonable certainty for death, in view of the survival rate of normal adults. To illustrate this point, in a group of 117 unoperated controls, the mean "adult age" (interval between emergence of the adult and death) was almost 13 months (387 days), with a maximum of 29 months in females and of 21 months in males. The gap between these normal survival rates and the "adult age" (a maximum of 48 days preceding nerve section plus up to 200 days postoperatively) of the tumor groups seems sufficiently large to minimize the danger of including in the analysis deaths not attributable to the tumors.

Among the animals in which the recurrent nerve had been severed, 54 tumor-bearing females and 28 tumor-bearing males died within the period of 200 days after the operation. The mortality rate in the two groups shows a marked difference in that the males survived considerably longer than the corresponding female group. For the male group, the mean survival time was 104.5 days, with a standard error of ± 11.43; that for the female group was 65.0 days, with a standard error of ± 6.71. Calculation of the standard error of the difference between these two means results in a critical ratio (C.R.) of 3, which indicates that such a situation would occur by chance alone 3 times in 1,000. The difference in survival time, therefore,

appears to be statistically significant.[3] The mortality rates are expressed on a percentual basis in Chart 1. The curves were obtained by plotting the percentage of survivors in each group at 10-day intervals.

These curves, as well as the mean values, clearly indicate a sex difference in tumor mortality. Special attention should be called to the first few weeks, which constitute the most significant period. Here the difference between the sexes is rather striking. While the first male tumor death did not occur until the 23d day after operation, ten females died of tumors between 2 and 23 days. The 2-day interval is remarkable in that it represents the earliest tumor death ever recorded in our work. The possibility that this case might have been a spontaneous tumor is exceedingly remote. Among approximately 500 normal adult animals examined, only two had "spontaneous" tumors, none of which had reached the size of those obtained experimentally by nerve severance.

The question arose whether or not the observed sex difference in tumor mortality had anything to do with hormonal differences. There is still no definite proof for the presence of sex hormones in insects, although there are certain indirect indications for it (5). The demonstration of a possible influence of the male and female gonads on resistance to tumors would in turn constitute additional indirect evidence for the existence of sex hormones in insects. Therefore, it seemed to be of interest to test the effect of castration on tumor mortality.

Gonadectomy was performed, in either newly emerged adults or old nymphs. In order to allow ample time for the effect of this operation to manifest itself, subsequent nerve severance was not performed until at least 3 weeks had passed after castration. As can be seen from Chart 1, the two curves representing the survival rates of tumor-bearing castrates are very similar and constitute values intermediate between those of noncastrate tumor-bearing males and females. The mean value for the survival rate of the male castrate group was 80.6 days (±6.18), that for the female castrates, 86.0 days (±7.40). The two values do not differ significantly (C.R. = 0.56; P = 0.58). For the difference between male castrate and male noncastrate tumor-bearing animals, the C.R. is 1.84 (P = 0.06), and for that between female castrate and female noncastrate tumor-bearing animals the C.R. is 2.10 (P = 0.04).

The comparison of the four experimental groups of animals studied leads to the following conclusion: Since tumor-bearing noncastrate males and females show a difference in survival rate which

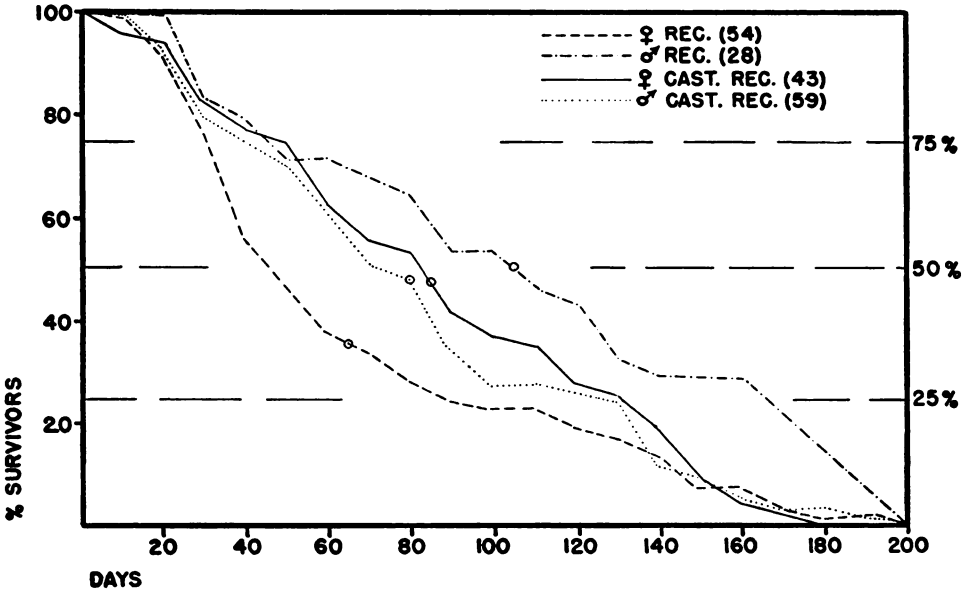

CHART 1.—Mortality rates of adult tumor-bearing Leucophaea males and females during a period of 200 days following nerve severance. The animals of the groups marked "REC." (54 females and 28 males) had the recurrent nerve cut on day 0. The animals of the groups marked "CAST. REC." (43 females and 59 males) had been castrated several weeks prior to day 0, on which nerve severance was performed. The curves show the survival rates of the four groups of animals on a percental basis. The means are indicated by small circles.

[3] I am grateful to Dr. Edith Boyd, Child Research Council, for her assistance in the statistical evaluation of these results.

can be abolished by castration, this difference must in some way be attributed to the gonads.

DISCUSSION

It has been shown that the presence of the sex glands in tumor-bearing insects seems responsible for differences in the mortality rates of males and females and that this difference disappears after gonadectomy. This result lends support to the assumption, so far only based on indirect evidence, that there are sex hormones in insects. A somewhat closer analysis of the survival rates in the four groups of experimental animals represented in Chart 1 shows that, while gonadectomy decreases the mortality in females, it increases it in males. The effect on the females might be explained in terms of metabolic changes. Normally, a substantial amount of reserve substances is needed for the development of the young. When the demand is abolished by ovariectomy, these substances become available for the needs of the female. These needs are increased by tumor development, which affects the vitally important alimentary canal. If tumor death were merely a consequence of starvation due to pathological changes in the gut, the change in tumor mortality after castration could be explained, at least as far as females are concerned, by changes in their metabolism, and no direct hormonal influences would need to be postulated. That the situation is not quite so simple is indicated, however, by metabolic studies which were undertaken to clarify this question (7). This work will be reported later in more detail. The results indicate that death in tumor-bearing females may occur at a time when their body reserves (especially fatty acids) are still intact. The causes of death must, therefore, be other than metabolic. In other words, in the female group the survival rate cannot be taken as a mere reflection of the body content in reserve material and cannot, therefore, be explained on this basis alone.

As far as the males are concerned, it is even more unlikely that metabolic changes caused by castration should themselves be responsible for the decrease in resistance to tumor growth. Even if changes in metabolism could be made responsible for the changed tumor resistance due to castration, the manner in which these metabolic changes are brought about suggests for its explanation the action of sex hormones.

Little need be said about the reported data on tumor incidence. They indicate that a large proportion, approximately $\frac{3}{4}$, of the specimens in which the recurrent nerve has been severed succumb to tumors. Evidence is not sufficient to postulate that the sexes differ with respect to the number of induced tumor cases. The seemingly higher incidence in females as compared to males of the same noncastrate group could be explained, at least in part, on the basis of the shorter survival time in females, which permits the identification of tumor cases with a somewhat greater degree of certainty. Furthermore, the observation that castration has no essential influence on tumor incidence makes a sex-linked difference in tumor susceptibility unlikely.

The higher frequency of tumors in the midgut, as compared to other locations, may be due to the fact that midgut tumors develop faster and reach lethal proportions before other tumors had a chance to manifest themselves.

SUMMARY

1. In the insect *Leucophaea maderae* the incidence of tumors developing after severance of the recurrent nerve is high—i.e., roughly 75 per cent. The incidence does not seem to differ significantly in males and females. Accordingly, castrates respond to the nerve transection in approximately the same way as noncastrate animals. Among the organs involved tumors appear most frequently in the midgut (stomach), an observation which may perhaps be explained by a more rapid and more destructive growth pattern of these as compared to tumors in other sites (salivary organs, foregut).

2. The mortality rates of tumor-bearing animals differ significantly: males as a group survive longer than females. The sex difference in resistance to the presence of tumors disappears after castration. This observation offers indirect evidence, but no proof, for the presence of sex hormones in insects.

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