tioning'' and that they provide evidence that ''*Pleurobranchaea* is capable of higher forms of learning.''

Turning to the earlier experiments (*1*), we generally agree with Lee's procedural comments, and we agree that the experimental design was less adequate than in the more recent study since the ''unpaired'' control group did not receive the CS and US alternately and within the span of time when the experimentals were conditioned. However, the ''unpaired'' control group did receive both the CS and US separately. Of these experiments, Lee raises three major issues dealing with nonassociative phenomena: sensitization, shock-induced inhibition, and facilitated extinction. We mentioned that sensitization may have been a factor underlying the behavior changes of the animals. In both the classical and avoidance conditioning procedures we attempted to minimize sensitizing the experimental or control animals differentially by seeking to apply equivalent amounts of the US; we felt that similar responses executed over the same period of time could be taken as a measure of equivalent stimulus strengths. For example, in the classical conditioning experiments we presented the food US to the control animals differently than to the experimentals in order to avoid exposing the control animals to tactile stimuli concomitantly with the food. Since the US in both cases elicited roughly the same number of feeding responses over the same period of time, we felt that the US's were equivalent. While this does not completely eliminate the possibility that the two methods of US presentation differentially sensitized the animals, it provides some evidence that the experimental-control differences were caused by CS-US pairing.

We also considered the possibility of shock-induced inhibition as the cause of the behavior changes of the experimental animals during avoidance conditioning. However, there are several lines of direct and indirect evidence that support the argument against it. Figure 2C in our earlier report (*1*) shows two groups of animals that were switched from conditions of experimental-avoidance to touch-control, one group on day 11, which Lee mentions, and another on day 15. Both groups of animals continued to withdraw from tactile stimuli for several days after the switch, indicating that some long-term effect had been conditioned. Figure 2, A and C, in (*1*) shows shock-control groups that received as much electrical stimulation as the experimental animals but continued to exhibit vigorous feeding responses to tactile stimulation for many days. The lack of long-term inhibition in these animals is inconsistent with the hypothesis of shock-induced inhibition. Our argument against shock-induced inhibition is indirectly supported by published observations that the normal feeding behavior of *Pleurobranchaea* is not suppressed even after strong electrical stimulation (*6*), and by the behavior of the control animals in the aversive conditioning experiments, which continued to feed despite the fact that they were exposed repeatedly to great amounts of electrical stimulation.

The statement that ''even if the experiment were improved with proper control groups, what would be demonstrated is only the facilitation of the extinction of a classically conditioned—or perhaps, sensitized—response'' is inconsistent with Lee's own use of the ''required control,'' and contains the unsupported implication that conditioned responses cannot be superimposed on previously altered behaviors: if Lee's proper control group were conducted, differences between the experimentals and controls would have to be accepted by definition as true associational conditioning, regardless of how the original response was established before the avoidance conditioning.

In conclusion, we feel that our reports, especially the more recent one, provide evidence that *Pleurobranchaea* is capable of associative learning. In his closing remarks on the recent report, Lee asks for objectivity and automated procedures. It was, in fact, for the purpose of objectivity that we specifically selected strong, identifiable, and reproducible behaviors, measured them quantitatively under blind conditions, and repeated the measurements thousands of times. Automation would certainly be helpful, and we have begun to develop some appropriate techniques. In the meantime, we believe that much of the fundamental biology has already been uncovered objectively and effectively by means of simple and inexpensive techniques.

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Of Stress, Vitamin A, and Tumors

In two recent *Science* reports (*1, 2*) there are serious omissions of references to prior literature directly related to the work reported.

Riley (*1*) demonstrated an increased tumor incidence in mice infected with the mammary tumor virus as a result of chronic exposure to environmental stresses. He hypothesizes that the host response to stressful stimuli results in adrenal cortical hypersecretion of corticosterone which has marked thymolytic and lympholytic actions in mice and results in a depression of cell-mediated immune reactions. The immunodepression is then held responsible for increased tumor development in stressed mice exposed to an oncogenic virus.

My colleagues and I have tested this hypothesis directly and have demonstrated that in mice inoculated with a murine sarcoma virus (MuSV-M), physical stress increases the incidence and severity of tumor development (*3*). We discussed the importance of prior history and environmental conditions on tumor incidence in mice exposed to oncogenic viruses (*3, 4*). We demonstrated the influence of stress on thymic size and cellularity and hypothesized that stress reduced immunocompetence through increased adrenal corticosterone secretion.

Furthermore, we showed that metyrapone, a chemical that inhibits corticosterone production, prevents the typical stress reaction of adrenal hypertrophy and thymic involution and increases the resistance of stressed and nonstressed mice to MuSV-M (*5*). Also, steroids such as deoxycorticosterone, which can compete with corticosterone for some tissue receptor sites, prevent the adverse effect of stress in mice inoculated with MuSV-M (*6*). Finally, vitamin A, which blocks some host responses to stress or cortisone administration, is remarkably protective against both thymic involution due to these agents and tumor development following inoculation with MuSV-M (*4, 7*). Thus, some of the main

elements of the hypothesis expressed by Riley had been amply demonstrated experimentally.

Felix *et al.* (*2*) state: "To date, however, multiple attempts to show that vitamin A inhibits the growth of transplanted tumor cells have been unsuccessful." They then demonstrate that vitamin A has such an action on a transplanted murine melanoma. We too (*8*) have demonstrated that vitamin A, employed without any other chemotherapeutic agent, inhibits growth of C3HBA transplanted tumor cells.

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Rettura *et al.* (*1*) reported that vitamin A–treated mice had a temporary decrease in the growth rate of injected adenocarcinoma cells, with prolonged survival but ultimate death from progressing tumor. Our findings differed in that tumor incidence was decreased in our system and treated animals usually remained tumor-free, whereas untreated mice developed and died of melanoma. We did not reference Rettura's article, which appeared after our report was submitted and accepted for publication. We acknowledge its relation to our work and thank Seifter for his comment.

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Since there are more than 200 published articles dealing with stress and its influence upon various pathological processes, including cancer, our citations were regrettably incomplete. The papers by Seifter and his colleagues are of relevance, not only in supporting experimentally the hypothetical aspects of my report (*1*), but the implications of their studies for therapeutic approaches to the adverse effects of stress are important and deserve attention.

These and related studies (*2*) are of special significance since they emphasize the critical aspects of stressful environmental conditions on immunological competence, and thus on the course of various incipient pathologies including cancer. The relationships between observed modulations of pathological processes and the effect of stress on the thymus and on circulating T and B cells deserve serious concern by investigators.

When mice are maintained under conventional housing conditions and handled in the usual manner, they are under constant or intermittent stress (*1*, *2*). The consequences of this stress are plasma corticosterone values two to ten times higher than normal, accompanied by thymus involution and lymphocytopenia. Such animals are abnormal and thus probably unsatisfactory for experiments that require a known immunological status. As a result of these findings, and of the reports of Seifter and his colleagues, some of the earlier work may have to be reexamined with animals maintained under quiescent environmental conditions that are free of stress and its adverse physiological consequences.

The potential use of vitamin A or other suitable substances that might effectively block the dangerous aspects of stress, without causing alternate abnormal or pathological conditions, is of obvious importance.

In addition to the classical stress studies of Selye, the early work of Santisteban, Dougherty, and White in establishing the relationship between stress, adrenal corticoids, and the thymus must be cited (*3*). They clearly showed that elevation of plasma corticoids produced involution of the thymus, and that this could be used as an index to quantitate the amount of circulating adrenal corticoids. The significance of those early observations is only now being fully appreciated, largely as a consequence of understanding the vital role of the thymus and T cells in the immunological process.

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