ment 1 but not experiment 2. Repeat studies with the cells of experiment 2 treated with higher concentrations of methotrexate and azacytidine showed that the cells were still resistant to killing. In experiments not shown here, prior treatment of line-1 cells with actinomycin D, puromycin, or mitomycin C showed increased susceptibility to tumor-specific antibody plus guinea pig complement. The data also show that antibody alone is not sufficient to kill drug-treated line-1 and line-10 cells. In experiments not reported here, we found no killing of drug-treated line-1 cells with antibody to line 10 plus guinea pig complement and of drug-treated line-10 cells with antibody to line 1 plus guinea pig complement (specificity controls).

Some experiments indicate enhanced sensitivity of cells to killing mediated by antibody plus complement after preliminary drug treatment is dependent on drug dose and is reversed within several hours by culturing in medium free of the drugs. Line-10 cells held at 4°C in the presence of the drugs show little or no increase in sensitivity to killing by antibody plus guinea pig complement. Preliminary data also indicate that the effect is not due to an increase in the number of Forssman or tumor-specific antigen sites or to increased fixation of guinea pig C4 and C3 onto the cell surface. Other investigators have shown that prior treatment of tumor cells with various drugs may increase or decrease their absorbing capacity for transplantation or antiviral antibody (*12*).

Others have shown that the presence of chlorambucil during exposure of polyoma-transformed hamster cells to antibody alone decreased the colony-forming efficiency of these cells (*13*). The relation of this observation to ours is not clear since it is difficult to explain mechanisms whereby antibodies become cytotoxic without complement.

The biochemical mechanisms of action of the drugs in our experiments have been studied extensively, but little is known of how tumor cell destruction is effected in vivo. These inhibitors may affect the synthesis of surface areas susceptible to the attack of terminal components of complement or reduce the effectiveness of cell surface repair mechanisms. Regardless of mechanisms, the beneficial effect of various drugs and metabolic inhibitors in the treatment of cancer might be due, in part, to their ability to increase the susceptibility of tumor cells to killing by antibody plus complement.

Some success has been reported in treating mouse leukemias with antibodies to which chlorambucil was coupled (*14*). O'Neill and Davies also reported that injection of melphalan into mice with EL4 leukemia before administration of antibody to EL4 resulted in 100 percent of the mice being free of disease at 150 days (*15*). Our results may furnish a rational explanation for their observations.

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Prolactin Receptors in Rat Liver: Possible Induction by Prolactin

Abstract. *A prolactin receptor, present in adult female rat liver, can be induced in males by estrogen. Hypophysectomy diminished receptor levels in the female and rendered males unresponsive to estrogen. A renal pituitary implant blunted the decrease in hypophysectomized females and induced the receptor in hypophysectomized males. The increased receptor level in hypophysectomized males with a renal pituitary implant was preceded by a sustained elevation of circulating prolactin. Our observations suggest that prolactin induces its own receptor.*

Adult female rat liver membranes possess a receptor specific for lactogenic hormones (that is, prolactin, placental lactogens, and primate growth hormones). Receptor levels are much lower in male than in female liver membranes. Estrogen administration to male rats induced the receptor. A role for the pituitary was suggested by the observations that hypophysectomy greatly decreased the hepatic receptor in female rats, and rendered males unresponsive to estrogen (*1*). In this study we have examined the role of the pituitary. The data suggest that prolactin can induce its own receptor in rat liver.

Adult male and female rats weighing 180 to 220 g were obtained from Fauna Breeding Company, Quebec. Animals were hypophysectomized by the parapharyngeal technique. Pituitaries from animals of comparable size were implanted beneath the renal capsule according to the schedules noted in the figure legends.

At the time of killing, animals were ether-anesthetized and decapitated. Blood, obtained from the cervical wound, was allowed to clot at 4°C. Serum was collected by centrifugation at 4°C and stored at −20°C. The methods employed for tissue fractionation, membrane preparation, the iodination of human growth hormone

(hGH) and insulin, and the measurement of the binding of ^{125}I-labeled hormones were as previously noted (*1*). Specific binding refers to the difference between radioactivity bound to the membranes in the presence of excess unlabeled hormone and that bound in its absence. It has been expressed as a percent of the total radioactivity added to the incubation. In previous studies we have shown that the specific binding of [^{125}I]hGH measures the level of lactogenic receptor in rat liver membranes (*1*). Rat prolactin was measured by a double antibody radioimmunoassay with materials supplied by the National Institute of Arthritis, Metabolism, and Digestive Diseases. The reference standard was RP-1 (11 international units per milligram).

Figure 1 demonstrates that by 3 days after hypophysectomy there was a marked reduction in [^{125}I]hGH specific binding by female rat liver membranes. In contrast, the specific binding of [^{125}I]-insulin underwent an increase significant at $P < .001$ (*2*). In those animals with a renal pituitary implant the decrease in specific binding of [^{125}I]hGH after hypophysectomy was small but significant at $P < .001$. This level of specific binding was much greater than the level in the animals without an implant ($P < .001$).

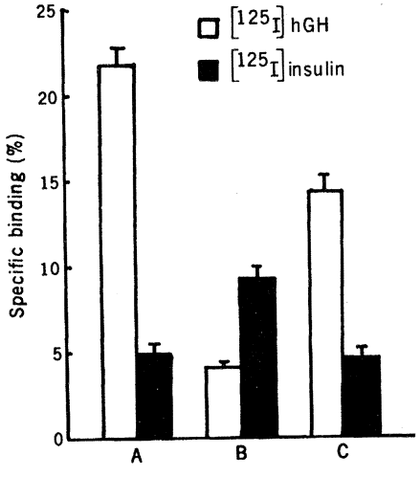

Fig. 1. Effect of hypophysectomy in the presence and absence of an implanted renal pituitary on the specific binding of [^{125}I]hGH and [^{125}I]insulin by female rat liver membranes. Females were hypophysectomized 2 days after sham operation or implantation of a renal subcapsular pituitary, obtained from a comparable female rat. All the rats, along with a group of control females, were killed 3 days after hypophysectomy. The line above each bar represents the standard error of the mean. (A) Controls, $N = 6$. (B) Hypophysectomized, $N = 7$. (C) Hypophysectomized and renal pituitary implant, $N = 5$.

Figure 2 demonstrates the influence of a renal pituitary implant in the hypophysectomized male rat. As previously established, a single dose of estradiol valerate markedly increased [^{125}I]-hGH specific binding (*3*). The low level of such binding in hypophysectomized rats (2.3 ± 0.5 percent) was completely uninfluenced by estrogen. However, those animals bearing a renal pituitary implant showed significantly greater [^{125}I]hGH specific binding (11.3 ± 1.8 percent) than control males ($P < .01$). Estrogen administration to the hypophysectomized rat with a renal pituitary implant increased [^{125}I]hGH specific binding to 28.4 ± 2.7 percent. The presence of the renal pituitary implant had no influence on the substantially reduced body weights consequent to hypophysectomy.

Figure 3 shows that the introduction of a renal pituitary implant into hypophysectomized male rats resulted in a marked increase in rat prolactin levels from < 2 ng/ml to 68.3 ± 17.4 ng/ml (mean $\pm$ standard error of the mean) at 3 days and 89.7 ± 17.7 ng/ml at 6 days after implantation (*4*). The change in specific binding of [^{125}I]hGH is characterized by a lag before increasing to maximal levels of 16.0 ± 2.2 percent by 12 days after implantation.

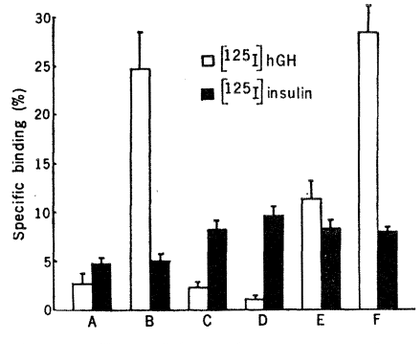

Fig. 2. Effect of hypophysectomy and estrogen administration in the presence or absence of an implanted renal pituitary on the specific binding of [^{125}I]hGH and [^{125}I]-insulin by male rat liver membranes. Pituitaries from animals of comparable size were implanted under the renal capsule. Two days later hypophysectomy was performed. Estradiol valerate (1 mg) was injected 3 days after hypophysectomy. All six groups of animals were killed 10 days after hypophysectomy. Membranes from all six groups were prepared and assayed for binding at the same time. The line above each bar represents the standard error of the mean. The groups, number of animals in each group, and the body weights, expressed as mean $\pm$ S.E.M., were as follows: (A) controls ($N = 4$, weight 294 ± 6 g); (B) estradiol valerate–treated ($N = 5$; weight 249 ± 5 g); (C) hypophysectomized ($N = 3$, weight 178 ± 8 g); (D) hypophysectomized plus estradiol valerate ($N = 3$, weight 180 ± 14 g); (E) hypophysectomized plus renal pituitary implant ($N = 5$, weight 159 ± 6 g); (F) hypophysectomized plus renal pituitary implant plus estradiol valerate ($N = 8$, weight 179 ± 11 g).

The extrasellar pituitary functions poorly, as evidenced by its incapacity to maintain body growth and prevent the atrophy of the thyroid, adrenals, and ovarian follicular and interstitial tissue (*5*). It does, however, produce an increase in serum prolactin levels, as confirmed in our study, owing to removal from the tonic inhibitory influence of the hypothalamus (*6*). The influence of the extrasellar pituitary on the level of hepatic lactogen receptors is striking. The renal pituitary implant prevents to a considerable, although not complete, degree the decline in hepatic lactogen receptor level consequent to hypophysectomy. The implant results in a striking increase in hepatic lactogen recep-

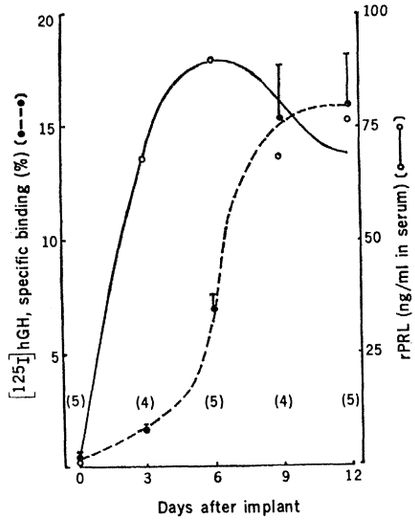

Fig. 3. Time course of change in serum prolactin and specific binding of [^{125}I]hGH by liver membranes in hypophysectomized male rats after renal pituitary implantation. All the animals were hypophysectomized and received estradiol valerate (1.5 mg per animal) 20 days prior to the time of renal pituitary implantation. The abscissa denotes the time after implantation at which the animals were killed. Membranes were prepared from all the tissues and assayed for specific binding at the same time. The numbers in parentheses denote the number of animals killed at each time. The rat prolactin values are the means of the individual animals. The specific binding is denoted as mean $\pm$ S.E.M.

tor levels in hypophysectomized male rats. This increase in receptor levels is preceded by a marked and sustained elevation of circulating rat prolactin levels. These observations taken together strongly suggest that the lactogen receptor is being induced by prolactin itself. They do not exclude a direct hepatic role for estrogen, although they do suggest that at least part of estrogen's inductive effect is through its capacity to stimulate an increase in circulating prolactin in the rat.

Several studies have emphasized that tissue receptor levels undergo modulation (*1, 3, 7*). This modulation may be an important way of regulating peripheral sensitivity to hormones. The repression of receptors by chronic elevation of hormone levels has been established for insulin and suggested for other hormones such as calcitonin (*8*). Our observations suggest that chronic elevation of hormone levels can induce receptors. Whether induction or repression occurs seems to depend, in part, upon the hormone and the tissue involved.

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Evidence for Origin of Insect Sex Pheromones: Presence in Food Plants

Abstract. *Compounds identified as sex attractant pheromones in a number of phytophagous insects were found in a variety of host plants. These agents vary in chemical composition in different plant species, which suggests that dietary factors may provide an evolutionary mechanism for diversification of certain insect species. A theoretical framework to explain this phenomenon is postulated on the basis of experiments with the oak leaf roller moth.*

Little is known about the origin or metabolic production of insect sex attractants. Other insect chemical signals such as aggregation pheromones are derived from plants; some insect defensive chemicals are also of extrinsic origin. For example, chemical communication in some species of bark beetles (*1*) is enhanced by synergistic aggregation substances found in host pine trees. Some insects may carefully sequester plant-produced chemicals and use them for their own defense (*2*). However, to our knowledge, the question of the origin of insect sex attractants remains unanswered. Moreover, the mechanisms by which these chemical signals contribute to species diversification (*3, 4*) are unclear. In this report, these questions are addressed in light of results in the study of insect pheromones in our laboratory, and a possible theoretical framework to explain these phenomena is proposed.

We have isolated and identified insect sex pheromones in a wide variety of host plants. The primary chemical communication system under investigation is the sex pheromone complex of the oak leaf roller moth (OLR), *Archips semiferanus* Walker (Lepidoptera:Tortricidae), an exceedingly destructive pest which has seriously damaged oak forests in the northeastern United States (*5*). A series of 21 isomeric tetradecenyl acetates (*6, 7*) were identified in the active sexual attractant fraction of the adult female; all isomers were active when tested on male OLR antennae by the electroantennogram (EAG) method (*8*). Seventeen of the 21 isomers proved attractive to OLR males when tested in field traps (*8, 9*).

During field testing, wild OLR male moths congregated near host oak trees and became sexually stimulated when near the leaves. Moreover, males frequently attempted to copulate with host leaves that had been damaged by larval feeding. The behavior was identical to the characteristic male sexual response to crude female extracts (*10*) and included extension and bending of the abdomen, rapid wing fluttering, projection of the hair pencils, and repeated brushing of these structures on the undersides of the leaves. This phenomenon suggested that the OLR pheromones could be present in the plant (*11*). In order to test this hypothesis, crude foliage extracts of several tree species were made by grinding fresh leaves, which were collected in areas having no visible insect defoliation (*12*), in redistilled spectrograde methylene chloride. The extracts were subjected to thin layer chromatography on silica gel with methylene chloride : hexane (50 : 50) as the developing solvent. A band having the same R_F value as standard 14-carbon unsaturated acetates and tetradecyl acetate was then eluted and rechromatographed on a nonpolar 5 percent SE-30 gas chromatographic (GC) column (*13*). A region having a retention time near that of standard tetradecenyl acetates was collected and subjected to computerized gas chromatography–mass spectrometry (GC-MS) (*14*) on 10 percent diethylene glycol succinate (DEGS). The GC-MS was programmed to scan only for discrete ions that were present in standard 14-carbon acetate standards. Our use of this technique, known as mass fragmentography or multiple ion detection, has been described (*6*). Analyses were made with the following diagnostic ions: *m/e* (mass to charge) 196, molecular ion of tetradecyl acetate (M_A) – $HOOCCH_3$; 194, molecular ion of tetradecenyl acetates (M_B) – $HOOCCH_3$; 166, M_B – $HOOCCH_3$ – C_2H_4; and 61, $H_2OOCCH_3^+$. Mass fragmentography of each extract was repeated with the same ions on a 5 percent SE-30 column to ensure against overlapping impurities. Optimum operating sensitivity of the GC-MS was in the range of 50 to 150 pg per component. If peaks having the same retention times (scan numbers) and the same quantitative ratios of these ions as standard tetradecyl acetate and the tetradecenyl acetates were observed,