The findings with respect to coding appear similarly conclusive: intersensory coding yields significantly shorter RT's than intrasensory coding ($P < .01$). The objection may be raised that since confusions occurred in the intrasensory situation, the differences obtained may be attributable to discrimination difficulty. To resolve this question, an extension of the study was carried out in which two-choice, rather than three-choice, RT's were measured under both coding schemes. Intersensory coding was restricted to the middle intensity on all sense modalities. Intrasensory coding involved only the highest and lowest intensities, thereby reducing the difficulty of the intensity discrimination to a minimum. The results indicated overall shorter RT values under the two-choice than under the three-choice conditions, thus supporting the relationship of RT to number of alternatives suggested by Hick (*4*). However, the differences attributable to coding were still present at a high level of statistical significance ($P < .01$).

The conclusions to be drawn from this study are as follows: (i) the classical relationships between RT and stimulus strength, number, and sense modality are supported for stimuli equated in loudness across the senses and presented under conditions of inter- as well as intrasensory coding; (ii) all stimuli yield shorter RT's when involved in choices among sense modalities than when involved in choices among levels of the same modality.

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Evaluating Reflux and Drainage in Foam Fractionations

Abstract. Extreme care must be exercised in assigning an observed increase in enrichment to the beneficial effects of reflux or drainage, particularly in a single-solute system. The use of a system containing two or more solutes is recommended.

A recent publication (*1*) has emphasized quite correctly the beneficial effect to be expected from reflux in enhancing enrichment ratios obtained in foam fractionations. Unfortunately, the contribution of drainage is difficult to isolate from that of reflux (*1, 2*). For example, an early study yielded an enrichment of about 9-fold for methyl orange under conditions in which 3 ml of condensed foam were collected in 10 minutes (*3*). On the other hand, collection of 1 ml during a 30- to 40-minute interval gave an enrichment just under 90 (*4*). [The slow collection rate was made possible by substituting for the 24-cm Vigreaux column (*3*) an inverted 250-ml conical separatory funnel. The small amount of foam that survived to pass slowly into the collection vessel was not colored, and it had a gossamer appearance.] Under the circumstances, one might be inclined to attribute much of that increase to improved drainage. However, the contribution of reflux could not safely be assumed to have remained constant.

An examination of the results of a fractionation of two substances provides a means for better evaluating the contributions of reflux and drainage. Thus, if reflux were the overriding factor, one might expect not only the individual enrichment ratios to increase but also the quotient of the enrichment ratios. The chief factor operating to oppose such an increase in the quotient would be the usual tendency of a solute to be enriched to a greater extent when the foam is in contact with a more dilute solution.

In the earlier study (*3*), enrichment ratios of 5.4 and 4.1 were obtained for the 2- and 1-naphthoates, respectively, when each was present alone, giving a quotient of 1.3; for an initially equimolar mixture, the quotient was about 2. When this work was repeated with the separatory funnel to improve drainage, enrichment ratios of 70 and 40 were obtained for the pure compounds; for a mixture having the same initial concentrations of each substance, the enrichment ratios were 43 and 21, and the quotient was 2.0. These results emphasize that one must be extremely cautious in drawing conclusions about the contribution of a single factor, whether it be reflux or drainage, from the change in enrichment of a one-solute system. In addition, it illustrates how competition for the surface by two solutes in a mixture can lower the enrichment ratios of each. At the same time, it shows that competition for the surface, aided by limited reflux, did not increase the enrichment quotient for the mixture over that calculated from the single-solute systems. In this case, better drainage must have been the reason for the changes in enrichment observed for the single-solute systems.

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Irradiation of Bacterial Luciferase in vivo

Abstract. The fading of in vivo luminescence which is characteristically displayed by resting cell suspensions of the luminous bacteria *Photobacterium fischeri* is partly accounted for by a gradual loss of active luciferase. This luminescence is further diminished by exposure of such suspensions to gamma radiation, but the irradiated cells yield more, rather than less, active luciferase than do cells of the non-irradiated suspension. These and similar results with menadione-treated cells indicate that, while luciferase is gradually inactivated by its catalysis of light production, it can resist inactivation by radiation better than can some component of its supporting metabolism.

In 1954 one of us (*1*) demonstrated that the luminescence of resting cell suspensions of the luminous bacteria *Photobacterium fischeri* could be significantly depressed by doses of radiation as low as 500 rad. At about the same time, the enzymatic nature of bacterial luminescence was being proved by Strehler and co-workers (*2*) and

by McElroy and co-workers (*3*), who identified the reaction components required for cell-free luminescence. As a first step in locating the radiation-sensitive component of bacterial luminescence, we sought to determine whether the radiation-induced depression resulted from an inactivation in vivo of the enzyme system itself or primarily from an effect on its supporting metabolism. The work of McElroy and Strehler cited above enabled us to develop a method of estimating cellular luciferase activity (*4*) which we subsequently applied to irradiated luminous bacterial cells.

As in the earlier work (*1*), *P. fisheri* cells were irradiated and incubated in buffered saline (nonproliferating) suspension. A report on the methods of preparing these suspensions, of measuring their luminous intensity, and of assaying them for active luciferase is in preparation. Our assay reliably distinguishes between cell extracts differing by ± 10 percent in luciferase activity. In a typical experiment, a single suspension held at 12° to 14°C was prepared for exposure to cobalt-60 gamma radiation. Aliquots were removed after successive exposure intervals, including zero-time for controls, and transferred for incubation to a 25°C shaking water bath. After about 4 hours of incubation, glucose was added to a final concentration of 1 percent to half the suspensions given each radiation treatment. Periodically during incubation the cells in each suspension were assayed for luciferase activity.

The results are shown in Fig. 1. Upon incubation, in which the suspensions are raised in temperature from 14° to 25°C, luminescence quickly appears. The nonirradiated suspensions rapidly attain maximum spontaneous (endogenous) luminous intensity which, except for the variable occurrence of secondary maxima, characteristically fades with time. It is assumed that this luminous intensity is directly related to the rate at which the luciferase reaction is proceeding within the cells of the suspension. Despite the fading of endogenous luminous intensity, additional (exogenous) luminescence can be induced by the addition of glucose, but once a new maximum is reached, this exogenous luminous intensity also fades with time. Exposure of the cells to the radiation dose shown considerably reduces the initial peak luminous intensity as well as that prevailing during the remainder of the incubation period. Exogenous luminescence is suppressed more than is endogenous; at the two highest doses it is virtually extinguished.

The results of the luciferase assay applied to these suspensions are also shown in Fig. 1. The fading of luminescence in the nonirradiated suspensions may be partially accounted for by the loss of active luciferase in the cells. As shown by other experiments (*4*), the induction of exogenous luminescence increases the rate of inactivation of luciferase over that prevailing during exogenous luminescence alone. Unexpectedly, however, the loss of luciferase from irradiated cells was less, rather than more, than that from the nonirradiated cells. At the end of the incubation period it appears that the more the luminous intensity is depressed, the greater is the amount of enzyme which can be recovered from the cells. In other experiments involving cell suspensions differing in cell density and in the stage of the growth of the cells harvested for preparation, the irradiated cells always yielded more active luciferase.

The results are similar to those obtained from cells treated with menadione in place of radiation. When buffered-saline cell suspensions are treated with $10^{-4}M$ menadione (added as the bisulfite for solubilization), endogenous luminescence is virtually extinguished in 40 minutes. Two hours later, however, when the nontreated suspensions display about a third of their original luminous intensity, they yield an average of 1.5 units of active luciferase per 10^9 cells, whereas the menadione-treated cells yield 2.1 units per 10^9 cells, or nearly 50 percent more luciferase.

Menadione has been shown by Cormier and Totter (*5*) to inhibit the luciferase reaction in vitro because of the presence in cell-free extracts of a menadione reductase which competes with luciferase for their common substrate, reduced flavin-mononucleotide. The results of the above experiment show that this inhibition can be produced in vivo. Since the loss of luciferase activity can be apparently minimized simply by lack of substrate, it would appear that the catalysis of light production may be involved in the inactivation of the enzyme.

We suggest that an analogous mechanism explains the results found in irradiated cells. In this case, radiation dosages up to 24.6 kilorad damage a substrate or the supporting metabolism for the luciferase reaction but not the enzyme itself. Recent evidence in our laboratories indicates that irradia-

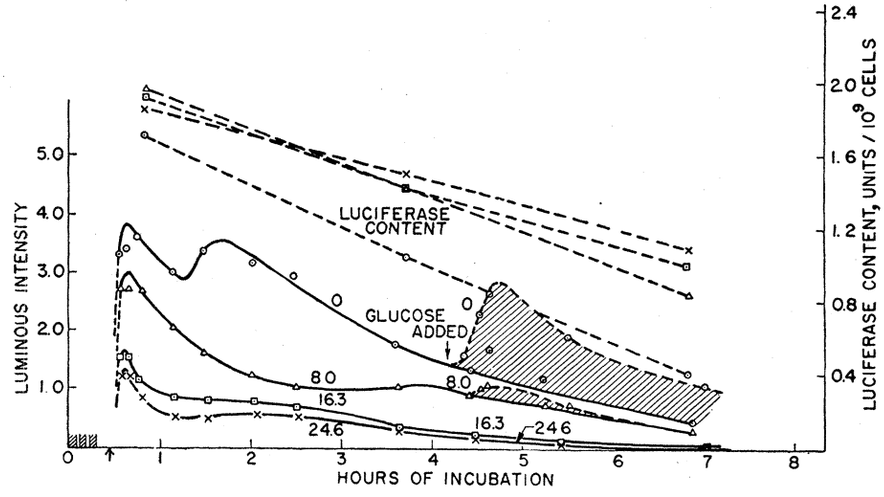

Fig. 1. Effect of 8.0, 16.3, and 24.6 kilorad of cobalt-60 gamma radiation on the luminescence and enzyme content of *Photobacterium fischeri* cells suspended in buffered saline. Luminescence is expressed as luminous intensity, measured as the current generated in a photometer, and is shown in the figure by the solid lines. The shaded portion of the curve represents the additional luminescence produced upon addition of glucose at the time indicated. The shaded bars in the lower left corner represent the successive radiation exposure times. The arrow at 0.5 hour represents the start of incubation. The dotted lines represent the luciferase content of the cells in units of luciferase per 10^9 cells (see right-hand ordinate). The radiation doses associated with the dotted lines are identified by the symbols employed on the solid lines.

tion accelerates the loss of the aldehyde component required for the luciferase reaction. The enzyme thus escapes inactivation from radiation as well as from its catalysis of light production. Since the latter is an oxidative reaction, it is indeed curious that the enzyme can withstand oxidative destruction from radiation but not from the oxidative chemical steps it catalyzes (*6*).

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Changes in Titer of Ecdysone in Bombyx mori during Metamorphosis

Abstract. The amount of ecdysone extracted from *Bombyx mori* rose precipitously immediately before, and fell immediately after, transition from the larval to the pupal stage, and thereafter a secondary rise in titer was observed. These observations correlate very well with physiologic sequences of metamorphosis, critical period, and oxygen uptake.

Ablation and transplantation experiments have demonstrated clearly that metamorphosis in insects is under hormonal control. Both Fukuda (*1*) and Williams (*2*) were able to demonstrate that the growth and differentiation hormone is secreted by the prothoracic gland and that this gland is activated by neurosecretion from the brain (*3*). Recently, Kobayashi and Burdette (*4*) have demonstrated synergism between ecdysone and brain hormone, a finding which indicates a direct action of the latter in addition to its tropic function. By means of a modification of the method of Butenandt and Karlson (*5*) to isolate ecdysone, the level of this hormone in the tissues has been followed before and after pupation in samples of *Bombyx mori* (*6*).

Samples (*7*) of full-grown larvae, silkworms in the prepupal stage, and silkworms at 1, 2, 3 to 4, 5 to 6, and 6 to 7 days of age (*8*) were procured in large enough quantities to assure a yield of active hormone. The *Calliphora* test was used for bioassaying the material, and different concentrations were tested until activity was detected or until the amount of crude extract was so great as to preclude additional injection because of the discrepancy in size between the *Calliphora* larvae and the volume to be injected.

Activity was obtained in the bioassays in five of the seven samples. The results are indicated in Table 1. No hormone was detected in full-grown larvae, and the greatest amount was found during the prepupal stage. The amount of hormone then declined until the pupae were 2 days of age, when the sample was not active. Activity was found again in subsequent samples.

When the activity is calculated on the basis of the number of *Calliphora* units per gram (wet weight) of tissue, it is found that approximately 6 *Calliphora* units per gram were present immediately before pupal moulting. When the titer is calculated in terms of the mean wet weight of *Bombyx* at this stage, it is found that 8 units were extracted from each silkworm of this strain. For an accurate expression of actual level of hormone at a given stage, the number of units should be multiplied by a constant, *K*, which takes into consideration the efficiency of the extraction procedure.

When one considers the titer of hormone in relation to the life cycle of the insect, it is apparent that metamorphosis occurs shortly after a large amount of hormone is secreted, and that afterward the level of hormone falls rapidly. A more gradual increase in the secretion of hormone then occurs, presumably preparatory to emergence. The critical period in *Bombyx* and the oxygen uptake also fall into a logical sequence with respect to the observed changes in titer of ecdysone (*9*).

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Table 1. Titer of ecdysone during metamorphosis of *Bombyx mori*.

Stage (25°C equivalent)	Wet weight (g)		Yield of crude hormone (g)	Bioassays (No.)	Maximum pupation (%)	C.U.*	
	Total sample	Mean				Per gram	Per silkworm
Full-grown larvae	4589.4	2.589	0.1760	8	25	I†	I†
Prepupae	7061.0	1.359	0.0655	3	56	5.8	7.9
1 day	6865.3	1.113	0.0628	3	67	1.9	2.1
2 days	7498.8	1.118	0.0628	6	35	I	I
3–4 days	7026.7	1.243	0.1230	4	80	3.7	4.6
5–6 days	7164.2	1.142	0.1339	6	59	1.6	1.8
6–7 days	7111.2	1.213	0.1064	4	80	3.1	3.7

**Calliphora* units. †Inactive.

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Prezygotic Selection in ABO Blood Groups

Abstract. A statistical method was devised to test whether prezygotic selection was operating in ABO blood groups, and it was demonstrated, with data from Japanese families, that heterozygous AO and BO fathers transmitted more than 50 percent O-bearing sperm (approximately 55 percent) to their children. Neither sperm incompatibility nor reproductive compensation could account for the results.

Selective mechanisms operating on ABO blood groups have so far been found to act only at postzygotic stages (*1*), while little attention has been paid to prezygotic selection, for which evidence is reported here.

Three different mechanisms of prezygotic selection are possible: (i) meiotic drive (*2*) or unequal production of gametes carrying different alleles in heterozygous parents; (ii) sperm competition, occurring independently of female genotype; (iii) sperm competition as a result of serological incompatibility between sperm carrying the A or B gene and the anti-A or anti-B antibody in the uterine secretion (*3*). The first two possible mechanisms occur in animals and plants (*4*).