possess the sexual agglutination reaction. This mating mechanism is fairly common among yeasts, especially among species evolved in terms of recent geological time.

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Hormonal Control of Onset of Corneal Reflex in the Frog

In a previous study (1) it was reported that the corneal (wink) reflex is unelicitable in the tadpole until just before the period of metamorphic climax. In *Rana pipens* the onset of the reflex normally precedes forelimb emergence from the branchial chamber by an average of 4 days (range, 0 to 10 days). In *R. catesbeiana* this is also true, except for rare instances of earlier onset. It was further established (*1, 2*) that the onset of the reflex was strictly related to metamorphosis; it never appeared in the nonmetamorphosing hypophysectomized tadpole, and the time of onset could be moved forward by treatment of the whole normal tadpole with thyroxine, but the extent of acceleration was less than for other metamorphic changes. However, local stimulation of the reflex center in the medulla oblongata of large midlarval tadpoles with pellets containing thyroxine produced a premature maturation of the center, as well as an unusually early onset of the reflex; maturation occurred as much as 18 days before forelimb emergence. These results demonstrated the dependence of the reflex center upon thyroid hormone for its final maturation.

It has been shown that the later stages of induced metamorphosis in thyroidless or hypophysectomized tadpoles are passed through progressively more slowly than the early stages, at a constant thyroxine dosage level (*3*), and that successive events or stages of metamorphosis tend to have a higher thyroxine concentration requirement or threshold (*4*). Hence it may be assumed that the later metamorphic event, forelimb emergence, displays a higher threshold than does the onset of the corneal reflex, the earlier metamorphic event.

To test this assumption, concentrations of thyroxine were sought which would in fact permit the establishment of the corneal reflex without concomitantly stimulating the rupture of the skin windows through which the forelimbs emerge. In tests of over 150 *R. pipiens* and a few *R. catesbeiana,* over a large range of thyroxine concentrations in the surrounding water (from 0.002 to 2 μg/lit., water and food being changed daily, with thyroxine added immediately thereafter), the validity of the assumption has been demonstrated in two instances (see data in Table 1 for 108- and 177-day animals). In six other instances a related and very significant lengthening of the interval between the onset of the reflex and forelimb emergence has been recorded. In general, it has been found that, at 25°C, concentrations of *dl*-thyroxine of 0.6 μg/lit. invariably bring about forelimb emergence, if permitted to act for a long enough time. Concentrations of 0.4 μg/lit. rarely produce forelimb emergence, although incipient thinning of the skin-window area is usually obtained. A concentration of 0.2 μg/lit. is insufficient to initiate the corneal reflex or forelimb emergence. At 15°C, even 1.0 μg/lit. is ineffective in producing rupture of the skin window.

The study discussed in this report provides further evidence in support of the belief that most metamorphic changes in the frog tadpole are separable events, capable of being brought about individually by local hormone treatment (*2, 5*), or capable of being separated from succeeding metamorphic events by careful manipulation of hormone concentration and temperature (*6*).

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Table 1. Record of treatment of hypophysectomized tadpoles, demonstrating separability of the onset of the corneal reflex from the emergence of the forelimbs. Thyroxine was added to the water in which the animals were raised.

Species (*Rana*)	Form of thyroxine	Thyroxine concn. (μg/lit.)	Temperature (°C)	Treatment time in days: Before reflex onset	Treatment time in days: Between reflex onset and forelimb emergence
R. pipiens	*dl*	1.0	25	73	41
R. pipiens	*dl*	1.0	15	161	37*
R. pipiens	*dl*	1.0	25	71	45
R. pipiens	*dl*	1.0	25	47	44*
R. pipiens	*l*	0.2	25	71	47
R. pipiens	*l*	0.4	15	170	108*
R. pipiens	*l*	0.4	25	87	43
R. catesbeiana	*dl*	0.4	15	70	177†

* Tadpole died prior to emergence of forelimbs.
† The metamorphic progress came to a complete standstill at 15°C; skin windows thinned only slightly. The tadpole was transferred to a 25°C bath after 164 days; forelimb emergence occurred 13 days later.

Factors Affecting the Relative Deposition of Strontium and Calcium in the Rat

Abstract. Varying the calcium, phosphorus, carbonate, and lactate content of the diet was shown to affect the deposition in bone of Sr^{89} to a degree quite different from concurrent effects on Ca^{45} deposition. The influence of these findings on the evaluation of the Sr^{90} fallout hazard is discussed.

In a recent report in *Science* we presented evidence against the commonly accepted concept that the deposition and retention of Sr^{90} in bone is simply related to the concomitant deposition and retention of calcium (*1*). This concept has been widely employed in the evaluation of the hazard to human beings of Sr^{90} from fallout. We now wish to report further experiments, of an admittedly preliminary nature, which support our earlier position and which suggest an explanation for the varied results which have been reported by investigators in this field (see *2*).

Six groups of four rats each were maintained for 8 days on diets which varied in one or more of the following constituents: calcium, phosphorus, car-

Table 1. Summary of experiment and results.

Group No.	Composition of diet (%)					Ingested isotopes per gram of bone ash (%)		
	Ca	P	Ca/P	Carbonate	Lactate	Sr^{89}	Ca^{45}	Sr^{89}/Ca^{45}
1	0.1	0.12	0.8		0.38	1.8 ± 0.2	8.1 ± 1.0	0.22 ± 0.02
2	0.1	0.12	0.8	0.13		2.3 ± 0.3	9.1 ± 1.0	0.25 ± 0.02
3	0.1	0.12	0.8	3.1		1.5 ± 0.3	4.4 ± 0.9	0.34 ± 0.02
4	2.0	0.12	17		8.8	1.0 ± 0.1	1.7 ± 0.2	0.60 ± 0.02
5	2.0	0.12	17	3.0		0.74 ± 0.16	1.6 ± 0.2	0.46 ± 0.07
6	2.0	2.4	0.8	3.0		0.34 ± 0.04	0.84 ± 0.19	0.41 ± 0.04
A†	0.1	0.5	0.2		0.38	0.87 ± 0.19‡	4.4 ± 0.7	0.20 ± 0.02‡
B†	2.0	0.5	4.0		8.8	0.43 ± 0.15‡	0.79 ± 0.28	0.55 ± 0.09‡

* All values are for an average of four animals, plus or minus one standard deviation.
† Results of previously reported experiment (see *1*).
‡ Strontium-90 rather than Sr^{89} was employed in these experiments (see *1*).

bonate, and lactate. The composition of the diets with respect to these variables is indicated in Table 1. For the 3 days prior to sacrifice, Sr^{89} and Ca^{45} were added to all of the diets. Other details of diet and procedure were as previously described (*1*). The rats employed were mature females of the Sprague-Dawley strain.

The concentrations of Sr^{89} and Ca^{45} in the femur, at sacrifice, expressed as percentages of total isotope fed, are recorded in Table 1. Also shown, as groups A and B, are comparable data from the previously reported experiment (*1*). Animals in groups A and B were sacrificed after 3 days on a Sr^{90}, Ca^{45} regimen; however, their period on the experimental diet prior to the addition of radioisotopes was 30 days, rather than the 5-day conditioning period employed for groups 1 through 6.

The previously reported effect of dietary calcium level on the ratio of Sr^{90}/Ca^{45} deposition in bone (see data for groups A and B) is confirmed by the results from groups 1 and 4. The animals fed a 2.0-percent calcium level diet show a Sr^{89}/Ca^{45} ratio nearly three times that of the animals fed the 0.1-percent calcium level diet. The lower phosphate content of the diets of groups 1 and 4 as compared with the diets of groups A and B had no apparent effect on the Sr^{89}/Ca^{45} ratio but did increase by a factor of two the absolute deposition of both Sr^{89} and Ca^{45}.

In groups A, B, 1, and 4, supplementary calcium was added as the lactate. Groups 2 and 5 correspond, respectively, to groups 1 and 4, except that supplementary calcium was added as the carbonate. The effect of dietary calcium level on the Sr^{89}/Ca^{45} ratio in bone is again evident in groups 2 and 5, although the difference in the ratios is somewhat less with calcium carbonate supplementation than with calcium lactate supplementation. The effect of carbonate (added as Na_2CO_3), independent of changes in calcium level, is seen in the comparison of group 3 with group 2. Added carbonate reduces deposition of both Sr^{89} and Ca^{45}, but the effect on Ca^{45} deposition is significantly greater than the effect on Sr^{89} deposition.

The effect of phosphate (added as Na_2HPO_4), independent of changes in level of calcium or carbonate, is seen in the comparison of groups 5 and 6. A 20-fold increase in phosphate reduced both Sr^{89} and Ca^{45} deposition by a factor of about two, leaving the ratio of Sr^{89} to Ca^{45} in bone essentially unchanged. At a constant phosphate level and a constant high carbonate level, the effect of variation in calcium level on the Sr^{89}/Ca^{45} ratio in bone is greatly reduced (compare groups 3 and 5), and with a constant Ca/P ratio, and high carbonate, the increase in the Sr^{89}/Ca^{45} ratio, with increased calcium level, is even smaller (compare groups 3 and 6).

Experiments reported by Wasserman *et al.* (*2*) were performed at a constant Ca/P ratio; this may explain in part the absence in their experiments of effects of dietary calcium level on the ratio of Sr to Ca deposited. It should also be noted that the rats in our experiments were mature, nongrowing animals, while those employed in the experiments of Wasserman *et al.* were rapidly growing animals which deposited much larger fractions of the administered radioisotopes.

Our present results, while they can hardly be said to clarify the situation, do serve to emphasize the complexity of the interrelationships involved. If, for purposes of hazard evaluation, this complexity makes expedient the temporary adoption of simplifying assumptions, such as the assumption that calcium will behave biologically as an isotopic diluent of Sr^{90}, the uncertainties introduced with such assumptions must be kept clearly in mind. Thus, in groups 2, 3, 5, and 6 of the present experiment, changes in the calcium, sodium, phosphate, and/or carbonate concentrations in the diet resulted in a nearly three-fold variation in the percentage ratio of Sr^{89}/Ca^{45} deposition in bone, and a nearly sevenfold variation in the absolute quantity of Sr^{89} deposited. Although the elevation of calcium levels in the diet did decrease Sr^{89} deposition, the effect of a 20-fold increase in calcium was only a twofold decrease in Sr^{89} deposition, and effects of similar magnitude were obtained by varying the phosphate and carbonate levels in the diet. While these are results of short-duration experiments, previous experiments involving feeding of Sr^{90} and Ca^{45} for periods of up to 24 days gave similar results with a more limited set of variables (*1*). For a reasonably adequate evaluation of this problem, additional variables must be studied over time periods embracing the life span of the experimental animals employed. Such experiments are being conducted in this laboratory (*3*).

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Phenylalanine Hydroxylation Cofactor in Phenylketonuria

Abstract. The enzymatic conversion of phenylalanine to tyrosine had previously been shown to require a nonprotein cofactor. It has now been demonstrated by direct assay that the cofactor is present in phenylketonuric liver samples. The lack of a functional phenylalanine hydroxylating system in phenylketonuria is not due to the absence of the cofactor.

Recent enzymological studies have strongly supported the suggestion, originally made by Jervis in 1947 (*1*), that in the disease phenylketonuria there is a block in the conversion of phenylalanine to tyrosine.

In 1953, results were reported of both in vitro (*2*) and in vivo (*3*) studies which showed that this reaction is completely missing or markedly decreased in phenylketonuria. After the system which catalyzes the hydroxylation of phenylalanine to form tyrosine was shown to require at least two protein fractions (*4, 5*), it was demonstrated by direct assay that only one of these enzymes was