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Phosphorylase b Kinase Inheritance in Mice

Abstract. *The gene for phosphorylase* b *kinase, a skeletal muscle enzyme, has been located on the X chromosome of mice. The inheritance of the enzyme through two generations from original matings between one inbred strain of mice, the I, which lacks the enzyme, and another strain, the* C_{57}, *follows the classical Mendelian pattern.*

The gene for phosphorylase *b* kinase, a skeletal muscle enzyme, has been located on the X chromosome of the mouse.

The inheritance of the enzyme was followed through two generations of offspring from matings between two inbred strains of mice, the I/FnLn and the C_{57}BL/FnLn. The I-strain mouse expresses no activity of the enzyme whatsoever in skeletal muscle (*1–3*), but is, otherwise, a normal, healthy laboratory animal with gross characteristics similar to those of the C_{57}-strain mouse.

The presence or the absence of phosphorylase *b* kinase in the skeletal muscle of the offspring from these matings was determined by two separate analyses. First, phosphorylase *b* kinase was assayed directly in a muscle homogenate by following the conversion of a highly purified preparation of phosphorylase *b* to phosphorylase *a* (*2, 4*). The substrate and the product may be conveniently differentiated by a cofactor requirement; the *b* form has an absolute requirement for 5′-adenosine monophosphate (5′-AMP) whereas the activity in the absence of the nucleotide is arbitrarily taken as the amount of phosphorylase *a* (*5*). Second, phosphorylase, which catalyzes the breakdown of glycogen in the muscle cell (*6*), was assayed with and without 5′-AMP (*1, 5*). The relative amount of phosphorylase *a* in a muscle homogenate is considered to be a direct reflection of the activity of this kinase, since phosphorylase *a* increases in the muscle following electrical stimulation or the administration of epinephrine (*7, 8*).

Furthermore, this specific kinase reacts with such facility that the *b* to *a* conversion will take place during the process of homogenization of the frozen sample of muscle unless the homogenizing vessel is cooled to −20° to −30°C (*8*). This precaution was observed when the effect of epinephrine was studied in mice of the F_1 generation. For confirmation of the presence or the absence of the kinase in mice of the F_2 generation, the conversion of phosphorylase *b* to the *a* form was allowed to proceed in a homogenizing vessel under controlled conditions. Samples of frozen muscle were homogenized in a ground glass unit (*9*) at 0° to 2°C for 18 to 24 seconds.

Owing to the lack of phosphorylase *b* kinase, the parental I-strain mouse exhibits no increase in phosphorylase *a* under any stimulus tried so far (*1–3*). One group of mice in the F_1 generation, the male offspring from I-strain females and C_{57}-strain males, resemble the I strain in this respect. Phosphorylase *b* kinase was absent (Table 1), and an intravenous dose of epinephrine failed to produce an increase in phosphorylase *a* in these males (Fig. 1). In contrast, F_1 males from matings of C_{57}-strain females and I-strain males responded to epinephrine with a rapid and sustained rise in phosphorylase *a* (Fig. 1). No data are given but all of the females in the F_1 generation responded to epinephrine as would be expected from the presence of the kinase (Table 1). Throughout these studies the absence of phosphorylase *b* kinase was always associated with an absence of phosphorylase *a;* the presence of the kinase was always associated with increases in phosphorylase *a*.

The two groups of male mice in the F_1 generation were identical in appearance, size, and vigor. Except for a slight dilution in the markings, all of the mice in the F_1 generation resembled the jet black C_{57}-strain parents. The fur of the I-strain mouse is predominately white; two large patches of sandy brown fur nearly cover the dorsal surface, and these two patches are separated by a band of white fur in the lumbar region.

This discrimination between the

Table 1. Distribution of phosphorylase *b* kinase in the F_1 and F_2 generations from matings of I- and C_{57}-strain mice.

Generation	Sex	No. of mice	Phos. *b* kinase*	Phos. *a* (%)†
		I-female × C_{57}*-male*		
F_1	♂	16	None	2.1±0.4‡
F_1	♀	10	476±72	24.7±2.6
F_2	♂	18	None	1.7±2.5
F_2	♂	26	883±47	38.2±3.3
F_2	♀	21	None	0.7±0.5
F_2	♀	19	500±43	26.0±1.8
		C_{57}*-female* × *I-male*		
F_1	♂	16	569±30	14.7±0.8
F_1	♀	17	394±41	23.4±2.1
F_2	♂	19	None	0.5±0.4
F_2	♂	21	784±49	37.7±3.4
F_2	♀	19	590±42	27.1±2.1

* Units (*4*) per milligram of protein (*12*). † (Units without 5′-AMP/units with 5′-AMP) × 100 (*5*). ‡ Standard error of the mean.

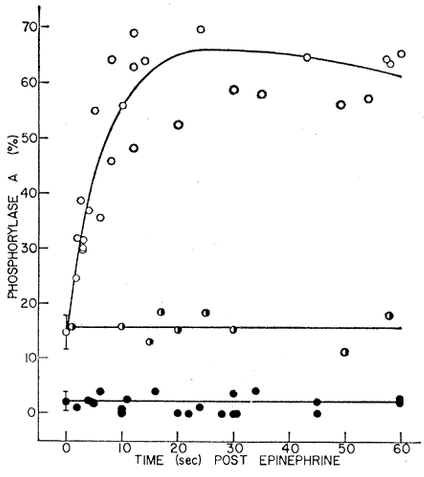

Fig. 1. The effect of epinephrine on phosphorylase in the gastrocnemius muscle of hybrid mice: F_1 males from C_{57}-females and I-males (open circles), and F_1 males from I-females and C_{57}-males (closed circles). One muscle was removed from the mouse under deep anesthesia (*2*), epinephrine (5 μl of a 5.46 μ*M* solution per gram of body weight) was injected into the tail vein, and the exposed contralateral muscle was removed within 1 minute. The muscles were frozen immediately in isopentane chilled in liquid nitrogen or between blocks of CO_2, and then stored in liquid nitrogen. Except at zero time each point represents one animal. The averages and the standard deviations (vertical bars) of the percent phosphorylase *a* (see legend of Table 1) in the muscles taken before epinephrine are plotted at zero time; each point represents 16 mice. The half-filled circles represent muscle taken from C_{57}-males after an injection of 0.85 percent NaCl (5 μl per gram of body weight) in the tail vein.

sexes in the inheritance of phosphorylase *b* kinase by mice in the F_1 generation suggests that the gene for this enzyme resides on the X chromosome. If the locus were on another chromosome, then all of the mice in the F_1 generation should have expressed the enzyme, since our observations indicate that the presence of the enzyme is dominant to its absence.

Accordingly, brother and sister matings within the two groups of the F_1 generation should yield offspring with two predictable patterns. The enzyme should be absent in 50 percent of the males and 50 percent of the females in the offspring, the F_2 generation, which were descended from original matings of I-strain females and C_{57}-strain males. In the F_2 generation from original matings of C_{57}-strain females and I-strain males all of the females should possess the enzyme and 50 percent of the males should lack the enzyme. The data in Table 1 confirm the predictions.

Therefore, the two groups of females in the F_1 generation may be considered to be heterozygous with respect to this kinase. In order to confirm this notion these females were mated with males from their particular paternal strain. The offspring from these matings, the first backcross (B_1), should also yield two predictable patterns. The studies are not yet complete but the initial determinations are as would be expected from the predicted results.

The concentration of glycogen in the resting muscle is 1.2 percent in I-strain mice and 0.5 percent in C_{57}-strain mice (*1*, *2*). No data are presented here (*10*), but in all offspring the absence of the kinase was associated with the same concentrations of muscle glycogen as found in the parental I strain, and, conversely, the presence of the kinase was associated with the same concentrations of glycogen as found in the parental C_{57} strain. This all-or-none phenomenon suggests a specific relationship, perhaps cause and effect, between phosphorylase *b* kinase and the stores of muscle glycogen in the resting muscle. Although this specific kinase is a key intermediate in a series of reactions which promote glycogen breakdown (*11*), this tempting conclusion must be considered as only tentative at this time.

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Voluntary Hypothermia in Reptiles

Abstract. *Studies on lizards in laboratory thermal gradients which are not shut down at night reveal complex thermoregulatory behavior. Maintenance of the high, characteristic levels of body temperature known for active lizards may be abandoned. Low levels, which incapacitate the lizards, are seleted. Evidence of complex responses of the "thermostat" at a reptilian level of organization suggests a reassessment of our theories concerning the evolutionary status of "torpidity" in birds and mammals.*

It is commonly assumed that diurnal reptiles become cold and inactive at night because the body cools as the environment cools. In this sense, a lizard's "torpidity" is obligatory and passive. The assumption would seem to follow the fact that few reptiles are able to achieve significant thermal homeostasis with metabolic heat, a python (*1*) being a notable exception. Rather, some reptiles are known to maintain body temperatures within narrow limits by behavioral exploitation of microclimatic thermal mosaics in their environments. It is the basking lizards of the arid areas of North America which have been most carefully (*2*) studied, and which form the context for generalizations (*3*, *4*).

The following experiments, however, suggest that low body temperatures are the result of a voluntary and actively initiated process in some lizards. The lizards may thus "prefer" and not simply tolerate low nocturnal temperatures. This would be significant to our conceptualization of the evolution of endothermy in birds and mammals (*5*).

Casual observations upon *Gerrhonotus* (*Anguidae*) and *Klauberina* (Xantusiidae) in substrate-heated thermal gradients prompted the experiments. Movement onto the warm surfaces was periodic and usually corresponded to the light periods in the observation room (photoperiod controlled). Inactive individuals would usually become responsive after feeding (*6*).

For experiments, animals which were judged to be active were placed, two each, in glass terraria (1 by 1 by 2 feet) (0.3 by 0.3 by 0.6 m) maintained in a constant-temperature room (set at 9° to 17°C in various experiments) with a controlled photoperiod (10 hours light, 14 hours dark). The ambient temperature established the cold end of the gradients, and one end of each terrarium was heated by a 250-watt, red-glassed heat lamp. This was suspended from above and provided each gradient with maximum temperatures which would be lethal to the species. Pieces of cardboard were scattered about so that the lizards could retreat

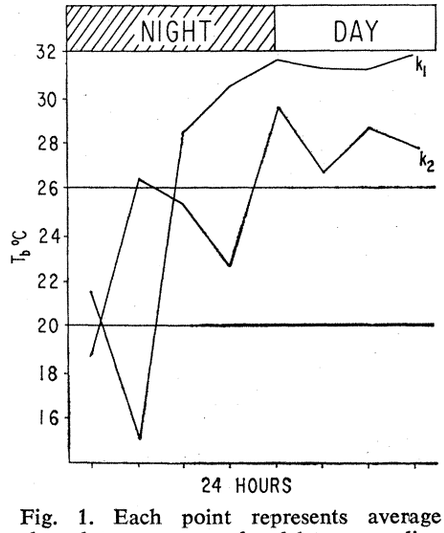

Fig. 1. Each point represents average cloacal temperatures for 3-hour sampling periods (two or three records each period) over 24 hours. K_1 is the larger of two male *Klauberina riversiana* lizards in the same terrarium. K_2 is a male in a terrarium with a female. The available temperatures in the gradient were constant through time, so that the nocturnal lows are achieved by voluntary movements of the lizards.