sucrose and trehalose depress the velocity of the enzyme-catalyzed reaction, their effects on the kinetic parameters of the enzyme should not cause any confusion in the interpretation of the induction experiments which test for a substrate-induced increase of the enzyme activity resulting from de novo protein biosynthesis.

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2 P. Desnuelle, Revue fr. Étud. clin. biol. *8*, 494 (1963).
3 J.M. Robert-Baudouy, R.C. Portalier and F.R. Stoeber, Eur. J. Biochem. *43*, 1 (1974).
4 W.M. Fitch and I.L. Chaikoff, J. biol. Chem. *235*, 554 (1960).
5 P. Desnuelle, Proc. 2nd Int. Coll. 'Automatisation and Prospective Biology', Pont-à-Mousson 1972, p. 22. Karger, Basel 1973.
6 M. Bounias, Bull. Soc. zool. Fr. *102*, 294 (1977).
7 M. Bounias, Comp. Biochem. Physiol. *66B*, 335 (1980).
8 M. Bounias, Experientia *36*, 157 (1980).
9 M. Bounias, Comp. Biochem. Physiol. *63B*, 407 (1979).
10 K.-B. Augustinsson, Acta physiol. scand. *15*, suppl. 52 (1948).
11 M. Dixon, Biochem. J. *55*, 170 (1953).
12 T.-C. Chou, J. theor. Biol. *59*, 253 (1976).
13 M. Bounias and M.R.J. Morgan, C.r. Soc. Biol. *175*, 12-20 (1981).
14 M. Bounias and M.R.J. Morgan, C.r. Acad. Sci., Paris, Ser. III 883 (1981).

The brachymorphic mutation of mice and altered developmental patterns of limb bud 3′:5′ cyclic adenosine monophosphate[1]

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Summary. Concentrations of cyclic AMP (cAMP) were determined in paired fore and hind limbs from day 12-16 of development in murine fetuses homozygous for the brachymorphic *(bm)* mutation and normal controls. A developmental rise in cAMP occurred 1 day earlier in *bm/bm* than in +/+ hind limbs and cAMP was higher in day-13 *bm/bm* than in +/+ fore limbs. Since cAMP is well documented to stimulate chondrogenic differentiation, premature cartilage determination secondary to altered levels of cAMP could play a role in *bm/bm* short-limbed dwarfism.

Homozygous brachymorphic mice *(bm/bm)* are characterized by a disproportionate shortening in the length of all bones where growth occurs by cartilage proliferation[2]. Studies by Orkin and co-workers[3] of epiphyseal cartilage indicated the presence of altered proteoglycans (reduced in size and number) in *bm/bm*. The majority of the proteoglycans appeared as a network of thin filaments associated with collagen in *bm/bm*, whereas the normal appearance consisted of polygonal matrix granules. On the other hand, the collagen in the extracellular matrix surrounding the cartilage chondrocytes appeared normal. In addition, *bm/bm* glycosaminoglycans (GAG) were identified as a type of chondroitin sulfate that was undersulfated[4]. Greene et al.[5] found alterations in GAG synthesis to be most severe in the proliferative zone of the epiphyseal growth plate of *bm/bm* limbs. In contrast, epiphyseal protein was found to be normal in *bm/bm*.

This under-sulfation in *bm/bm* has been hypothesized to be due to a deficiency in the enzymatic conversion of adenosine 5′-phosphosulfate (APS) to 3′-phosphoadenosine 5′-phosphosulfate (PAPS), a general sulfate donor[6]. In particular, the defect in *bm/bm* cartilage was related to a 93% decrease in levels of APS kinase. These observations were made on neonatal *bm/bm* mice compared to the +/+ mice from the congenic control strain (C57BL/6J) - dosage was not shown in heterozygotes. Interestingly, the metabolically-related compound, cyclic AMP (cAMP) was found to be altered in palatal shelves of *bm/bm* mice which were studied because of the high susceptibility to cortisone-induced cleft palate determined by homozygosity at the brachymorphic locus[7]. Since alterations in cAMP levels in limb mesenchyme appear to trigger chondrogenesis[8,9], we have studied concentrations of cAMP in developing limb buds of *bm/bm* and syngeneic normal (+/+) fetuses.

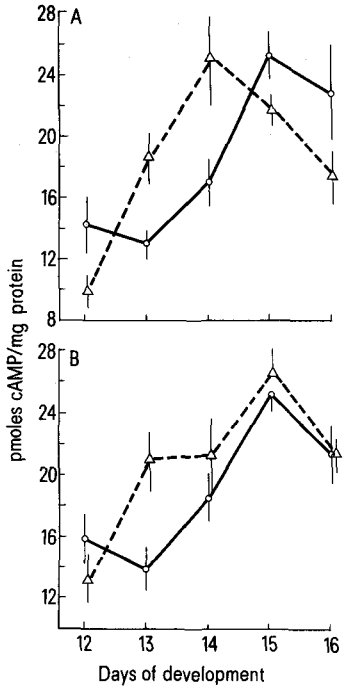
Materials and methods. Brachymorphic mice were obtained from K.S. Brown, N.I.D.R., N.I.H., and a colony was established. Pregnant *bm/bm* and C57BL/6J females (mated to their respective males) were sacrificed by decapitation at 12-16 days of gestation. Day 0 (zero) was determined by the discovery of a vaginal plug in the morning. Embryos removed from these females (110 *bm/bm* and 85 C57BL/6J fetuses were used in total; they were unevenly distributed among days) were double checked for the specific day of development using external characteristics as described in Theiler[10]. After sacrificing the mother, the uterus was immediately dissected out and placed in a warmed, high glucose media (0.2 M glucose, 0.05 M NaCl, 0.02 M Na_2HPO_4, and 0.0026 M KH_2PO_4). Each embryo was individually removed from the uterus and the 2 front and 2 back limb buds dissected off while in the high glucose media. The removed limb buds were placed in a test tube with 300 µl H_2O and immediately placed in a boiling water bath for 10 min. This method of deproteinizing tissue has been found to be superior to perchloric and trichloracetic acid precipitations[11]. After cooling on ice, the 2 samples from each embryo (front and back limb bud aliquots) in the litter were then centrifuged at 1300×g for 15 min at 4 °C. 200 µl from each supernatant was aliquoted, divided into 2×100 µl duplicates and stored for cAMP assay. The remaining 100 µl liquid and limb pellets were saved for protein determination by the method of Lowry.

cAMP levels were measured by a modification of the radioimmunoassay of Steiner et al.[12] using a commercially available kit (New England Nuclear) and expressed in pmol cAMP per mg of limb bud protein.

Results and discussion. Hind limb concentrations of cAMP increased from day 12 to day 14 in *bm/bm* fetuses and then decreased to day 16 (fig. A). Normal hind limbs showed a nearly identical developmental pattern which was delayed by 1 day, however (fig. A). Fore limb concentrations of cAMP were nearly identical to hind limb concentrations in normal fetuses (fig. B) while in *bm/bm* fetuses, they were

less distinguishable from those of normal fetuses except on day 13 were *bm/bm* fore limbs had higher cAMP levels than did the congenic C57BL/6J fetuses.

The prenatal differences in limb bud cAMP levels observed here between *bm/bm* and C57BL/6J may be related to the dwarfed phenotype observed in adult *bm/bm* mice. Using the model of Miller and coworkers[13], the results found here for back limb buds can be interpreted to explain the dwarfed limbs and undersulfated cartilage proteoglycans observed in postnatal *bm/bm*. The higher levels of cAMP on days 13 and 14 (back limbs) could be related to suppressed cell division of chondrocytes and the consequent dwarfed skeleton in adult *bm/bm* mice. The lower levels of cAMP on days 15 and 16 may relate to undersulfation of matrix proteoglycans observed later in *bm/bm*. Such a hypothesis assumes the presence of 'critical periods' and is less readily applied to the front limb bud data. However, these results are important in combining observations on postnatal *bm/bm* limbs (dwarfed with undersulfated cartilage proteoglycans) with models about cAMP's role in chondrogenesis derived from other systems. Deficient cartilage concentrations of cAMP have been reported in chicks homozygous for the nanomelia mutation[14] – a recessive lethal trait characterized by a severe reduction in size of all cartilaginous structures. The correlational nature of the results here and elsewhere on adenine nucleotides must always be remembered. Until extensive cause and effect results are obtained, differences in cAMP or APS levels must be viewed as merely suggestive.



Means and SE for concentrations of cAMP in paired limb buds of fetuses homozygous for brachymorphic (*bm/bm;* about 22 fetuses per day) or normal, syngeneic controls (+/+; about 17 fetuses per day) by day of gestation. *A* Hind limb buds; *B* fore limb buds; –△–, *bm/bm;* –○–, +/+.

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2 P.W. Lane and M.M. Dickie, J. Hered. *59,* 300 (1968).
3 R.W. Orkin, B.R. Williams, R.E. Cranley, D.C. Poppke and K.S. Brown, J. Cell Biol. *73,* 287 (1977).
4 R.W. Orkin, R.M. Pratt and G.R. Martin, Devl Biol. *50,* 82 (1976).
5 R.M. Greene, K.S. Brown and R.M. Pratt, Anat. Rec. *191,* 19 (1978).
6 K. Sugahara and N.B. Schwartz, Proc. natl Acad. Sci. USA *76,* 6615 (1979).
7 R.M. Pratt, D.S. Salomon, V.M. Diewert, R.P. Erickson, R. Burns and K.S. Brown, Teratogen. Carcinogen. Mutagen. *1,* 15 (1980).
8 M. Solursh, P.B. Ahrens and R.S. Reiter, In Vitro *14,* 51 (1978).
9 R.A. Kosher, M.P. Savage and S.-C. Chan, J. exp. Zool. *209,* 221 (1979).
10 K. Theiler, The House Mouse. Springer, Heidelberg 1972.
11 R.H. Cooper, M. McPherson and J.G. Schofield, Biochem. J. *127,* 143 (1972).
12 A.L. Steiner, C.W. Parker and D.M. Kipnis, J. biol. Chem. *247,* 1106 (1972).
13 R.P. Miller, M. Husain and S. Lohin, J. Cell Physiol. *100,* 63 (1979).
14 L.A. Bourret, P.F. Goetinck, R. Hintz and G.A. Rodan, FEBS Lett. *108,* 353 (1979).

Genetic analysis of modifier variability in *Drosophila subobscura*[1]

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Summary. The previously detected modifier variability acting on the expression of the Bare (*Ba*) locus in *Drosophila subobscura* is demonstrated to be due to polygenes situated along the chromosome O. From crosses between isogenic lines of high and low modifier effect we ascertained the presence of approximately 5 modifier loci.

Modifier variability is one type of genetic variability frequently used to explain many evolutionary changes which cannot be understood by considering only structural loci[3–5]. In spite of the great modifier variability acting on the expression of structural genes that has been found[6,7], little research has been devoted to the quantification of this type of variability in natural populations. With this in mind, we have studied the modifier variability in one of the chromosomes of *Drosophila subobscura* (the so-called O chromosome) in a natural population, inasmuch as it affects the expression of the dominant morphological mutant Bare (*Ba*)[8]. This mutant is precisely located on chromosome O of this species, and its phenotypic effect is to reduce variably the number of bristles[9].

The problem that arises is to define whether the variability we have studied is mainly due to the structural locus (isoallelic variability in wild O chromosomes) or to typical modifiers, namely allelic substitutions in loci on the chro-