three species of cartilaginous fish correspond to those described by Mezquita et al. for the dog shark *(S. caniculus)*[7]. All of the three species belonging to the orders petromyzoniformes and myxiniformes (class cyclostomata) have only one PGAM band in all tissues (fig. 2)., comparable to the single-banded pattern reported for birds. However, whereas the loss of isozymes in birds is secondary, the single-banded pattern in the cyclostomata may be caused by the existence of only one gene coding for PGAM, reflecting a primitive ancestral stage of vertebrate evolution. This hypothesis implies that the existence of two distinct PGAM loci is due to a duplication of the ancestral gene during the evolution of vertebrates possessing jaws. This fits well with data reported on lactate dehydrogenase and hemoglobin genes[13,14].

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Effects of parathyroid hormone and calcitonin on carbonic anhydrase location in osteoclasts of cultured embryonic chick bone[1]

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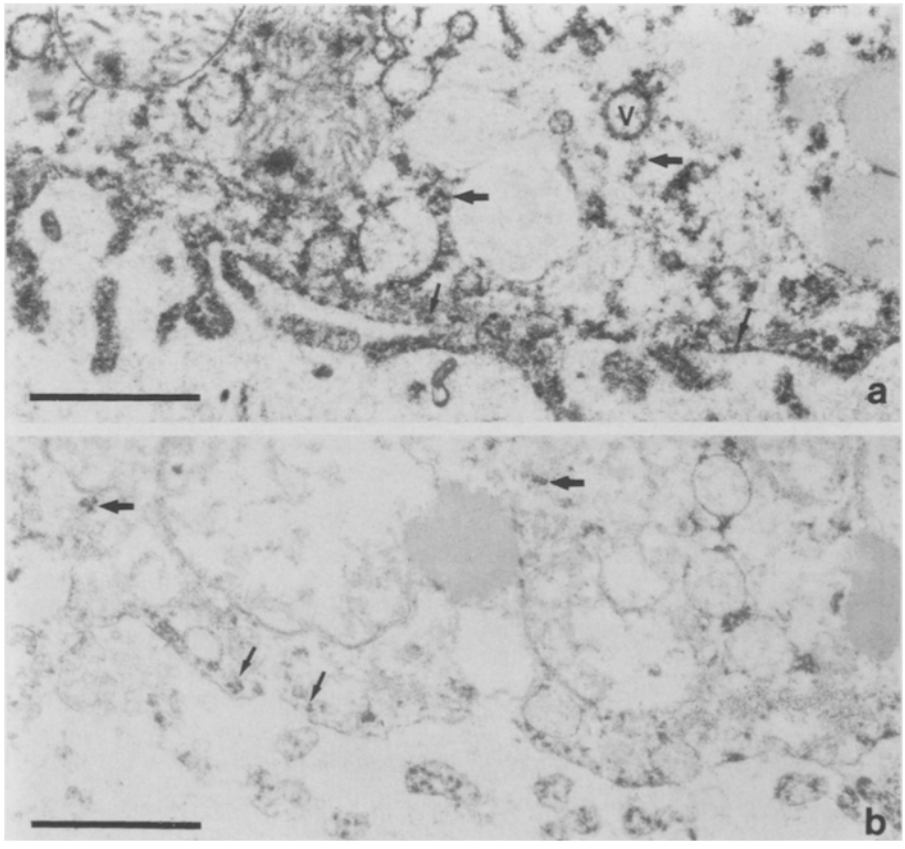
Summary. The ultrastructural location of carbonic anhydrase has been examined in osteoclasts of cultured embryonic chick metatarsi. In untreated cultures and in those to which parathyroid hormone (PTH) was added, the enzyme was found in close association with the plasma membrane of the ruffled border. After brief calcitonin treatment (10 min) the ruffled border disappeared and the association of carbonic anhydrase with the plasma membrane was diminished. The results indicate that the hormones employed act directly on embryonic bone to alter osteoclast structure and the intracellular location of carbonic anhydrase.
Key words. Osteoclasts; cultured bones; carbonic anhydrase; calcitonin; parathyroid hormone.

Evidence continues to be obtained that indicates that carbonic anhydrase is important in the function of osteoclasts. Recently, the ultrastructural localization of the enzyme was observed to change with different states of cellular activity[2]. A comparison of calcitonin-treated and untreated chicks showed that calcitonin inactivation was accompanied by a reduction of enzyme staining on the plasma membrane and endosteal surface of the bone beneath the osteoclast. Enzyme localization was accomplished in osteoclasts of chick long bones by an immunocytochemical method[3] combined with a modified preembedding staining procedure[4]. The occurence of carbonic anhydrase in osteoclasts from rat calvaria has been confirmed by Väänänen and Parvinen[5].

In the present study we extend our previous observations to an in vitro system employing embryonic chicks. An improvement in ultrastructure was also accomplished by the addition of osmium post-fixation to the immunocytochemical procedure.

Metatarsi from 18-day-old chick embryos were cut longitudinally and the marrow was removed. The pieces of bone were incubated (37 °C, 5% CO_2) on stainless steel grids in 2 ml BGJ_b medium (Gibco, Grand Island, New York) which contained 1 mg/ml bovine serum albumin, 100 µg/ml penicillin and 100 µg/ml streptomycin. The bones were cultured with salmon calcitonin (Sigma, St. Louis, Missouri) for 10 min or 1 h (150 µU/ml) or with bovine parathyroid hormone (PTH; see 'acknowledgments') for 12 h (400 µU/ml). Parallel cultures not treated with hormones served as controls. Bones from three to six embryos were examined in each treatment group. The times of exposure to hormones were selected on the basis of the ultrastructural preservation of the tissues which were fixed with phosphate-buffered glutaraldehyde (2.5%) and OsO_4 (1%). The osteoclasts were noticeably more fragile after calcitonin than after PTH treatment. For this reason and because the response of osteoclasts is known to be rapid[6], shorter exposure times were selected for calcitonin treatment.

Carbonic anhydrase was localized by peroxidase-antiperoxidase (PAP) immunocytochemistry as described by Anderson et al.[2]. The antibody directed against carbonic anhydrase was produced in rabbits injected with highly purified chicken carbonic anhydrase isoenzyme C, the only isoenzyme present in chicken red blood cells[7]. Antiserum specificity was reconfirmed and immunoglobulin G (IgG) was fractionated from whole antiserum by ammonium sulfate precipitation and DEAE cellulose chromatography as previously described[8]. For controls, the primary antibody was replaced with saline or a 1:50 dilution of normal rabbit immunogammaglobulin. Between 15 and 30 osteoclasts were



a Typical ruffled border region of osteoclast after culturing in the presence of PTH. The ruffled border has carbonic anhydrase associated with it in a nearly continuous line (e.g. thin arrows). Enzyme is also present in the cytoplasm (thick arrows) and is associated with vesicles (V). Staining in mitochondria, which also occurred in controls, is due to endogenous reaction product. × 23,000. Bar = 1 μm. *b* Region of an osteoclast after exposure to calcitonin for 10 min. The orientation of the cell is similar to *a*. Carbonic anhydrase is dissociated from the membrane in many places (thin arrows). Enzyme is present in the cytoplasm (thick arrows). Remnants of the ruffled border are present as extracellular vesicles. Bone (not shown) was present near the bottom of the figure. × 23,000. Bar = 1 μm.

examined in each treatment group. All sections were stained with saturated uranyl acetate in 70% ethanol and Reynolds' lead citrate.

For morphometric analysis, micrographs were enlarged to 36,300 × . The linear distance along the ruffled border region which was occupied by carbonic anhydrase and the total distance were measured using a Zeiss Videoplan image analysis system (Thornwood, New York).

When no hormones were added to the culture dish carbonic anhydrase was distributed in the cytoplasm in a diffuse manner and was associated with the plasma membrane, although not as extensively as with PTH treatment (table). This distribution was similar for both 1 h and 12 h culture periods.

In the presence of continued exposure to PTH the ruffled border was maintained and carbonic anhydrase in abundance was associated with it (table, fig., a). Staining for carbonic anhydrase may be identified by focal densities which approximate the size of PAP complexes. Due to the use of uranium and lead-containing stains in this study, the size of the focal denisities is slightly larger than the previously reported size of 10 to 20 nm[3]. When calcitonin was added the ruffled borders were greatly diminished in extent. Carbonic anhydrase was distributed diffusely in the cytoplasm and was not closely associated with the plasma membrane (table, fig., b). Immunocytochemical controls for all treatment groups were negative.

Percentage of ruffled border region with which carbonic anhydrase (CA) is associated following various hormone treatments using morphometric analysis

Treatment	Number of cells examined	Total membrane analyzed (mm)*	Length of membrane occupied by CA (mm)*	% of total occupied by CA
Parathyroid hormone	5	1609	1256	78.0 ± 10.9
None	4	1200	810	67.5 ± 20.6
Calcitonin (10 min)	7	1338	181	13.9 ± 3.0**

*Distance measured on micrographs. Means are ± SD. **Significantly different ($p < 0.01$).

This study of osteoclasts in 18-day-old chick embryo metatarsi adds verification to the earlier finding of plasma membrane-associated carbonic anhydrase in osteoclasts from 4-week-old chicks and it supports the finding that calcitonin plays a role in the rapid dissociation of the enzyme from the membrane[2]. In this manner the action of carbonic anhydrase in acid secretion by osteoclasts could be blocked through the action of calcitonin. Calcitonin receptors have been demonstrated in mature rat osteoclasts[9] and osteoclasts have been shown to respond to calcitonin in a number of ways, in addition to its effect on carbonic anhydrase location. Many investigators have observed that after treatment with calcitonin, osteoclasts round up and move away from the bone surface and the villi of the ruffled border shorten[10], secretion of hydrolytic enzymes stops[11], and ameboid motion ceases[6]. Calcitonin secretion has been detected in chick embryo plasma at 17 days of development[12] although there is histologic evidence that secretion begins sooner[13]. Osteoclastic activity appears to be influenced by calcitonin by day 12.5 of embryonic development[14]. The use of a culture system made it possible to observe the effect of administered hormones without the effects of endogenous hormones being present. In this study PTH had little detectable effect except perhaps to stabilize the association of carbonic anhydrase with the membrane (table). It is possible that the membrane association of carbonic anhydrase is important in maintaining the enzyme in a fully active state. Membrane-associated carbonic anhydrase has been re-

ported in a variety of tissues including oyster mantle[15], kidney[16], turtle bladder epithelium[17], and villus cavity cells of the chorioallantoic membrane[18]. This laboratory has also found that purified chicken and salmon erythrocyte carbonic anhydrases are stabilized in the face of rising temperature and pH when lipid vesicles are present[19]. The membrane-enzyme association may also be important in orienting the enzyme in an appropriate way for it to function, possibly in the secretion of hydrogen ions.

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Mobile gene localization and viability in *Drosophila melanogaster*

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Summary. The location of the mobile element mdg-1 was determined by in situ hybridization in salivary gland chromosomes of *Drosophila melanogaster*. The locations of mdg-1 are nonrandom and some 'hot spots' exist. Moreover, the spectra of mdg-1 locations vary with the viability values of the families from which the larvae originated. This suggests that particular frequency spectra are associated with lethality resulting from inbreeding.
Key words. Mobile elements; inbreeding; in situ hybridization; *Drosophila melanogaster*.

The genome is now known to contain many reiterated transposable elements that may be the cause of genetic instability of structural genes through activation, inactivation or regulation[1-3]. These elements may appear at different chromosomal locations in different strains and in different individuals from the same laboratory stock or the same natural population[4-9]. Only a little information about the amount of polymorphism of such elements in natural populations is available, though such information could shed light on their origin, their effects on the organisms carrying them and their possible role in the adaptation of populations. Indeed, though the molecular structure of some of these elements is now well known, we have little idea whether they do or do not provide benefit to the organisms. However, some transposable elements in bacteria do procure a higher adaptability to their carriers[10,11] and the number and location of mobile dispersed genes (mdg) in *Drosophila melanogaster* may be associated with fitness[5,12]. Here, we study the location of the element mdg-1 in the chromosomes of individuals from a natural population of *Drosophila melanogaster* in connection with fitness after inbreeding.

A laboratory population of *Drosophila melanogaster* was established from flies captured in Azerbaidjan (USSR) recently. The population so formed was maintained in the laboratory by mass culture at 25 °C for three generations before the experiment was started. 58 sib pairs, offspring of 58 pairs of unrelated flies of the stock, were set up and allowed to lay eggs. Egg hatchability and egg-to-adult survival were then determined in their offspring that developed at 25 °C[13]. Other subsequent samples of eggs laid by the same sib pairs were allowed to develop at 18 °C in order to lower the developmental rate and to obtain large larvae with chromosomes suitable for in situ hybridization[4]. The number and location of the mdg-1 element were then analyzed in the salivary gland chromosomes of these inbred larvae. 17 sib progenies were chosen for the cytological study according to their viability values, which ranged from 0.32 to 1 (see the distribution of viability values in figure 1). Low overall viability values are due to either a high embryonic mortality rate or a high larvo-pupal mortality, or to both[13]. We thus distinguished three classes of inbred progenies characterized by 1) low egg hatchability (overall viability values ranging from 0.38 to 0.61; five progenies), 2) low larval viability (with overall viability values ranging from 0.32 to 0.72; seven progenies), 3) high egg-to-adult survival rate (viabilities higher than 0.90; five progenies). We have also

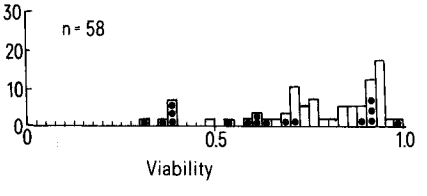

Figure 1. Distribution of the proportion of the fertilized eggs developing to the adult stage in F2 inbred progenies from sib pairs. ●indicates the families from which the larvae were taken. N: number of pairs.