were allowed to develop at room temperature overnight, after which they were hung to dry. One set of strips was sprayed with phenylacetylchloride containing a pyridine buffer so that the 6-APA would be converted back to benzylpenicillin for use in demonstrating biological activity. The well-aerated and dried chromatogram strips were later placed upon large agar plates seeded with *B. subtilis*. After overnight incubation, the strips were removed and the zones of inhibition were recorded in terms of mobility ($R_F$) from the origin.

Table 1 shows that both cultures completely destroyed cephalosporin C in the absence of 6-APA, but that the presence of the acid completely prevented destruction of cephalosporin C. This was corroborated by the chromatograms which showed zones of inhibition due to cephalosporin C at an $R_F$ of 0.16, and 6-APA at an $R_F$ of 0.40, after acylation to penicillin.

As this demonstrated that 6-APA blocked or prevented the action of cephalosporinase, 45 bacterial cultures that were known to actively destroy cephalosporin were subjected to the same procedure.

Nineteen of the cultures, or 42 percent, completely destroyed cephalosporin C in the absence of 6-APA, but the presence of the acid totally arrested the destruction of cephalosporin. The cultures (*7*) included these strains; *Aerobacter aerogenes* ATCC 884, *Aerobacter cloacae* ATCC 961, *Alcaligenes faecalis* ATCC 212, *Bacillus subtilis* MB-38, *Bacillus* sp., Bodenheimer MB-265, *Lactobacillus bulgaricus* MB-300, *Pseudomonas aeruginosa* ATCC 10145 and MB-1075, *Pseudomonas cichorii* ATCC 10857, *Pseudomonas primulae* ATCC 10860, *Pseudomonas* sp. AI MB-150, and the following strains of *Escherichia coli*: ATCC 10586, B, C, $K_{12}$, MB-217, MB-225, MB-648, and MB-711.

Table 1. The protective effect of 6-aminopenicillanic acid (6-APA) upon the destruction of cephalosporin C (C) by *Alcaligenes faecalis* ATCC 212 and *Alcaligenes viscosus* ATCC 337. Each component was identified on the chromatograms.

| *A. faecalis* C | *A. faecalis* C + 6-APA | *A. viscosus* C | *A. viscosus* C + 6-APA | Control C + 6-APA |
|---|---|---|---|---|
| | | *Bioassay** | | |
| — | 37.5 | — | 38 | 38 |
| | | *Chromatograms†* | | |
| — | 0.16 | — | 0.15 | 0.16 |
| — | 0.40 | — | 0.42 | 0.39 |
| | | *Control chromatograms* | | |
| — | 0.17 | — | 0.17 | 0.16 |

*Zone of inhibition measured in millimeters diameter. †After conversion of 6-APA to benzylpenicillin ($R_F$ zones of inhibition).

Two cultures, *Erwinia amylovora* MB-756 and *Serratia marcescens* ATCC 990, completely destroyed cephalosporin but, in the presence of 6-APA, only deacetylation of this antibiotic was observed. Deacetylcephalosporin C, although having considerably less bioactivity than cephalosporin C, can be demonstrated by an $R_F$ of 0.08 in the chromatographic system used. Therefore, 6-APA is not a general enzyme inhibitor but is probably specific for cephalosporinase.

Six cultures, or 13 percent, partially destroyed cephalosporin C in the absence of 6-APA, but the presence of this acid completely blocked the destruction. These cultures included the strains; *Alcaligenes faecalis* ATCC 213, *Escherichia coli* ATCC 9637, MB-931, MB-967, *Pseudomonas aeruginosa* MB-95, and *Pseudomonas fluorescens* ATCC 949.

In 15 cultures, or 33 percent, that completely destroyed cephalosporin C, the presence of 6-APA only partially prevented loss of biological activity. Such bacteria included a *Pasteurella* sp. MB-90, *Propionibacterium jensenii* ATCC 4867, *Proteus vulgaris* MB-1012, *Serratia marcescens* ATCC 264 and MB-531, *Vibrio comma* MB-581, and several different species of *Pseudomonas: P. aptata* ATCC 10205, *P. alcaligenes* ATCC 12815, *P. aceris* ATCC 10853, *P. coronafaciens* ATCC 9005, *P. maculicola* ATCC 11781, *P. synxantha* ATCC 796, *P. testosteroni* ATCC 11996, *P. tabaci* ATCC 11527, and *P. woodsii* ATCC 9655.

Two cultures, *Aeromonas salmonicida* MB-1166 and an *Alcaligenes* sp. MB-495 completely destroyed both cephalosporin C and 6-APA simultaneously. It is conceivable that in these two cultures both penicillinase and cephalosporinase were present, although it has been reported (*8*) that cephalosporin C competitively inhibits the action of penicillinase.

Only one culture, *Pseudomonas denitrificans* MB-580, completely destroyed cephalosporin C in both the presence and absence of 6-APA.

The results with these last three organisms may possibly denote yet another method by which microorganisms destroy cephalosporin—in addition to the accepted methods: by hydrolysis of the $\beta$-lactam ring, deacetylation, and, theoretically, by the action of amidase. No cephalosporin C amidase was demonstrated in these bacterial cultures. This inactivation of cephalosporin may be related to a similar unknown metabolic mechanism of penicillin resistance which has been reported previously (*9*).

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## Compact Bone in Chinese and Japanese

Abstract. *Measurements in vivo of compact bone in individuals of Chinese and Japanese ancestry, whether American-born or born abroad, show them to have less compact bone per unit length than Americans of European ancestry.*

In discussions of the nature of osteoporosis, much has been made of the apparently lighter skeletons of Asiatics as possibly indicating diminished bone formation or increased bone loss under restricted dietary conditions (*1*).

As a part of our continuing studies on the mechanisms of bone loss, we have had the opportunity to compare compact bone in 51 individuals of Chinese or Japanese ancestry with age and sex-specific standards for Americans of European ancestry (*2*). All measurements were made on the second metacarpal with P-A hand

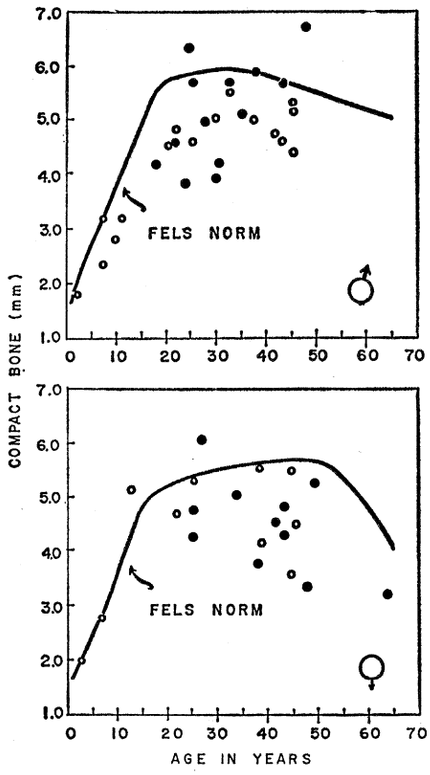


Fig. 1. Comparison of compact bone in American-born subjects of Chinese and Japanese ancestry (open dots), comparable subjects born abroad (black dots) and participants in the Fels Longitudinal Studies of Growth and Development (solid lines). Top, males; bottom, females. Both abscissas show age in years.

radiographs taken at a fixed (90 cm) tube-to-film distance. Replicability coefficients exceeded 0.98 (*2*).

As shown, the 51 Chinese and Japanese subjects had significantly less compact bone than the reference population at all ages ($\chi^2 = 24.0$). As a group they averaged 1.28 standard deviations below the Fels trend line. This tendency for diminished compact bone for subjects of Asiatic ancestry was true both for those born abroad (−1.37 S.D.) and those born in the United States (−1.19 S.D.). The nine children under 18 years of age similarly showed less compact bone at the middle-metacarpal site than the reference population.

In addition to the data on 51 subjects shown in Fig. 1, eight additional children of Chinese-Caucasian ancestry were studied. Though American-born, and from professional families of superior economic status, these children also fell below the Fels Institute averages for compact bone, though to a lesser degree (−0.66 S.D.) than the Chinese-Japanese group as a whole.

Compact bone formation is unquestionably impaired in children from Central America suffering from Kwashiorkor or in areas where native dietaries otherwise provide as little as 1.8 g of animal protein per day, as we have shown (*3*). Dietary deficiencies during the war years may account for the very low compact bone values here observed for seven of our Japanese subjects born between 1931 and 1946. However, the low levels of compact bone observed in both American-born and Asiatic-born Chinese and Japanese, their continuance in American-born children of Chinese families of superior economic status, and the intermediate levels of compact bone in children of Chinese-White ancestry obviously lead us to consider that the lesser compact bone of Asiatics is primarily a reflection of endogenous control mechanisms.

To this point we may cite parent-child and sibling comparisons of compact bone in more than 100 families in the Fels Longitudinal Studies, which indicate genetic mediation of compact bone in the second metacarpal, and further suggest mediation by the X chromosome (*2*).

Thus, while dietary studies of all subjects are currently in progress, and while occupation and handedness are being investigated further, we are inclined to attribute the lesser amounts of compact bone in Chinese and Japanese, here observed, more to genetic factors than to limiting nutrients.

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## Molecular and Submolecular Localization of Two Isoantigens of Mouse Immunoglobulins

Abstract. *Three related classes of immunoglobulins (7S γ-, $\beta_{2A}$-, and $\gamma_{1M}$-globulin) in normal C3H and C57BL/6 mouse serums were isolated and examined for the isoantigens Iga-1 and Iga-2. The results indicate that the genetic locus determining the Iga-1 and Iga-2 antigens affects only a portion of the normal mouse immunoglobulin population (that is, 7S γ-globulin) and only the γ-specific part ("F" piece) of the 7S γ-globulin molecule.*

The immunoglobulins are a group of heterogeneous, structurally related serum proteins with which antibody activity has been associated. Recently, genetic polymorphism of mouse immunoglobulins has been recognized with the use of isoimmune antiserums (*1*). Two isoantigens, Iga-1 and Iga-2, under the control of allelic genes, have been described (*2*). The present study was undertaken to determine which class of immunoglobulin carries the antigens specified by the Iga-1 and Iga-2 locus and what molecular subunit has these isoantigenic characters.

Serums from normal and immune C3H/He mice (Iga-1) and C57BL/6 mice (Iga-2) were used for the preparation of 7*S* γ-globulins, $\beta_{2A}$-globulins, and 18*S* $\gamma_1$-macroglobulins. The 7*S* γ-globulins were isolated by block (zone) electrophoresis (*3*); 18*S* $\gamma_1$-macroglobulins and $\beta_{2A}$-globulins were obtained by a combination of block electrophoresis and filtration through Sephadex G-200 (*4*). The preparations of normal $\beta_{2A}$-globulins were contaminated with small amounts of 18*S* $\gamma_1$-macroglobulins. Myeloma $\beta_{2A}$-globulins, which are closely related to normal $\beta_{2A}$-globulins, were prepared by zone electrophoresis and diethylaminoethyl-cellulose chromatography of serums from C3H mice bearing the transplantable plasma cell tumors, 5647 and SPC-1 (*5*). The identity and purity of the fractions were evaluated by immunoelectrophoresis with rabbit antiserums prepared by immunization with mouse immunoglobulin fractions or normal mouse serum. Starch-gel electrophoresis and ultracentrifugation were also used to characterize representative fractions.

Purified 7*S* γ-, $\beta_{2A}$-, and 18*S* $\gamma_1$-macroglobulins from C3H mice were