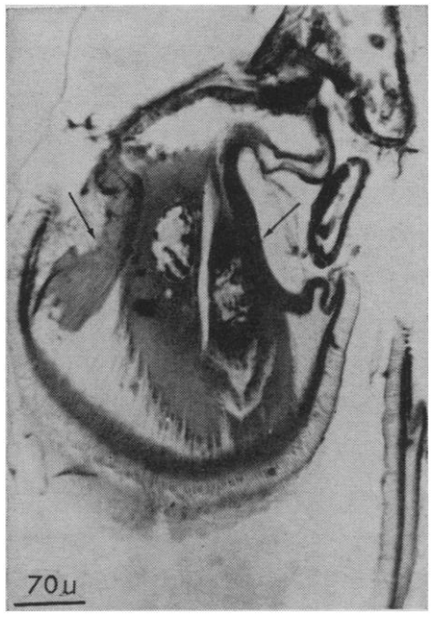

Fig. 2. Section through a footpad parallel with the dorsal cuticle. The previous section has removed the cuticle over the center of the pad exposing the giant nuclei and their cytoplasm; some of the dorsal cuticle remains at either side (arrows). On the distal and lateral margins, there are thousands of tenent hairs, each secreted by a single hypodermal cell. At the lower right, the edge of the second footpad is cut in vertical section and shows the ventral tenent hairs (left), dorsal cuticle (right), and narrow central lumen filled with haemolymph. Stained with methyl-blue-eosin; late pupal stage.

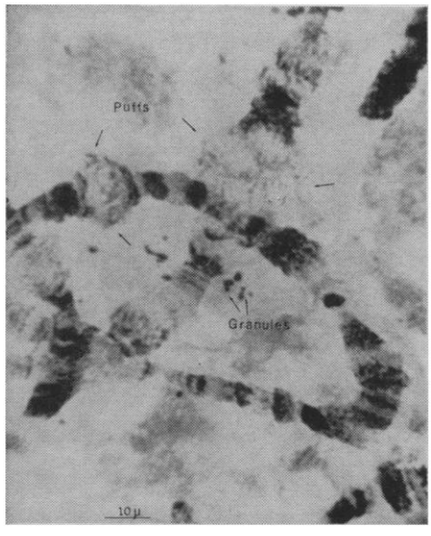

Fig. 3. Chromosomes from a giant hypodermal cell of the footpad at the time of cuticle secretion. Note the banding of the chromosomes, several large "puffs" representing areas of activity, and granules apparently of chromosomal origin. Lacto-acetic-orcein squash preparation.

also evident within the nucleus at the various developmental stages; some are shown in Fig. 3.

The changes occurring in the giant cells are closely paralleled by events taking place in the trichogen and tormogen cells which secrete hairs and sockets on the leg segments. These cells also possess banded chromosomes, but they are much smaller in diameter than the giant cell chromosomes, resembling those of the even smaller hair secreting cells of *Drosophila* (*4*). The very large size of the giant cells of the footpads in the Cyclorrhapha would seem to be correlated with the enormous volume of cuticle which they secrete. In the female *Sarcophaga bullata,* the footpads are not quite as large as in the male, and both the giant cells and their chromosomes are of correspondingly smaller dimensions.

The potentialities of these cells are considerable. They are superficially situated and easily accessible for experimental procedures. Being hypodermal cells they are almost certainly under the control of the molting hormone, and their secretory product remains as a permanent structure. The cells would be ideal material on which to observe possible changes in puffing pattern following the introduction of hormones (*5*). The cells and their nuclei are easily separable from the foot and it may be possible to transplant them, as has been done recently for salivary gland nuclei of *Drosophila* (*6*). *Sarcophaga bullata* is by no means the largest member of the Cyclorrhapha nor does it have the largest footpads; since there appears to be a direct correlation, for this particular cell type, between chromosome size and the volume of cuticle secreted, one may anticipate the discovery of flies having footpad cells and chromosomes with dimensions far exceeding those shown here.

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7. Supported by research grant 14544 from the National Science Foundation. Some of the results were presented at the 16th International Congress of Zoology, Washington, 21–27 August 1963.

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6-Aminopenicillanic Acid: Inhibition of Destruction of Cephalosporin C by Bacteria

Abstract. *6-Aminopenicillanic acid, from which all the true penicillins are derived, inhibits the destruction of cephalosporin C by certain microorganisms. This inhibition is probably specific for "cephalosporinase." The enzymatic deacetylation of cephalosporin C is not affected by the acid.*

Cephalosporin C (*1*) differs from the true penicillins in being derived from 7-aminocephalosporanic acid nucleus. It is not inactivated by penicillinase, although its biological activity is destroyed by another enzyme, "cephalosporinase" (*2*). It has been shown that a large number of microorganisms are capable of inactivating cephalosporin C by means of cephalosporinase or by deacetylation (*3*). Cephalosporin might also be inactivated by the action of cephalosporin-C amidase, if such an enzyme could be demonstrated.

All the true penicillins are derived from 6-aminopenicillanic acid (6-APA), which induces penicillinase formation (*4*). I have investigated the effect of this acid on the inactivation of cephalosporin C. Two bacterial cultures were selected, *Alcaligenes faecalis* ATCC 212 and *Alcaligenes viscosus* ATCC 337, because they both contain a very active intracellular penicillin amidase (*5*) and also completely destroy cephalosporin C in a very short time.

The cultures were grown submerged in Difco nutrient broth containing 0.2 percent yeast extract, at 28°C for 16 hours. The cultures were then centrifuged in duplicate and washed with 0.1*M* (*p*H 7.5) phosphate buffer. The cells were suspended in the phosphate buffer (1/10 the original volume) containing 4 mg of cephalosporin C per milliliter. To one of each pair of tubes 6-APA was also added, 4 mg/ml. The tubes were incubated for 4 hours in a water bath maintained at 37°C and were then centrifuged. Bioassays of the supernatants were performed by the usual penicillin assay-disk method (*6*). The zones of inhibition were recorded in millimeters of diameter (Table 1). The supernatants were also examined in duplicate by descending chromatography on Whatman No. 1 paper in isopropanol : pyridine : water (65 : 5 : 30), suitable antibiotics being used on control strips. The chromatograms

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.

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 β-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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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).

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6. The assay disks (No. 740-D, Schleicher and Schuell Co.) were saturated with the supernatant, placed on solidified penassay agar (Difco) which had previously been inoculated with *Bacillus subtilis* MB-35, and then incubated for suitable periods.
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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