quency of nonobserving responses and degree of general activity were independent of one another for each subject, and although both increased with time neither was related to performance on the vigilance task used (*10*).

C. H. BAKER

Defence Research Medical Laboratories, Defence Research Board, Toronto, Canada

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8. The possible total of 68,400 photographs was not achieved because of camera difficulties.
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Sex Chromatin in Mammalian Bone

Abstract. The presence of a sex chromatin body similar to that reported in other tissues has been demonstrated in the nuclei of osteoblasts, osteocytes, and periosteal cells from female dogs and cats.

Since 1949, when Barr and Bertram (*1*) first described a histologic sex difference in the neurons of the female cat, the presence of the nuclear sex chromatin body has been noted in a variety of cells from different species (*2–5*). In man, it has been studied extensively in the nervous system (*6*), polymorphonuclear leukocytes (*7*), epidermis (*8*), and mucous membrane (*9*). It has also been observed in the cells of the amniotic fluid, the placenta, and the fetal membranes (*10, 11*). In animals other than man an even greater number of cell types have been examined (*4*). Only a few species did not exhibit a visible nuclear sex difference (*2*). In any animals in which the sex chromatin body could be identified, it was demonstrable in all of the tissues that were investigated.

Table 1. Incidence of the sex chromatin body in the bone and periosteal cells of female animals (fewer than 10 percent of cells in male animals showed a similar structure).

No.	Cells with sex chromatin (%)		
	Periosteum	Osteoblasts	Osteocytes
		Adult cat	
4	76	53	48
		Adult dog	
4	72	52	50
		Puppy	
4	79	76	73

The presence of a sex chromatin body in bone has not been previously reported. We do not know whether this tissue has actually ever been studied, or if technical difficulties in fixation and decalcification have prevented the proper examination of nuclear detail. The purpose of this investigation was to disclose whether the sex chromatin body could be observed in the bone of animals known to exhibit a histologic sex difference in other tissues (*12*).

Eight adult dogs, eight cats and eight 6-week-old puppies, equally divided according to sex, were used for this study. The animals were killed with a lethal dose of sodium pentobarbital, and portions of rib and tibia, including the periosteum, were removed and immediately placed into modified Davidson's solution (alcohol-formalin-acetic acid). After fixation for 48 hours the specimens were transferred to an aqueous solution of ethylenediaminetetraacetic acid at *p*H 7.4. Periodic radiographic examination of the tissue samples was used to determine the degree of decalcification. After decalcification the specimens were washed, dehydrated in alcohol, and embedded in paraffin. Sections were cut at 7 to 10 μ with a rotary microtome. The tissues were stained with Stowell's modification of the Feulgen-Schiff technique (*13*) and counterstained with fast green dye.

The stained specimens were examined under oil emersion at magnifications of 900 and 1250. The criteria used for recognition of the sex chromatin body (size, shape, position, and Feulgen-positive staining properties) were based upon previous descriptions for other mammalian cells (*1, 5*). Identification of the sex chromatin was originally established in samples of bone tissue from four cats and four dogs of known sex. Subsequently, the sex determination of unidentified coded specimens was based upon the percentage of 100 well-preserved cells containing the sex chromatin body. Cell counts limited to one particular field could not be made in compact bone because of the difficulty in obtaining uniform cellular fixation.

The sex chromatin body could be identified in osteoblasts, osteocytes, and periosteal cells of the bone tissue from female dogs and cats (Table 1). In appearance, it was similar to that which has been seen in other mammalian tissues. Generally, it was the largest chromatin mass within the nucleus. It could be readily distinguished from the nucleolus by its differential staining properties. Due to the high deoxyribonucleic acid content, the sex chromatin body appeared red with the Feulgen stain whereas the nucleolus, containing mainly ribonucleic acid, was colorless. In counterstained specimens, the nucleolus was light green.

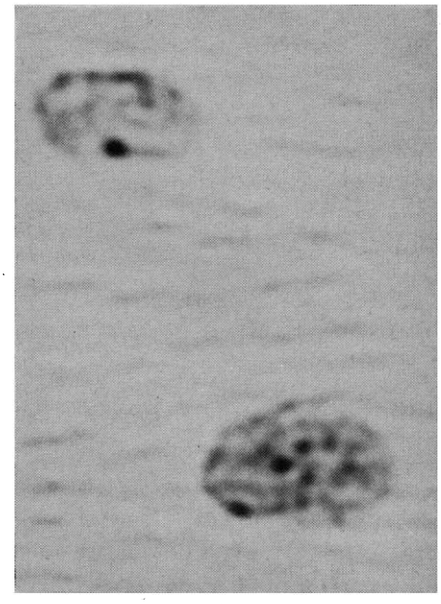

Fig. 1. Osteocytes in bone from female puppy. The sex chromatin appears as a planoconvex body at the nuclear-cytoplasmic junction. Feulgen stain (×1100).

In most instances, the sex chromatin body appeared as a centrally projecting, deeply stained, planoconvex-shaped mass approximating the nuclear-cytoplasmic junction (Fig. 1). Occasionally it assumed a triangular or flattened form. Although the sex chromatin body has sometimes been described as appearing in a more central position within the nucleus (*5*), contact with the nuclear-cytoplasmic junction was used as a criterion in this study.

The sex chromatin body was most readily observed in cells with well-

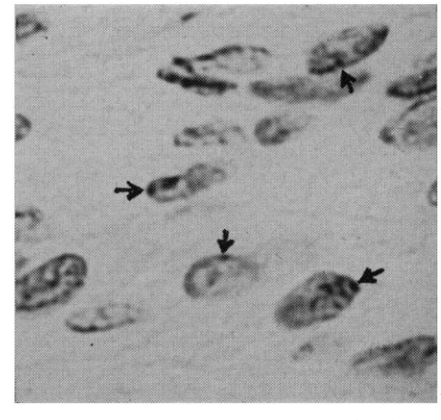

Fig. 2. Periosteum from female dog showing the high incidence of visible sex chromatin bodies. Feulgen stain (×1100).

preserved vesicular nuclei. Thus, periosteum and young bone provided the best material for study (Figs. 1, 2). In these tissues the sex chromatin body could be recognized in about 75 percent of the cells (Table 1). In adult bone, approximately 50 percent of the nuclei contained an identifiable sex chromatin body. These figures compare favorably with the reported observations in other tissues of the dog and cat (*2, 4*). In sections of bone from male animals less than 10 percent of the cells contained a nuclear chromatin mass of a size, shape, and position comparable to that which is found in the female. Of the unlabeled coded sections from the 16 animals that were investigated, all were correctly identified according to their sex source.

Although the significance of the sex chromatin body has not been established, its recognition in tissues has led to many important clinical applications. Thus, it has been used extensively in prenatal sex determinations, in studies of infertility, and in distinguishing the chromosomal sex of individuals with congenital errors in sexual development (*10, 14*). Experimentally, the presence of a histologic sex difference in cells also provides a useful physiologic tag for studying grafted tissues (*15*). As a result of the findings in the present investigation, this technique is now being used to evaluate the fate of bone homografts.

DANIEL M. VERNINO
DANIEL M. LASKIN
University of Illinois College of Dentistry, Chicago

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3 June 1960

Acrylic Acid, an "Antibiotic" Principle in Phaeocystis Blooms in Antarctic Waters

Abstract. An acidic algal substance has been isolated and characterized as the sodium salt. Synthetic sodium acrylate possesses chemical and antibacterial properties identical with those of the natural product. This observation identifies the algal substance in the stomach contents of euphausids, the diet of pygoscelid penguins, which inhibits the penguin gastrointestinal microflora.

A substance which inhibited the gastrointestinal microflora of pygoscelid penguins (*1*) was traced to the phytoplankton-laden stomach contents of their euphausid diet (*Euphausia superba*) and then to a green mucilaginous colonial alga (*2*). The purpose of this report is to describe the isolation of an antibacterial substance from the mucilaginous colonial alga, *Phaeocystis*, and the identification of this substance as acrylic acid (*3*).

The phytoplankton communities in the Gerlache and Bransfield straits off the west coast of Palmer Peninsula were studied in January and February 1959. Antibiotic assays and microscopic examination of concentrates of plankton gathered by net indicated that antibacterial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* is correlated with the predominance (40 to 90 percent) of a species of *Phaeocystis* (similar to *P. pouchetii*) in the blooms. Algal concentrates (5.1 percent dry wt.) of *Phaeocystis* were obtained with a number 2 net which retained these mucilaginous algae but permitted most of the diatoms to pass through. These preparations were stored in polyethylene bottles at −20°C aboard ship, transported in dry ice, and maintained in the laboratory at −55°C.

Air drying on glass plates, desiccation in a vacuum, and lyophilization of the algal concentrates caused an approximately 20-fold reduction in activity. Extraction of this dried material, as well as the wet algae, with acetone, ethers, and alcohols produced concentrates which lost their activity rapidly at 5°C. The formation of inactive gummy material usually occurred as a result of the numerous abortive attempts to concentrate and purify the antibacterial substance. Distillation of acidic algal supernatants produced antibacterial distillates which failed to leave an active residue after evaporation.

A virtually pure active solid has been obtained only by the following procedure. The supernatant fluid of thawed cells (*p*H 5.5) was adjusted to *p*H 3.0 with HCl or H_2SO_4. The acidic substance readily volatilized under reduced pressure (in a Rinco rotating vacuum-type evaporator) to yield an active condensate (*p*H 3.7) in the solvent recovery condenser. Barium chloride and silver nitrate tests on the condensate indicated that the mineral acids used for acidification were absent. After careful adjustment of the *p*H to the equivalence point (*p*H 7.5) with NaOH, evaporation in a vacuum yielded an active sodium salt. Recovery of the original activity was increased from 80 percent to virtually 100 percent by the use of additional ice traps in the vacuum line.

Titration curves on the acid condensate as well as the weight of the sodium salt formed at equivalence were used to calculate neutralization equivalents of 92 to 99. Analysis of the sodium salt was 35.39 percent carbon and 3.6 percent hydrogen. Sodium fusion elemental analyses were negative for sulfur, nitrogen, and halogens. Qualitative tests indicated the absence of carbonyl and hydroxy groups, while the rapid color changes in bromine and potassium permanganate solutions indicated unsaturation. These data suggested that acrylic acid ($CH_2{=}CH{-}COOH$), the sodium salt of which has a neutralization equivalent of 94 and a composition of 38.3 percent carbon and 3.2 percent hydrogen, was a likely possibility.

The monomer was extracted from a partially polymerized commercial preparation of acrylic acid and used to prepare sodium and calcium salts in the same manner as the algal material. The infrared spectra of the natural and synthetic preparations of sodium acrylate and calcium acrylate, determined by a Beckman IR-5 infrared spectrophotometer, were found to be identical. Filter paper disk antibacterial assays (on *p*H 6.5 heart infusion agar, Difco) of the natural and synthetic salts against 12 microorganisms were used to calculate the minimum inhibitory concentrations. The results presented in Table 1 indicate identical spectra of activity. Ascending filter paper chromatograms and x-ray diffraction analyses also

Table 1. Antibacterial spectrum of natural sodium acrylate from *Phaeocystis* and the synthetic salt.

Test organism	Minimum inhibitory concn. (mg/ml)	
	Synthetic	Natural
Pasteurella multocida	0.025	0.030
Corynebacterium pseudodiphtheriticum	0.21	0.21
Mycobacterium smegmatis	0.35	0.35
Streptococcus pyogenes	0.6	1.3
Staphylococcus aureus	0.8	1.6
Proteus vulgaris	2.5	2.5
Candida albicans	4.5	9.0
Escherichia coli	4.8	5.0
Sarcina lutea	5.0	5.0
Klebsiella pneumoniae	25.0	38.0
Torula lactosa	> 50.0	> 50.0
Aspergillus fumigatus	> 50.0	> 50.0