stretched beyond its elastic limit. Within a freshly stretched sheet remain discrete birefringent substructures in which the slow direction is parallel to the direction of the tensile stress. Stretched sheets of polyethylene or rubber also exhibit birefringence in which the slow direction is parallel to the applied force. Thus the birefringence of Fig. 1*B* is consistent with a film which has been thinned by tensile stresses.

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Alloocimene: Absence in Cigarette Smoke

Abstract. *Alloocimene was not found to be a constituent of cigarette smoke under test conditions that allowed 86 percent recovery when authentic alloocimene was added to the smoke. In the same experiments dipentene was found to the extent of 0.2 percent of the smoke condensate.*

Wynder and Hoffmann recently reported (*1*) the presence of "up to 0.5 percent alloocimene" in cigarette smoke. These authors also found that alloocimene (2,6-dimethylocta-2,4,6-triene) was an effective promoter of mouse skin carcinogenesis when applied in a concentration of 1 to 5 percent after an initiating dose of 300 μg of dimethylbenzanthracene.

Studies in our laboratory have failed to show the presence of alloocimene in smoke from cigarettes made with a blend of Bright (flue-cured) tobaccos or with a typical commercial blend of Bright, Burley, Turkish, and Maryland tobaccos. Our measurements indicate that not more than 2.4 μg of alloocimene could be present in the smoke from one 85-mm cigarette. This is equivalent to less than 0.006 percent of the smoke condensate.

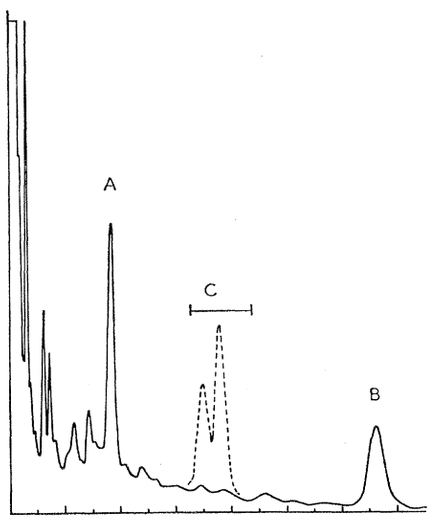

Fig. 1. Gas chromatogram of a volatile extract of cigarette smoke. *A*, Dipentene. *B*, Octyl iodide (190 μg per cigarette, added). *C*, Effluent range collected for ultraviolet examination. The dashed curve under *C* shows the response obtained when 186 μg of alloocimene per cigarette was added to the smoke traps.

Although we were unable to find any evidence for alloocimene in cigarette smoke, we did confirm the presence of dipentene to the extent of 76 to 108 μg in smoke from 85-mm cigarettes made with the commercial blend of tobaccos and 164 to 180 μg in smoke from 85-mm cigarettes made with a blend of Bright tobaccos. This is equivalent to average values of 0.2 and 0.3 percent of the respective smoke condensates. Dipentene was previously reported as a constituent of cigarette smoke by Johnstone *et al.* (*2*).

The cigarettes were smoked to a constant butt length of 30 mm in the Liggett and Myers' Model 4 smoking machine (*3*) that took ten puffs of 2 seconds each at 60-second intervals. The smoke was condensed in glass traps cooled with liquid air. Smoke condensate from 105 cigarettes (85-mm) was rinsed from the traps with three 25-ml portions of distilled water and four 25-ml portions of cyclopentane. Each of the cyclopentane rinses was used in turn to extract the combined water fraction. The cyclopentane extracts were then combined and concentrated at 25°C with a stream of nitrogen to a volume of 10 ml. This solution was distilled at 95°C at a pressure of 1.0 mm-Hg and the distillate was collected in an efficient glass-coil trap cooled in liquid air. The distillate was diluted to 10 ml with cyclopentane and a 5-μl portion was analyzed by gas-liquid chromatography at 80°C on a 0.5 cm (ID) by 1.8 m stainless-steel column containing 80 to 100 mesh Gas-Chrom P (*4*) coated with 6 percent silicone rubber, SE-30 (*4*). Helium carrier gas at 2.6 atm inlet pressure was used to provide an exit flow rate of 90 cm^3/min. The instrument used for the gas-liquid chromatography was equipped with dual columns and flame ionization detectors (*5*).

Octyl iodide was added to the smoke traps before collection of the sample, to provide an internal standard from which completeness of recovery could be estimated. For several determinations the recovery of octyl iodide ranged from 84 to 88 percent. No definite indication of alloocimene was obtained in the gas chromatogram at a retention time typical of authentic alloocimene (Fig. 1). Since there was some nonspecific background material eluted from the gas-liquid chromatographic column at the retention time (R_t) for alloocimene, this material was collected (R_t, 6.6 to 8.8 minutes) and examined for intensity of ultraviolet absorption at 220 to 370 mμ. The spectrum for the collected sample was not indicative of alloocimene (*6*). A calculation of amount was made based on the absorbance at 273 mμ. With a value for an intensity of E_{mol} equal to 41,500 at 273 mμ for authentic alloocimene [30 percent *trans*-(C_4)-*trans*-(C_6), 70 percent *trans*-(C_4)-*cis*-(C_6)] and correcting for losses sustained in the collection, we determined that no more than 2.4 μg of alloocimene per 85-mm cigarette could be present in the smoke sample.

In several experiments we added a measured amount of authentic alloocimene (186 μg per cigarette) to the smoke traps before collecting the smoke, to establish the efficiency of our analytical procedure and to determine whether alloocimene was sufficiently stable in the presence of other smoke components. The added alloocimene was recovered from the gas-liquid chromatography to the extent of 86 to 89 percent based on peak area measurement. The *trans-trans* and *trans-cis* isomers emerged after 7.0 and 7.6 minutes, respectively, under the chromatographic conditions used.

The authentic sample of alloocimene

(*7*) contained, as measured by gas-liquid chromatography and ultraviolet, infrared (*6*) and mass spectrometry, a mixture of 30 percent *trans*-(C_4)-*trans*-(C_6)-alloocimene and 70 percent *trans*-(C_4)-*cis*-(C_6)-alloocimene (*8*).

The identification of dipentene was made on the basis of identical retention times (3.7 minutes) and mass spectra for the smoke isolates and for an authentic sample (*9*). The major ion fragment had a ratio of mass to charge of 68 which is typical of dipentene and distinguishes it from several of the isomeric terpenes (*10*).

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Radium-226, Radium-228, Lead-210, and Fluorine in Persons With Osteogenic Sarcoma

Abstract. *Concentrations of the naturally occurring alpha-emitting radioelements, radium-226, radium-228, and lead-210, and of stable lead and fluorine were determined in bone specimens from 32 individuals having a verified osteogenic sarcoma. Comparison of these results with those for the average person showed no significant differences in either the absorbed dose (rad) from the accumulated radioisotopes or in the concentrations of the elements studied.*

As part of a program to determine the toxicity to humans of internally deposited radioelements, we have studied the uptake and retention of Ra^{226}, Ra^{228}, Th^{228}, and Pb^{210} arising from the environment (*1, 2, 3*). Since large amounts of Ra^{226} or other bone-seeking radioelements have been shown to produce a high incidence of osteogenic sarcoma (*4*), it is conceivable that the normal natural environmental radiation may have a direct effect on the spontaneous incidence of this disease.

Natural levels of radium, however, are more than a factor of 10^3 lower than the levels known to be toxic and the range of the variations in dose is small. In addition, exposure is lifelong, and the dose is distributed more uniformly throughout the skeleton than that at the known toxic levels. In spite of these differences in deposition and dose, it has been presumed by some that the variation in natural radiation dose will result in a corresponding variation in the incidence of osteogenic sarcoma.

Naturally occurring radioisotopes which contribute significant radiation doses to bone include K^{40}, Ra^{226}, Ra^{228}, and Pb^{210}. The metabolism of K^{40} is such that there is little individual variation within a given age group (*5*). We have, therefore, compared the Ra^{226}, Ra^{228}, and Pb^{210} content of the bone from normal individuals with that in proven cases of osteogenic sarcoma. In addition, we have determined the concentrations of two stable bone-seeking elements, lead and fluorine.

The concentrations of these elements depend on the environmental concentrations. Radium-226 is acquired through both food and water, but since most of the world's population consumes water with no significant concentration of radium, food is the primary source. In these individuals the Ra^{226} concentration is low and the values span a relatively small range (*6*). In some local areas, however, the drinking water comes from wells which have a high Ra^{226} concentration, and individuals consuming this water may have a body burden ten or more times greater than those whose only source of radium is food (*1*). The Ra^{228} content of the body is similarly controlled, but because of the short half-life of the Ra^{228} (5.8 years) (*7*) the concentration in the body varies with age. Thorium-228, a decay product of Ra^{228}, is not appreciably absorbed from either food or water and hence its presence in bone is due to the decay of its parent, Ra^{228}. Fluoride, like radium, enters the body primarily through food and drinking water, whereas Pb^{210} and lead are acquired from both food and air (*3, 8*).

For this study undiseased bone was obtained from the area adjacent to the tumor. When received, the samples were cleaned of soft tissue and bone marrow, dried at 110°C, and stored in sealed polyethylene bags at a temperature of −27°C until analyzed. The Ra^{226}, Ra^{228}, Pb^{210}, stable lead and fluorine were determined as already described (*9*).

The samples chosen to represent bone from subjects with osteogenic sarcoma were specimens taken from bone removed in the treatment of this disease at the Mayo Clinic over the period of about a year. These subjects, with one exception, had been exposed only to natural environmental radium. The one exception, who had received the equivalent of an intravenous injection of about 400 μg of radium, has been excluded from this study and is discussed elsewhere (*10*).

An osteogenic sarcoma is defined as a malignant tumor, the malignant cells of which produce osteoid tissue even if in only small foci. These sarcomas may be classified as osteoblastic, chondroblastic, or fibroblastic depending on whether there is a predominance of osteoid, chondroid, or fibromatoid differentiation. Osteogenic sarcomas were graded from 1 to 4, the larger values corresponding to the greater degree of cellular dedifferentiation. The distribution of the grades of malignancy (1 to 4) in this series of 32 cases was 0, 22, 44, and 34 percent, respectively, which is very similar to the 1.6, 17.2, 54.4, and 26.7 percent, respectively, found in a series of 430 cases (*11*). There is a corresponding similarity in histologic types: osteoblastic, chondroblastic, and fibroblastic types were, respectively, 63, 12, and 25 percent in this series and 50, 27, and 23 percent in the larger series. The ratio of 19 males to 13 females, and the anatomical distribution of skeletal involvement are also typical for any group of cases of osteogenic sarcoma. The group of subjects selected for this study, therefore, appears to be a representative sample.

The measurements of the Ra^{226}, Ra^{228}, Pb^{210}, total Pb and F in bone from these individuals having verified osteogenic sarcomas are summarized in Table 1.