rather than by pollen mother cell wall retention. Exine material (sporopollenin) deposited in the shared tectum during wall development binds adjacent grains together. All pollen walls that exist as free surfaces demonstrate normal wall patterns and suggest that contiguity is a necessary requisite for cohesion. The events leading to the establishment of a shared tectum are being sought in an ontogenetic study.

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14 January 1963

Marihuana: Tetrahydrocannabinol and Related Compounds

Abstract. *Marihuana was analyzed for its major constituents, cannabidiolic acid, cannabidiol, tetrahydrocannabinol, and cannabinol, by treating the petroleum ether extract with diazomethane. The methyl esters so produced, together with the unchanged components, were subjected to gas chromatography on a polar silicone column.*

Some 20 years ago three major constituents of marihuana (cannabidiol, tetrahydrocannabinol and cannabinol) were separated and identified by research groups in the United States (*1*) and in England (*2*). At the same time theories were formulated about the relationship of these compounds to one another in the maturing marihuana plant (*3*).

Recently there has been renewed interest in marihuana as a result of three separate but related developments. The first (*4*) development was the isolation of a fourth major constituent, cannabidiolic acid (*5*), an antibacterial substance, which has been postulated as the biological precursor of both cannabidiol and tetrahydrocannabinol. The second was the use by criminals of an extract of marihuana plant on tobacco to reduce the ease with which illegal marihuana cigarets are recognized by law enforcement officers (*6*). The third was the possibility that detailed analyses of marihuana could be correlated with its origin and thus help control the international traffic in marihuana (*7*, *8*).

In this report a simple, rapid method of determining simultaneously, cannabidiolic acid, cannabidiol, tetrahydrocannabinol (the active component of marihuana), and cannabinol is presented; no such method has been described in the literature (*9*).

Gas chromatography was tried on petroleum ether extracts of marihuana but as expected, and in accordance with the work of others (*8*), cannabidiolic acid was not detected. High molecular weight organic acids, in general, are strongly associated and not amenable to gas chromatography as such. Therefore, the marihuana was extracted with petroleum ether (saturated with nitrogen gas). The petroleum ether was removed under partial vacuum in a stream of nitrogen and the residue was treated with a mild methylating agent, diazomethane (*10*). The resulting product was dissolved in anhydrous, peroxide-free, ethyl ether and subjected to gas chromatography in an argon ionization-gas chromatograph (Electronic Instruments Research) operated at 180°C, in which the flow of argon gas was 80 ml/min. A cyanoethyl silicone gum (General Electric 949) in 0.5 percent concentration on a 120 mesh silanized Chromosorb-W support was used as the column. Retention times are listed in Table 1 for the major components. The relative amounts of these components in certain samples of marihuana, red oil, and hashish are indicated (*11*).

The retention times were established for the identified components with known materials of high purity (*12*). The retention times for cannabidiol, tetrahydrocannabinol, and cannabinol were not affected by the diazomethane treatment and each of these components was recovered unchanged, after gas chromatography (*13*), as shown by the infra-red spectrums of the eluted material (*14*). Quantitative results can be obtained by integrating the areas under the peaks with a disc integrator calibrated with known compounds.

Table 1. Major components in marihuana from various sources. The number after the component is the retention time (in minutes from solvent emergence) on the chromatograph. The retention time of the methyl ester of cannabidiolic acid diacetate was 35.7. Relative contents: S, small; M, medium; L, large.

Hashish	Red oil*	Marihuana		
		America	Africa	Thailand
		Unknown A, 2.7		
S	S	S	S	S
		Unknown B, 7.1		
S	S	L	M	S
		Cannabidiol, 10.6		
L	L	S	S	
		Unknown C, 12.2		
		L	S	S
		Tetrahydrocannabinol 14.6		
L	L	L	L	L
		Cannabidiolic acid, methyl ester 17.4		
		S	S	L
		Cannabinol 24.8		
M	M	M	S	M
		Unknown D, 28.3		
S	S	S	S	S

* Red oil is a marihuana concentrate.

Quantitative variations among different marihuana samples from the same geographic region are often quite large and seem to depend on the fertility of the soil, the maturity of the plant when harvested, and the length of time between harvest and analysis. To avoid composition changes after receipt, all marihuana samples in this laboratory are kept at −18°C.

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References and Notes

1. Roger Adams and coworkers at the University of Illinois in the period 1940 to 1949 synthesized cannabinol, isolated cannabidiol, and produced tetrahydrocannabinol; tetrahydrocannabinol was isolated from a sample of marihuana resin by Wollner, Matchett, Levine, and Lowe in 1942.
2. A. R. Todd *et al.* at the University of Manchester, 1940 to 1943, synthesized cannabinol and prepared tetrahydrocannabinol.
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11. Tobacco samples containing marihuana resin showed, when subjected to this procedure, a characteristic marihuana chromatogram with two additional peaks at 1.7 minutes and 5.0 minutes.
12. I thank Dr. G. Hoffman of the Army Chemical Center Research Laboratory for the pure materials. He obtained the cannabidiolic acid diacetate from Dr. O. E. Schultz, Kiel, Germany. The results for this reference compound, which does not occur naturally in marihuana, are included in Table 1. I thank A. L. Mills for the petroleum ether extractions, and L. W. Haddaway for the diazomethane treatment.
13. M. Lerner, A. L. Mills, S. F. Mount, *J. Forensic Sci.* **8**, 126 (1963).
14. Methylation of the marihuana extract often produces a small peak with a retention time of 1.3 minutes in addition to the peak attributable to the methyl ester of cannabidiolic acid. Aside from these two peaks the marihuana chromatogram is unchanged by methylation.

21 February 1963

Strontium-90 Content of Deciduous Human Incisors

Abstract. *The concentrations of strontium-90 in deciduous incisor teeth of children born in St. Louis between 1949 to 1957 are in accord with estimated bone levels, suggesting that human deciduous teeth are useful as an index of strontium-90 accumulation during the time the teeth are formed.*

In 1958 Kalckar suggested that the radioactive content of deciduous teeth could be used as an index of the accumulation of radioactivity and the body burden of various nuclides in children (*1*). More recently, Reiss has presented preliminary data to indicate the feasibility of such measurements (*2*). We now present findings for the strontium-90 content of noncarious deciduous incisor teeth of children born in St. Louis during the years from 1949 through 1957.

Strontium-90 analyses were performed on ashed samples by the method developed by the New York Operations Office of the U.S. Atomic Energy Commission (*3*). For teeth of children born between 1949 and 1952, when strontium-90 concentrations were low, samples weighing approximately 10 g (70 incisor crowns) were used. The weight of the samples was successively decreased to 2 to 3 grams for the period 1955–57. The collection procedure, classification, and preparation of the teeth have been described (*2*). Pooled samples consisting of teeth from the first and last 6 months of each year were analyzed, but the data obtained for the entire year were averaged. Enamel and dentin were separated by the flotation method of Battistone and Burnett (*4*). To obtain sufficient material for enamel-dentin analysis, samples for the birth years 1952 and 1956 contained 400 and 150 teeth, respectively. Calcium determinations were performed on each sample by oxalate precipitation and permanganate titration.

Human incisor crowns develop during a 6-month prenatal and 5-month postnatal period (*2*), and at the birth date of the child the crown of the tooth is approximately 70 percent calcified (*5*). Figure 1 shows that the strontium-90 content of incisor teeth from bottle-fed children increased slowly between 1949 and 1953 but began to increase markedly between 1954 and 1955. The strontium-90 content between 1955 and 1957 continued to increase but at a slower rate than that for 1954 and 1955. The sharp increase in strontium-90 content of teeth between 1954 and 1955 coincides with a period of extensive nuclear testing begun in 1953. One factor which has not been adequately measured and which may account for the small amount of strontium-90 for teeth of children born before 1952 is that of external strontium-90 accretion during the 5 to 7 years the teeth remained in the body before they were shed. This factor, although presumably small, may account for part of the concentration (0.18 pc/g of calcium) in the teeth of children born in 1949.

Table 1 compares the strontium-90 distribution between the enamel and dentin of incisor crowns for teeth of children born in 1952 (three samples) and 1956 (five samples). Because there were no significant differences for strontium-90 distribution for these age groups, the data for the samples have been pooled. Enamel, which contains 38.5 percent of the tooth-crown calcium, contains a smaller percentage (32 percent) of the crown strontium-90, while dentin, containing 61.5 percent of the crown calcium, contains 68 percent of the crown strontium-90. The differences between strontium-90 content of enamel and dentin may be due to the continual formation of secondary dentin and to exchange with blood strontium-90 during a period of increasing strontium-90 concentration. Because many of the teeth had undergone various degrees of normal attrition, the dentin in these samples represents a somewhat greater proportion of the total mass of the crown than would be expected for crowns in which no enamel attrition had occurred. Nonetheless, the

Table 1. Distribution of calcium and strontium-90 between dentin and enamel of deciduous incisors. Values are given as percentage distribution of the substances in the entire crown on a dry weight basis. The ratios of 1.20 and 0.90 are significantly different from 1.00 with a probability of $\ll .01$.

Calcium	Sr^{90}	Ca/Sr^{90}
	Enamel	
38.5 ± 3.3	32.0 ± 3.5	1.20
	Dentin	
61.5 ± 3.3	68.0 ± 2.9	0.90

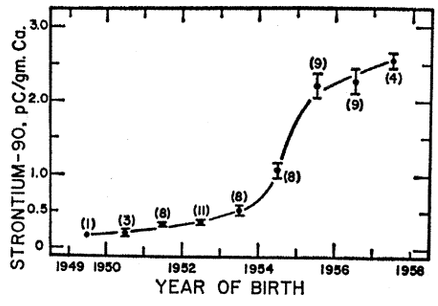

Fig. 1. Strontium-90 content of deciduous incisors from bottle-fed children versus year of birth. Vertical lines are standard error of the mean for number of samples in parentheses.

Table 2. Comparison of strontium-90 content of noncarious incisors from breast-fed and bottle-fed children born from 1951 to 1957. Values are average values ± standard error of the mean. Numbers of samples in parentheses. Differences between teeth of breast-fed and bottle-fed children for the years 1953–56 are significantly different at $P = <.01$. Values for bone are taken from published papers (*7*).

Strontium-90 (pc/g Ca)		
Teeth of children fed by		Bone
Breast	Bottle	
	1951	
0.27 ± 0.05 (3)	0.30 ± 0.03 (8)	0.28
	1952	
0.33 ± 0.02 (7)	0.36 ± 0.02 (11)	0.38
	1953	
0.43 ± 0.04 (7)	0.54 ± 0.03 (8)	0.56
	1954	
0.79 ± 0.05 (8)	1.04 ± 0.10 (8)	0.67
	1955	
1.30 ± 0.11 (9)	2.21 ± 0.18 (9)	1.04
	1956	
1.84 ± 0.21 (6)	2.26 ± 0.16 (9)	1.8
	1957	
	2.56 ± 0.11 (4)	2.1