Table 1. Analysis of variance on number of bar presses under the two treatments with trials pooled.

Source	df	MS	F
Between subjects	9	315.1	3.63
Between sessions	1	57.8	
Between treatments	1	5313.8	61.3*
Remainder	8	86.7	

*$p < .001$.

and Kjaer noise-measuring system, consisting of a condenser microphone cartridge type 4131, a cathode follower (type 2613), an audio spectrum analyzer (type 2109), and a level recorder, type 2304. The tape loop could be played back, through a power amplifier, and an Altec 604 speaker. A white-noise generator, Grason Stradler No. 455.3, could be switched into the system instead of the tape recorder, the output being 80 db relative to 0.002 dyne/cm^2. The speaker enclosure was mounted in such a way that the experimental box could be placed directly under the speaker.

The results of the experiment are shown in Fig. 1. The average number of times the bar was touched for white noise is roughly the same for both groups after the first two trials, and is consistently higher than that for squeals, which is also roughly the same for both groups. Group I, exposed initially to white noise, takes three trials to reach asymptote. The analysis of variance (Table 1) shows a significant difference between the "noise" and "squeal" treatments.

The results show clearly that there is no specific component in the squeal of a distressed rat which evokes what might be called altruistic behavior, when this behavior is defined as pressing a bar to stop the squeal. On the other hand, when the sound of white noise can be stopped by pressing a bar, rats learn to do this very quickly, and maintain a comparatively high level of responding. Therefore, the squeals and the white noise must be regarded simply as two sources of auditory stimulation, the latter giving rise to more behavioral activity than the former.

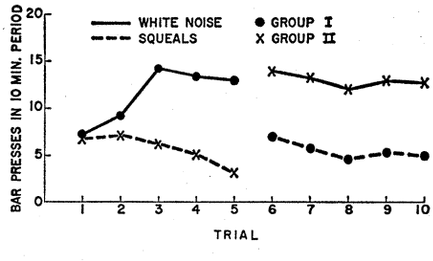

Fig. 1. Average number of bar presses in a 10-minute period, for two groups of rats, one group being exposed to white noise on five successive days followed by exposure to "distress" squeals on another five successive days, the other group being exposed to "distress" squeals, followed by white noise.

Further, the fact that the group exposed to white noise on the first five trials reaches asymptote only on the third trial suggests that this increased activity is directed rather than undirected. This might indicate that the stimuli are noxious in differing degrees. However, comparison between data from the present study and that of Rice and Gainer shows that the number of bar presses reported by the latter authors lies between those of the recorded squeals and the white noise. This adds support to the activation explanation, since increased arousal could be expected in the Rice and Gainer situation where squeals were provided by a wriggling rat (on a hoist), prodded by an experimenter, in a compartment very close to the bar. On the other hand, one would not expect the squeals to be more noxious simply because the experimenter and a live rat are present. Furthermore, in the Rice and Gainer situation, bar pressing did not suppress the presence of either the rat or the experimenter. The comparison is also more difficult to explain if the bar-pressing behavior is interpreted as altruistic, since, in that case, both situations with squeals—either live or recorded—should yield more frequent bar pressing than the white noise.

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18 February 1963

Nature of Cohesion within Pollen Tetrads of Typha latifolia

Abstract. *Pollen tetrads in* Typha latifolia *result from the fusion of the outermost portion of the exine (the tectum) where microspores within the meiotic tetrad are contiguous. Exine stratification is discussed.*

The electron microscope has been used successfully by a number of investigators (*1*) to study the nature of pollen exine stratification and fine structure. However, none has investigated pollen which remains in tetrads or polyads at maturity. Pollen tetrads and polyads are common in a number of families (*2*) and have been used in systematic treatments to separate genera and species (*3*); they have also been treated as phylogenetically advanced over monads (*4*). The genetic basis for pollen tetrad formation is little known, though Levan (*5*) has reported a *Petunia* line containing a recessive gene for pollen tetrad formation. In an attempt to obtain an insight into the nature of cohesion in pollen tetrads a sample of *Typha latifolia* L. pollen was obtained for study. Wodehouse (*6*) has reported that pollen tetrads of *T. latifolia* are generally of the isobilateral or rhomboidal types with a number of other orientations occurring. Wodehouse characterized the ornamentation of *T. latifolia* pollen grains as finely reticulate, with the reticulate pattern present on all surfaces including those involved in cohesion. While Wodehouse was not able to discern the presence of columellae supporting the ornamentation, Erdtman (*7*) indicates that columellae are present in *T. capensis*.

Pollen of *T. latifolia* was collected fresh and placed in 70-percent ethanol for 24 hours to clean pollen surfaces of oil droplets and remnants of tapetal materials. The cleaned sample was divided into two portions. The first was stained by 1-percent OsO_4 at room temperature for 2 hours; the second portion was acetylated (heated to 100°C in a mixture of 9 parts acetic

Table 1. Exine thicknesses of *Typha latifolia* pollen. Thicknesses are averages of representative wall areas, as measured from electron micrographs.)

Component	Thickness (μ)
Free-wall surface	
Tectum (including spinules)	0.6
Columellae	.2
Foot layer	.45
Endexine	.1 or less
Cohesion surface	
Shared tectum	0.14
Columellae	Barely perceptible
Foot layer	.15
Endexine	.1 or less

anhydride to 1 part concentrated sulfuric acid), washed, and stained in 2-percent $KMnO_4$ for 2 hours at room temperature. Both portions were dehydrated through a graded alcohol series and embedded in Araldite casting resin M. Sections were cut with a diamond knife and observations and micrographs were made with an RCA electron microscope model EMU 3-D. To complement electron microscopy, optical observations were made of fuchsin-stained, acetylated pollen tetrads in glycerine-water mounts.

As seen in the light microscope, pollen tetrads of *T. latifolia* are not consistently of isobilateral or rhomboidal form (*8*); approximately 17-percent of the tetrads in our sample were of linear, decussate, T-shaped, and asymmetrical form. Exine ornamentation is reticulate, muri surfaces appear smooth, and in linear tetrads ornamentation modifications on presumptive cohesion surfaces were not observed. Each grain in the tetrad had a single pore oriented randomly on exposed surfaces. In the light microscope, pore and exine stratifications are difficult to analyze.

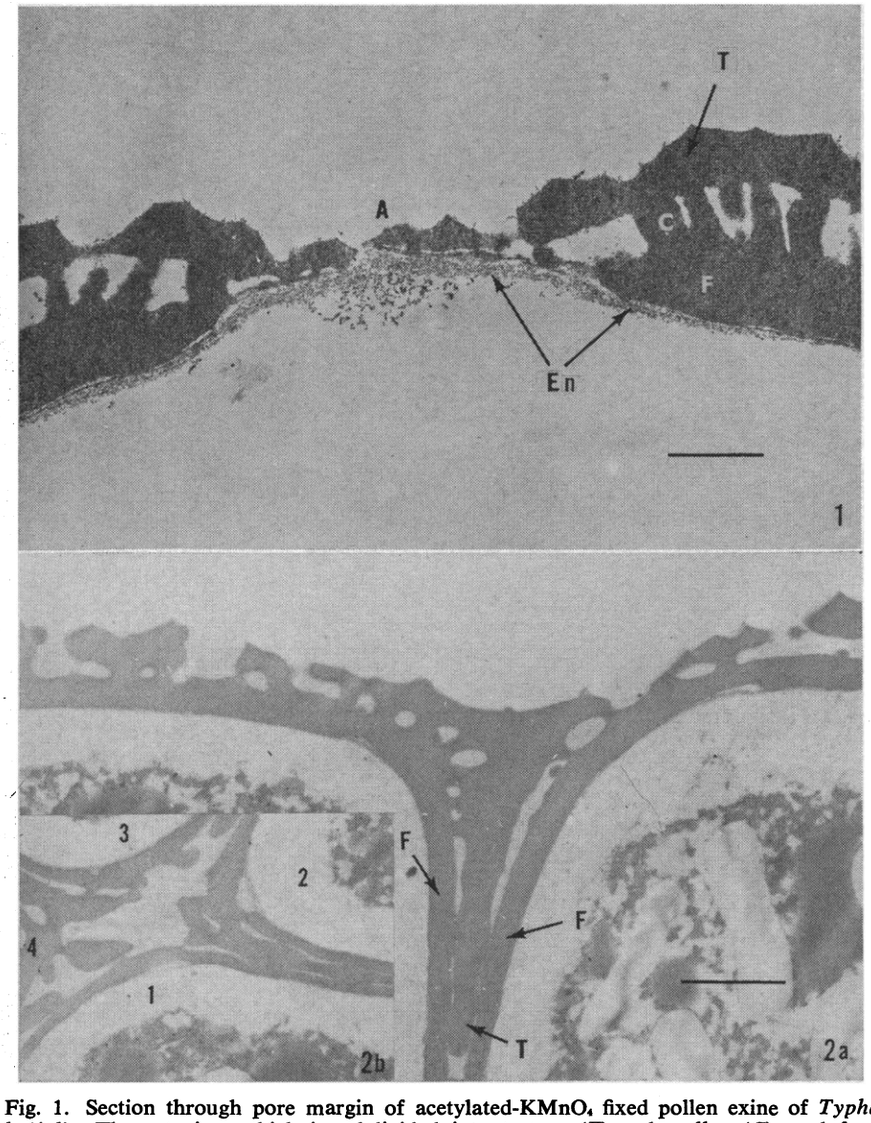

Fig. 1. Section through pore margin of acetylated-$KMnO_4$ fixed pollen exine of *Typha latifolia*. The ectexine, which is subdivided into tectum (*T*), columellae (*C*), and foot layer (*F*), is well developed on the aperture flanks and highly reduced over the aperture proper (*A*). The endexine (*En*) is shown to increase in thickness into the pore (× 13,000). Fig. 2*a*. Section through portions of two members of a *T. latifolia* pollen tetrad demonstrating cohesion. The shared tectum (*T*) and thinned foot layer (*F*) are characteristic of the cohesion surfaces. Note that the columellae are greatly reduced. Fixation of OsO_4 (× 13,200). Fig. 2*b*. Section through center of OsO_4-fixed pollen grain tetrad of *T. latifolia*, showing the four pollen grains (numbered 1 to 4) and the relationship of the shared tecta. Note lack of total fusion (× 13,200). Line equals 1 μ, in both figures.

Electron micrographs of acetylated pollen tetrads of *T. latifolia* demonstrate that the exine is composed of an ectexine whose structure consists of (i) a tectum forming the reticulate pattern, (ii) columellae which support the tectum, and (iii) a thick foot layer (Fig. 1), and beneath this well-developed ectexine, an extremely thin, fine-granular endexine. Similar exine stratification has been reported for *Zeo mays* pollen (*9*). The exine on exposed surfaces is considerably thicker than on cohesion surfaces (Table 1). The tectum itself is ornamented with fine spinules on noncohesion surfaces.

The ectexine often displays a gradual thickening in the regions adjoining the pore; however, it decreases rapidly in thickness at the pore margin (Fig. 1). Over the pore proper the ectexine is represented by a thin foot layer with an occasional insula supported by columellae. The endexine thickens underneath the thinning ectexine to become the major exine layer of the pore membrane. In nonacetylated grains the intine can be seen to enlarge considerably below the pore.

Cohesion of pollen grains in the tetrads results from the sharing of a common tectum along fusion surfaces (Fig. 2). At the very margin of fusion surfaces the tectum also exhibits cohesion often in the form of a relatively massive unit. As seen in cross sections, the reticulate pattern is continuous on the fusion surfaces but columellae are extremely reduced in height, and, as indicated in Table 1, total exine thickness of the fused walls is reduced to 0.54 μ; this reduction is most apparent on extensive internal surfaces. Exine thickening increases as exposed surfaces are achieved. In electron micrographs of linear tetrads no indication of presumptive fusion surfaces or breakage have been noted.

Our opinion, derived from the data available, is that variations in pollen tetrad form are better explained on the basis of spindle orientations during meiosis II and the resultant patterns of microspore position rather than on chance disruption of isobilateral microspore tetrads. Cohesion is due to the possession of a common exine layer by contiguous grains within the tetrad

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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