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9. Partly supported by a Grass Foundation Fellowship to J.E.B., by grant 5 RO1 NB-06251-02, by USAF contract AF 33(615)-3885, by Bell Telephone Laboratories, Inc., and by ONR (Nonr 3962(22)). We thank Prof. Ali Javan for his kindness and assistance in the laser work. Part of the research was carried out at the Marine Biological Laboratory, Woods Hole, Mass.

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Chemical Modification of Yeast Alanine-tRNA with a Radioactive Carbodiimide

Abstract. *Yeast alanine-tRNA was reacted with 1-cyclohexyl-3-[2-morpholinyl-(4)-ethyl] carbodiimide ^{14}C-methoiodide in the presence of magnesium ion. The carbodiimide formed addition products with bases in the sequences ψpGp, UpCp and UpUpIpGpCp. The expected bases in the sequence TpψpCpGpApUp did not react, although this region is postulated to be in a loop that is not hydrogen-bonded. The capacity of the alanine-tRNA to accept amino acids decreased after reaction with the carbodiimide.*

Although the primary sequences of several transfer RNA molecules have been determined (*1*, *2*), the secondary and tertiary structures of these molecules and the relationship which these structures have to biological activity need further investigation. For this reason, a tRNA (*3*) of known sequence —yeast alanine-tRNA—was treated with 1-cyclohexyl-3-[2-morpholinyl-(4)-ethyl]carbodiimide methoiodide (CM CMI), a reagent which reacts with the bases Up, ψp, Gp, and Ip, but not with Ap and Cp (*4*). The reactivity of the CMCMI with these bases in the intact tRNA molecule depends upon the conformation in the presence of Mg^{++} (*5*). The reacted tRNA was digested with pancreatic ribonuclease, which normally cleaves polyribonucleotides at the 3′-phosphoryl bond of the pyrimidine bases, and ribonuclease T_1, which cleaves at the 3′ phosphoryl bond of the bases G and I; two-dimensional oligonucleotide patterns (fingerprints) were made of the digests. Since the CMCMI derivatives of bases are not susceptible to the action of pancreatic ribonuclease (*6*), the oligonucleotides containing CMCMI could be located by their new positions in the fingerprint as well as by their radioactivity. We also measured the effect which the chemical modification of these bases had on the ability of the tRNA to accept alanine.

The CMCMI was synthesized by treating excess 1-cyclohexyl-3-[2-morpholinyl-(4)-ethyl]carbodiimide (redistilled, Aldrich Chemical Co.) with ^{14}C-methyl iodide (New England Nuclear Corp.). The CMCMI was then crystallized from a mixture of acetone and ether. The purified alanine-tRNA was obtained by the method of Apgar *et al.* (*7*). The purity of the alanine-tRNA is thought to be greater than 80 percent (*8*).

In one series of experiments, mixed yeast tRNA (General Biochemicals Corp.) was treated with the radioactive CMCMI under the following conditions: 25 mg of CMCMI, and 2.5 mg of tRNA per milliliter in 0.01*M* borate, *p*H 8.0, and 0.02*M* $MgCl_2$. The reaction was stopped by precipitating the tRNA with two volumes of cold 95 percent ethanol.

To determine the quantity of ^{14}C-CMCMI attached to the tRNA, 20-μl portions of an aqueous solution of the precipitate were spotted onto washed Whatman DE 81 paper and eluted with *p*H 1.9 buffer (*9*) for 3 hours to remove excess reagent. The paper was dried; the spots were cut out, placed in scintillation vials, moistened with seven drops of NE219 liquid scintillator (Nuclear Enterprises), and counted on an Ansitron scintillation counter.

At 38°C (Fig. 1) nearly 2 moles of CMCMI attach per molecule of tRNA in the first 60 seconds. The reaction then slows down so that after 8 to 10 hours 6 to 7 moles of reagent have

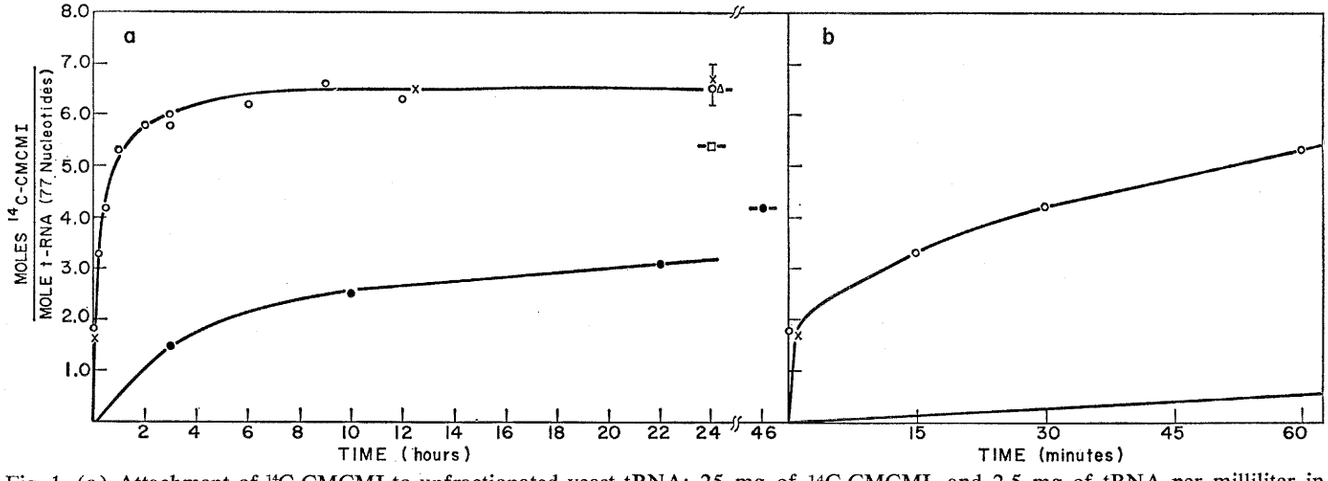

Fig. 1. (a) Attachment of ^{14}C-CMCMI to unfractionated yeast tRNA; 25 mg of ^{14}C-CMCMI, and 2.5 mg of tRNA per milliliter in 0.01*M* borate, *p*H 8.0, 0.02*M* $MgCl_2$. Solid circles, 2°C; open squares, 16°C; open triangles, 30°C; open circles 38°C. Attachment of ^{14}C-CMCMI to alanine-tRNA (conditions as above); crosses, 38°C. (The 24-hour point of alanine-tRNA is average of four experiments at 38°C, the range being indicated by the extent of the vertical lines.) (b) Expansion of the 0 to 1-hour segment of Fig. 1a.

attached to each molecule of tRNA, and this level of attachment persists for the remainder of a 24-hour period. Addition of fresh reagent at 9 hours does not result in further reaction. At 2°C the reaction proceeds more slowly and continues to increase throughout the remainder of a 46-hour period.

When purified alanine-tRNA is treated under the same conditions at 38°C the results are the same as those for the mixed yeast tRNA (Fig. 1). One might speculate that, as far as reactivity toward this reagent is concerned, both the mixed and the alanine tRNA's have common structural features.

The CMCMI-alanine-tRNA samples that were used for subsequent fingerprinting and measurements of biological activity were prepared in a manner different from the procedure used for mixed tRNA. After precipitation with ethanol, the reacted tRNA was passed through a column (0.9 by 25 cm) of SE-SEPHADEX C-50 equilibrated with 0.2*M* acetate buffer, *p*H 4.6, to remove the excess reagent. The tRNA fractions were then pooled and dialyzed overnight against water at 2°C. There was no reversal of ^{14}C-CMCMI attachment during dialysis.

The ^{14}C-CMCMI-alanine-tRNA was digested simultaneously with pancreatic and T_1 ribonuclease for 90 minutes in 0.1*M* borate buffer, *p*H 7.5, at 38°C. The digests were fingerprinted on Whatman 3MM paper by electrophoresis in one direction in *p*H 1.9 buffer (*9*) and by ascending chromatography in the other direction in a *t*-butanol, *p*H 1.9 buffer system (1:1). The fingerprints were then radioautographed; Ansco nonscreen x-ray film was used.

Figure 2 shows the radioautograph and contact print of the fingerprint of the "24-hour" sample of ^{14}C-CMCMI-alanine-tRNA. The most noticeable differences with the fingerprint of unreacted alanine-tRNA are the appearance of three new oligonucleotide spots (Fig. 2a, white arrows) and the lessening in intensity of the spot that normally contains a mixture of ψp and Ip in the upper right portion of the fingerprint (Fig. 2a, ψp spot). The three new oligonucleotide spots correspond exactly to the three darkest spots in the radioautograph (Fig. 2b, black arrows), an indication that these oligonucleotides do have the radioactive reagent attached to them.

An examination of the radioautograph of the fingerprint of the "60-second" sample of ^{14}C-CMCMI-alanine-tRNA shows a different pattern of prominent spots. Five spots are clearly visible in the "60-second" radioautograph, the darkest of which are spots *X* and *Y* (Fig. 2c). Thus oligonucleotides X and Y are the first to react in the molecule.

The compositions of the oligonucleotides were determined by alkaline hydrolysis, a procedure which removes the CMCMI and hydrolyzes the oligonucleotides to mononucleotides. These compositions are shown in Fig. 2c; quantitative values as determined by ultraviolet absorption or radioactivity estimation, or both, are given in Table 1. The spots were eluted from the paper and the ultraviolet absorptions of the eluates were read at 260 mμ in a Zeiss spectrophotometer.

The sequence UpCp is given in that order since only Up reacts and would thus not be hydrolyzed by pancreatic ribonuclease. The sequence ψpGp is given in that order since ψp and Gp appear together in the molecule only in the sequence MeIpψpGp. The sequence Ap,?,Cp cannot be fully determined because after alkaline hydrolysis some of the mononucleotides appear to be recovered as breakdown products. The position of this oligonucleotide in the fingerprint indicates that it is at least a trinucleotide.

The ψp spot in the upper right portion of the fingerprint contains a mix-

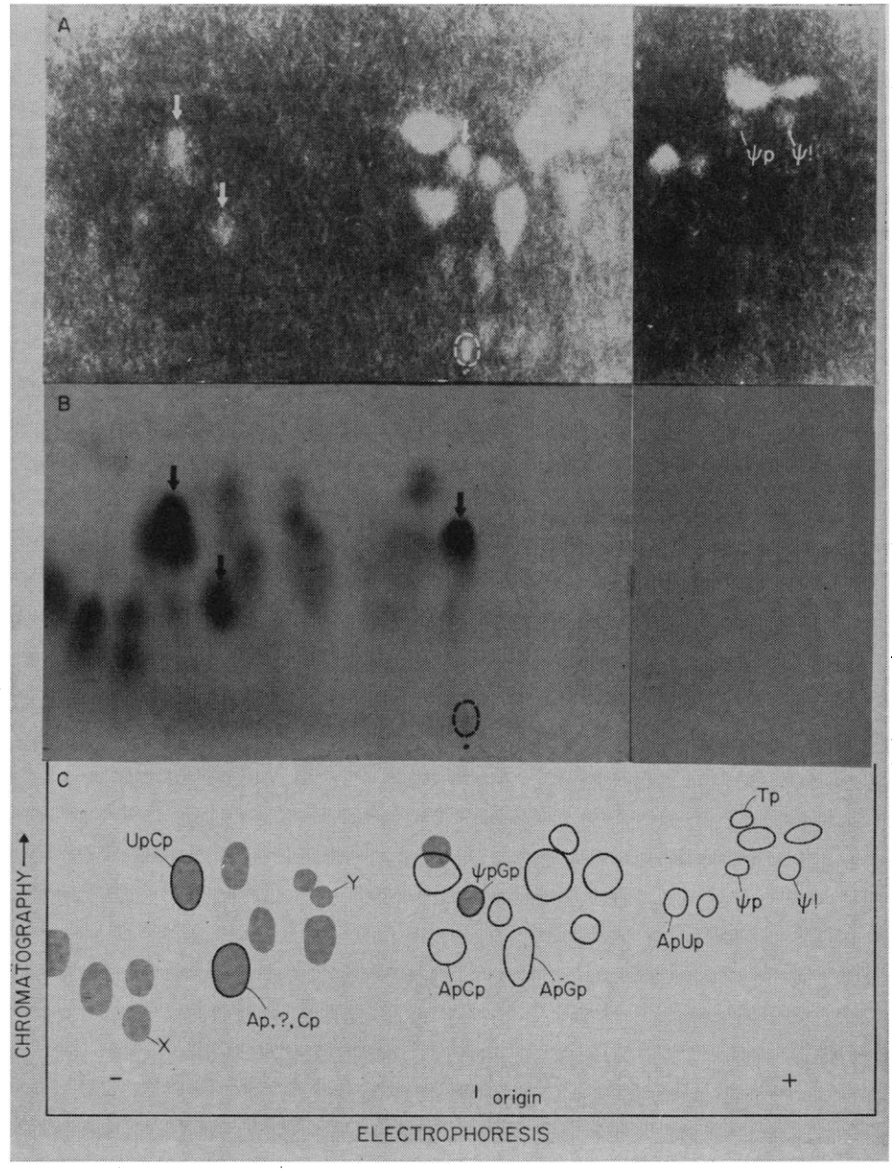

Fig. 2. Nucleotide pattern (fingerprint) of ^{14}C-CMCMI-alanine-tRNA (6 to 7 moles of ^{14}C-CMCMI per mole of tRNA) digested simultaneously with T_1 and pancreatic ribonucleases. (A) Contact print showing ultraviolet-absorbing spots; (B) radioautograph; (C) composite tracing of A and B.

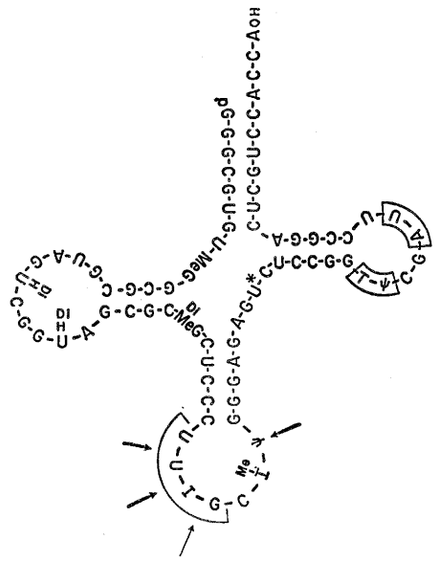

Fig. 3. The sequence of yeast alanine-tRNA arranged in the cloverleaf structure proposed by Holley (*1*). The arrows point to the areas of the sequence which react with ^{14}C-CMCMI. The bases enclosed in boxes do not react.

ture of ψp and Ip in the digest of unreacted alanine-tRNA. In the digest of the "24-hour" sample of CMCMI-alanine-tRNA, the Ip is no longer present in that spot. Ip is found, however, in the alkaline hydrolyzate of the combined minor radioactive spots, including *X* and *Y* (Fig. 2c). Therefore, either Ip or the bases near Ip have apparently reacted.

There are two moles of ψp per molecule of yeast alanine-tRNA. The ψp from the sequence TpψpCpGp appears as free ψp after digestion of alanine-tRNA with the two ribonucleases. In the CMCMI alanine-tRNA, this free ψp is present in the usual place in the fingerprint (Fig. 2) and in the normal amount (Table 1). The ψp from MeIpψpGp appears only in the dinucleotide MeIpψp in alanine-tRNA owing to the inability of ribonuclease T_1 to hydrolyze bonds after MeIp. The attachment of CMCMI to the ψp apparently enables this bond to be hydrolyzed in the CMCMI-alanine-tRNA so that this ψp appears in the dinucleotide ψpGp.

In addition to the free ψp, Tp and ApUp are present in the usual places in the fingerprint (Fig. 2) and in the normal amounts (Table 1), an indication that the TpψpCpGpApUp sequence does not react with CMCMI although Gp,Up,Tp, and ψp themselves would be expected to react, if available. This result is consistent with the enzymatic studies of Armstrong *et al.* (*10*) which showed that Tp and ψp are less susceptible to the action of pancreatic ribonuclease than expected. It is also consistent with the work of Nelson and Holley (*11*) which showed that the TpψpCpGpApUp sequence is less susceptible than other areas of the molecule to bromination.

The ^{14}C-CMCMI-alanine-tRNA was also digested with pancreatic ribonuclease alone and fingerprinted; most of the radioactivity remained at the origin. When the origin spot was digested with T_1 ribonuclease and fingerprinted again, both the UpCp and ψpGp spots appeared in the radioautograph. Thus, at least some of the UpCp must come from GpUpCp.

Only 1.8 moles of ^{14}C-CMCMI are recovered in the three major spots (Table 1). Some of the other 4 to 5 moles of reagent are attached to the sequence UpUpIpGpCp, and the rest are probably generally distributed. Reaction of ^{14}C-CMCMI with the sequence UpUpIpGpCp could result in more than one radioactive spot since one or more of the bases in that sequence could react.

The assay for the acceptor activity has already been described (*8*). The acceptor activity of the alanine-tRNA decreases as more CMCMI is attached. After 60 seconds of reaction, the molecule retains 71 ± 4 percent of its ability to accept alanine, as compared with a control sample treated in a similar manner. After 24 hours of reaction only 51 ± 5 percent of its activity remains.

Figure 3 shows the proposed cloverleaf structure of alanine-tRNA. It is evident that the CMCMI attaches to bases in the lower loop which contains the sequences UpUpIpGpCp and ψpGp. However, the right loop containing TpψpCpGpApUp does not seem to react at all. If it is not hydrogen-bonded, as proposed by this model, then one would have to modify the model to explain why the reagent does not react with this area. Perhaps this loop is buried or folded over onto the left loop.

The source of Ap,?,Cp can only be guessed at. Since ApCp and ApUp are present in normal quantities, the Ap,?,Cp probably comes from one or more of the following sequences: ApGpdiHUp Cp, diHUpApGpCp, and ApGpUp*Cp (*12*).

Thus, certain sequences in the alanine-tRNA molecule, including the sequence UpUpIpGpCpMeIpψpGp, are more exposed and available to reaction with CMCMI than other sequences, notably TpψpCpGpApUp. The exposed nature of the IpGpCp region—the proposed anticodon region—of the molecule is consistent with the enzymatic studies of Penswick and Holley (*13*) and Armstrong *et al.* (*10*). It is also evident that the modification of bases in the exposed regions hinders the ability of the molecule to accept amino acids.

Table 1. Quantitative values of spots eluted from a fingerprint of CMCMI-alanine-tRNA (6 to 7 moles of CMCMI per mole of alanine-tRNA) digested simultaneously with both pancreatic and T_1 ribonucleases. The number of millimicromoles (ultraviolet) of the oligonucleotides and mononucleotides were calculated from the optical density measurements of the spots, with the use of extinction coefficients of Stanley and Bock (*14*). For radioactivity estimation, portions of the radioactive oligonucleotides were spotted on planchets and counted on a low-background counter. The number of millimicromoles of the CMCMI-oligonucleotides (radioactivity) was calculated from the molar-specific radioactivity of the CMCMI. The ratios of the number of millimicromoles of the mono- and oligonucleotides to the number of millimicromoles of tRNA were determined by dividing the former by the number of millimicromoles of TP (ultraviolet), since there is one Tp per alanine-tRNA.

Sequence	Yields (mμ moles)		Ratios of No. of moles to No. of moles of tRNA		Ratio of expected No. moles to moles tRNA
	Ultraviolet	Radioactivity	Ultraviolet	Radioactivity	
		CMCMI-oligonucleotides			
UpCp	21.0	21.8	1.1	1.1	
Ap,?,Cp	(5.9)*	6.2	(0.3)*	0.3	
ψpGp	7.5	6.0	0.4	0.3	
		Oligonucleotides, mononucleotides			
ApGp	75.3		3.9		4.0
ApCp	40.1		2.1		2.0
ApUp	27.7		1.4		1.0†
Tp	18.5		1.0		1.0
ψp+ψ!	19.3		1.0		1.0

*The number of millimicromoles of Ap,?,Cp was calculated from the ultraviolet data by using an approximate molar extinction coefficient of 30×10^3, which is appropriate for a trinucleotide. †ApUp is always present in more than the expected ratio of 1.0 mole per mole of alanine-tRNA (*8*, *15*).

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3. Abbreviations: Ap, adenosine 3′-phosphate; Cp, cytidine 3′-phosphate; DiHUp, 5,6 dihydrouridine 3′-phosphate; DiMeGp, N^2-dimethylguanosine 3′-phosphate; Ip, inosine 3′-phosphate; MeGp, 1-methylgaunosine 3′-phosphate; MeIp, 1-methylinosine 3′-phosphate; ψp, pseudouridine 3′-phosphate; ψ!, pseudouridine 2′, 3′ cyclic phosphate; Tp, ribothymidine 3′-phosphate; tRNA, transfer RNA; Up, uridine 3′-phosphate.
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24 July 1967

2,5-Dimethoxy-4-methyl-amphetamine (STP): A New Hallucinogenic Drug

Abstract. *We have assessed the effects in normal control volunteers of 2,5-dimethoxy-4-methyl-amphetamine, the chemical present in the hallucinogenic drug STP, in two independent trials. In low doses, this compound produces a mild euphoria. Doses greater than 3 milligrams may cause pronounced hallucinogenic effects lasting about 8 hours and similar to those produced by hallucinogenic doses of lysergic acid diethylamide, mescaline, and psilocybin. 2,5-Dimethoxy-4-methyl-amphetamine, which is chemically related to mescaline and amphetamine, is about 100 times more potent as a hallucinogen than mescaline and only one-thirtieth as potent as lysergic acid diethylamide. Its psychological effects are not accentuated by chlorpromazine.*

A new hallucinogenic drug, STP, has been used extensively by "hippie" populations in recent months. There have been reports that this drug produces hallucinogenic reactions lasting up to 72 hours and that these reactions are intensified by chlorpromazine, the tranquilizer commonly used as an antidote to other hallucinogens. Chemists at the U.S. Food and Drug Administration have identified black-market preparations of STP as identical with DOM (2,5-dimethoxy-4-methyl-amphetamine) and have estimated that black-market preparations of this drug contain about 10 mg of DOM in each pill (*1*). To clarify the effects of DOM in man, we have, at the request of the Food and Drug Administration, examined the physical and mental actions of DOM in normal control volunteers. Two independent studies have been performed, one at the Johns Hopkins Hospital in Baltimore and one at the Veterans Administration Hospital in Palo Alto, California.

Five normal control male volunteers aged 21 to 35 were obtained through the office of financial aid at the Johns Hopkins University. They were interviewed by an experienced clinical psychiatrist (L.F.) and were administered a Minnesota Multiphasic Personality inventory and a Thematic Apperception Scale. Applicants with a history of frequent use of marijuana or other mental stimulants were rejected. Before receiving the drug, each subject was given a physical examination, chest x-ray, electrocardiogram, electroencephalogram, tests of blood chemistry, and hematological tests. Subjects were told that they would receive a drug called DOM, which was presumably identical to the hallucinogen STP, but that their dose would be considerably smaller than that thought to occur in STP tablets.

At 9 a.m., after fasting since the preceding midnight, subjects received DOM at dosages of 2.0, 2.4, 2.4, 2.8, and 3.2 mg as the hydrochloride dissolved in distilled water. Before receiving the drug and 2, 4, and 6 hours thereafter, tests of free recall, associative organization, subjective mood scales, and an LSD symptom specific scale were administered. Results of these tests will be reported separately (*2*). Urine collections before administration of the drug and 3, 6, 9, and 24 hours thereafter were assayed for unchanged DOM by a specific and sensitive spectrophotofluorometric method (*3*). At hourly intervals, pulse, blood pressure, oral temperature, and pupillary diameter were measured. Interviews with subjects were tape-recorded at various intervals.

Pupillary dilatation of about 15 percent occurred between 1 and 6 hours after administration of the drug. Pulse rate increased (mean increase of 15 beats per minute) as did systolic blood pressure (mean increase of 15 mm-Hg) between 1 and 6 hours, with a maximum effect at 4 hours; however, diastolic blood pressure was unaffected. The mean oral temperature had increased by a maximum of 1.2°F 4 hours after administration of the drug.

Certain features were common to the experiences of all five subjects. Subjective effects began between 1 and 2 hours after administration of the drug, with a peak between 3 and 5 hours and subsidence of effects by 7 to 8 hours. All subjects initially experienced a moderate euphoria. Perceptual effects varied but were present to some degree in all.

The subject who received 2.0 mg had the mildest reaction. He began to feel "a little high" 2 hours after taking the drug. At 3 hours he felt "a little weird; there is like a blank space between my head and body." Four hours after taking the drug, he felt normal and described his experience, "I felt good, more than usual. With my eyes closed I was pretty relaxed, and there was lots of visual imagery while listening to music. . . . It is like a half-way decent pot experience."

The three subjects who received 2.4 and 2.8 mg experienced effects similar to those described above, but the maximum effects lasted until 5 hours after the drug was taken; the effects had subsided by 8 hours. One of the subjects who received 2.4 mg first felt a change 1.5 hours after taking the drug, when he noticed that things on the wall were slightly shifting and surfaces were rippling. He described it, "Things were creeping, waving, a sort of corrugated effect. . . . I felt a little lightheaded and high. . . . Sometimes the ceiling had patterns of dots which turned into real faces . . . and when I closed my eyes it was like dreaming