range, with most of the means reported being between 400 and 600 μg per 24 hours for control subjects (*1*). Our metabolic balance study indicated a positive balance of 5.1 mg or 38 percent retention. Most reported zinc balance studies indicate that normal adults are in near equilibrium (*1*). However, no definitive conclusion can be drawn as to whether this condition of hyperzincemia is accompanied by an abnormal zinc absorption since the zinc balance study lasted only 8 days. Conclusions must await additional balance studies on the propositus as well as his siblings.

There is a strong indication that the zinc binding capacity of one or more of the serum proteins is increased since the propositus has normal concentrations of the major zinc binding proteins—albumin, α_2-macroglobulin, and transferrin (*7*), as well as total protein. The increase of the plasma zinc after the oyster meal and the absence of any ill effects from it demonstrates that the binding capacity of the serum proteins is not saturated under usual circumstances even though they are binding three to four times normal quantities of zinc. The possibility that the subject has an uncharacterized protein with the capacity to bind excess zinc could not be supported by electrophoretic studies, which indicated normal serum protein patterns. However, protein fractionation with ammonium sulfate indicated that the excess zinc was present in the precipitate which consisted mainly of albumin. These data suggest that most of the excess zinc is being bound by albumin.

Genetically, the familial condition appears in five of the seven members tested in one generation and was present in the second generation (Fig. 1). Therefore, it appears to be inherited as an autosomal dominant factor that is not sex-linked. Both parents of the propositus are deceased as well as 13 of the mother's siblings. However, five paternal sisters and their offspring are living and are being evaluated.

Whether this condition is accompanied by metabolic defects not yet apparent remains to be determined. We recommend that when extremely elevated plasma zinc values are noted, they should not be casually dismissed as sample contamination since it is possible that heritable hyperzincemia exists in other families.

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6. Extreme precautions were taken to exclude sample contamination. We used all plastic syringes for blood collection and glass- and plasticware was soaked in 1 percent disodium ethylenediamine tetraacetate and rinsed thoroughly with deionized water.
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Markers for Detection of Supplementation in Narcotic Programs—Deuterium-Labeled Methadone

Abstract. *Specific deuterium labeling of methadone and use of gas chromatography–mass spectroscopy technique permits rapid and quantitative determination of the ratio of the labeled to unlabeled drug in body fluids. A trideuteriomethadone (methadone-d_3) was shown to have exactly the same analgesic activity and toxicity in mice as methadone. The rates of absorption, distribution, and excretion of methadone-d_3 and methadone were identical in rats. These observations suggest that methadone-d_3 may be used as an in vivo marker for monitoring methadone intake of patients, and thus may improve the effectiveness of methadone treatment programs.*

Methadone is widely used in well-organized treatment programs for heroin addiction (*1*). One of the significant problems in the methadone treatment programs is that the therapist has no idea whether the patient is taking his methadone as prescribed, or supplementing his dose with the same drug obtained illegally. No current method of analysis permits this determination. The routine urine screening is only qualitative since the sample from a single voiding is analyzed rather than a sample from the 24-hour excretion. The use of creatinine or some other markers would not solve the problem since the excretion profile of these substances in the urine does not follow the excretion profile of methadone or its metabolites. However, if a deuterium-labeled analog of the drug which is indistinguishable metabolically from methadone can be prepared, the use of a gas chromatography–mass spectroscopy (GC-MS) technique would provide a simple, sensitive, specific, and reasonably inexpensive method for the detection of illicit supplementation. We now report the development of just such a method.

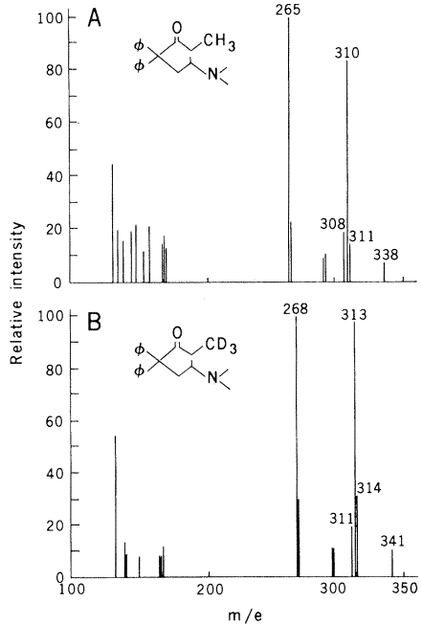

Fig. 1. Chemical ionization mass spectrum of (A) methadone and (B) methadone-d_3. The data were recorded on a Biospect mass spectrometer; methane was used as the ionization gas via the direct probe mode; the probe temperature was 250°C. The quasi-molecular ions (M + 1) of methadone and methadone-d_3 are at 310 and 313, respectively; m/e, ratio of mass to charge.

In designing a stable isotope-labeled methadone, we decided to replace the hydrogen atoms at C-1 with deuterium atoms. The C-1 position of methadone is the least crucial in terms of its pharmacological properties (*2*). Methadone-d_3 (*dl*-[1,1,1,-$^2H_3$]methadone) was prepared by a modification of the published procedure (*3*). First, methadone-d_5 (*dl*-[1,1,1,2,2,-$^2H_5$]methadone) was obtained by reacting the Grignard solution prepared from magnesium and ethyl bromide-d_5 (Merck Sharp and Dohme, Montreal; isotopic purity > 99 percent) with *dl*-γ-dimethyl-α,α-diphenylvaleronitrile. Conversion of methadone-d_5 to methadone-d_3 was achieved by ex-

Table 1. Isotopic ratios (methadone-d_3 and methadone) of urines and tissues of four rats as determined by gas chromatography–mass spectroscopy (GC-MS). Rats 1, 2, and 3 were killed 3 hours after subcutaneous injection (10 mg/kg; 0.2 ml in 0.9 percent saline) and rat 4 was killed after oral administration (50 mg/kg; 0.4 ml of distilled water) of a mixture of methadone-d_3 and methadone (1 : 1). Major organs and urines were isolated, and unchanged methadone-d_3 and methadone were extracted (*5*). This extract was purified by thin-layer chromatography before GC-MS analysis by two solvent systems: (i) chloroform and (ii) ethyl acetate, *n*-butanol, ethanol, and ammonium hydroxide (70 : 10 : 15 : 1). The drug ratios were determined as the ratio of the cumulative ion current of mass fragment 313 (methadone-d_3) relative to that of 310 (methadone) on a mass spectrometer (Biospect) with repetitive scan (*m/e* 306 to 315). The drug mixture was separated on a 3 percent SE-30 Chromosorb W column (1.8 m) at 235°C. The retention times of methadone-d_3 and methadone are 2 minutes 20 seconds and 2 minutes 21.5 seconds, respectively. The areas under the mass fragment 313 and 310 were integrated manually with the use of a planometer. The extracts from the site of injection or the injection solution were used as the reference standards.

Tissues*	Isotopic ratios							
	Rat 1		Rat 2		Rat 3		Rat 4	
	Sample	Standard	Sample	Standard	Sample	Standard	Sample	Standard
Urine	0.92	0.91	0.97	0.96	0.97	0.97	1.11	1.11
Spleen	1.01	0.99	0.98	0.97	1.02	1.02	1.10	1.11
Liver	0.98	0.96	0.97	0.96	0.97	0.97	1.10	1.11
Lung	0.96	0.94	0.97	0.96	0.98	0.97	1.12	1.14
Kidney	0.96	0.94	0.96	0.96	0.98	0.97	1.10	1.11
Heart	0.96	0.94	1.02	1.02	1.00	1.02	1.11	1.11

*The isotopic ratios for tissues and standards were identical in the blood sample of rat 1; the brain and small intestine samples of rat 2; the small intestine sample of rat 3; and the blood, brain, and small intestine samples of rat 4.

changing the two acidic deuterium atoms at C-2 in dilute aqueous sodium hydroxide. The methadone-d_3 obtained was found to be analytically pure. The chemical ionization mass spectra of methadone (Lilly) and of methadone-d_3 are shown in Fig. 1.

Analgesic activities of methadone-d_3, methadone, and their 1:1 mixture were identical in mice (Fig. 2A). These results support our hypothesis that the deuterium substitution at C-1 does not interfere with the drug-receptor interactions. The toxicities of the three drug preparations were the same; the median lethal dose (LD_{50}) was 41 ± 0.5 mg/kg (Fig. 2B). Therefore, we show that the acute toxicity of methadone is not affected by deuterium substitution at C-1.

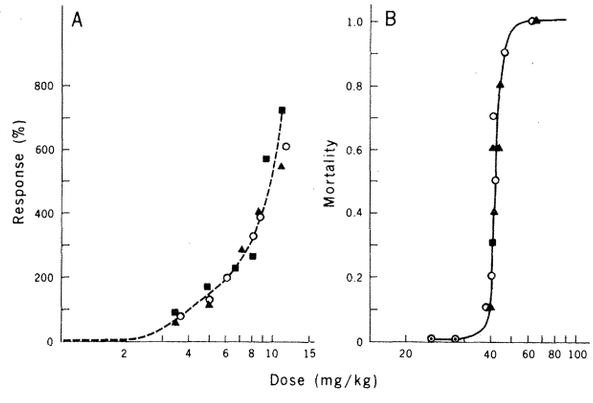

Fig. 2. Comparison of the analgesic activities and toxicities of methadone (○), methadone-d_3 (▲), and their 1 : 1 mixture (■) in male albino Swiss strain mice. The drugs were administered intraperitoneally in 0.9 percent NaCl solution (0.5 ml). All drug concentrations are expressed in terms of the hydrochloride salt. (A) The dose-response curves of analgesic activities were measured as an elevation of the pain threshold by the hot-plate method (55°C). Each data point represents the average of four animals. The response time was greater than 60 seconds for dosages exceeding 12.5 mg/kg in the three drug preparations. (B) The relative toxicities were determined by comparing the acute mortality within 24 hours. Mortality was obtained from ten animals for each data point. The symbol ⊙ indicates points at which mortality was the same for all three drugs.

In order to determine that the rates of absorption, distribution, and excretion of methadone-d_3 and methadone were the same in rats, the ratios of the two were monitored as a function of route of administration. A 1:1 mixture of the labeled and unlabeled methadone was administered to four rats; the ratios of methadone-d_3 and methadone in major organs, blood, and urine were identical (Table 1).

The equivalency of the analgesic activities, toxicities, and pharmacokinetics of methadone and methadone-d_3 in these laboratory animals suggests that the deuterium-labeled drug may be used as an in vivo marker for detecting illicit methadone supplementation. In principle, a suspected patient may be maintained on a fixed ratio of methadone to methadone-d_3. Deviation from the prescribed isotopic ratio in the patient's routine urine analysis would be indicative of illegal supplementation, and the degree of change would give some indication of the amount of supplementation. With this information, the therapist can advise cessation of the supplementation on penalty of being dropped from the program or may decide to increase the dosage given by the program if there is evidence that adjustment of the client's prescription is warranted.

To combat inter- or intraprogram diversion or diversion of methadone into the illicit drug market, other deuterium-labeled methadones have been synthesized ([1-$^2H_1$]methadone-d_1 and [1,1-$^2H_2$]-methadone-d_2) (*4*). With three different labeled methadones and many different ratios it may be possible to vary the ratios of labeled to unlabeled methadone for each patient. Thus, diversion of drugs may be determined, and appropriate measures may then be taken to discourage the abuse. It should be noted that in this case labeled methadone need not be given all the time, but only to certain patients as a spot check.

The principal objective of an effective monitoring system for the methadone treatment program is to relax the control of the current program in order that more liberal take-home policies can be implemented. As a result, a majority of addicts (~ 95 percent) may be encouraged to enroll in the treatment program rather than become dependent on the supply of the illicit heroin market and organized crime. Thus, heroin addiction may be more effectively combated.

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A Multistable Movement Display: Evidence for Two Separate Motion Systems in Human Vision

Abstract. *Two competing sensations of apparent movement were produced by the rapid alternation of two multielement stimulus frames. Either sensation could be made dominant by appropriate manipulations of the stimulus display. The results suggest that there are two systems capable of generating movement signals in man. One system depends on preliminary processing of form, and the second system does not.*

Sensations of stroboscopic movement were produced by a cyclic alternation of two stimulus frames in a tachistoscope. Frame 1 contained three black dots (a, b, c) arranged in a horizontal row on a white background. Frame 2 contained three identical dots (d, e, f), also arranged horizontally but shifted to the right, so that the positions of dots d and e of frame 2 overlapped those of b and c, respectively, of frame 1. With a frame duration of 200 msec and an interval of approximately 40 msec between frames, the spatiotemporal display gave rise to a multistable percept; either the observer perceived a group of three dots moving in toto back and forth (group movement) or he perceived the overlapping dots of each frame as stationary and a third dot as moving back and forth from one end of the display to the other (element movement) (*1*). On the average, the two movement sensations alternated spontaneously about eight times per minute, and the rate of reversal remained stable over a 10-minute period. However, we have been able to bring the multistable percept under stimulus control, that is, to cause either the group movement sensation or the element movement sensation to predominate, by manipulating the duration of the interval between frames, the type of viewing (binocular or dichoptic), or the contrast of the stimulus frames (*2*). The results suggest that there are two systems or channels for generating movement signals in humans, each with different functional properties.

A three-channel Gerbrands tachistoscope was used to superimpose the two alternating stimulus frames (provided by two separate channels) on a continuously illuminated, uniform background (provided by a third channel). The viewing distance was 81 cm; at this distance each stimulus frame and the uniform background subtended a visual angle of 9° horizontally and 6°15′ vertically. The diameter of each black dot was 40′ with a center-to-center separation of 60′ between a pair of adjacent dots. The luminance of the black dots when superimposed on the uniform background was 0.15 millilambert; that of the white area of each stimulus frame when superimposed on the uniform background, 0.35 mlam. During the interval between stimulus frames, only the uniform white background (0.10 mlam) was visible. In all experimental conditions the duration of each stimulus frame was 200 msec. The interval between stimulus frames (ISI) was varied.

In the first experiment there were 12 different stimulus conditions resulting from the factorial combination of six ISI's (5, 10, 20, 30, 50, or 70 msec) and two types of viewing (binocular or dichoptic). In the binocular condition the observer viewed both stimulus frames with both eyes. In the dichoptic condition, with appropriately arranged Polaroid filters, one stimulus frame was presented to the observer's right eye, the other to the observer's left eye (*3*). In both viewing conditions the uniform background provided by the third channel of the tachistoscope was visible to both eyes and allowed the observer to maintain a constant degree of accommodation and convergence.

The dependent measure was the type of movement reported by the observer (either element or group) after he watched four cycles of one of the 12 experimental stimulus sequences (one cycle: frame 1–ISI–frame 2–ISI). While viewing each sequence, the observer was instructed to direct his gaze toward the center of the stimulus display (no fixation point was used) and at the same time to attend to (be aware of) the entire display. Each of the 12 stimulus sequences was presented 10 times, following an order determined by block randomization. Eight observers participated in the experiment (*4*).

The number of times that each observer reported group movement in each of the 12 experimental conditions was converted to a percentage. The pattern of results was the same for all observers. The observers reported group movement very infrequently (most always saw element movement) at short ISI's in the binocular condition (Fig. 1). With binocular viewing and long ISI's (50 or 70 msec), the observers almost always saw group movement. In addition, the transition from the element movement sensation (few group movement responses) to the group movement sensation occurs abruptly at about 40 msec. In contrast to the results with binocular viewing, the mean percentage of group movement responses in the dichoptic condition is equal to or near 100 at all ISI's. The element movement sensation could not be obtained with dichoptic viewing.

If one looks only at the percentage data in the binocular condition, it is not possible to determine whether or not the absolute strength of the group movement sensation changed with ISI. The percentages show only that the strength of the group movement sensation decreased relative to that of the element movement sensation as the ISI was made shorter. It is not clear whether the absolute strength of the group movement sensation changed with ISI. The dichoptic data suggest that the absolute strength remained constant. However, supplementary observations indicate that the group movement sensation is more fragile at the short ISI's. When the stimulus sequences were extended beyond four cycles, the group movement sensation adapted within a few seconds with short ISI's (that is, the sensation of movement ceased and was replaced by a sensation of "on-off" flashing of the stimulus frames) but continued indefinitely with long ISI's.

The procedure of a second experiment was identical to that of the first experiment with the following exceptions. Two different stimulus displays were used in the second experiment. In one condition (positive-positive), there were black dots on a white background in both stimulus frames. In the second condition (positive-negative), the dot-to-background contrast was reversed in the two stimu-