Table 1. *Aspergillus flavus* infection of corn kernels from selected, freshly harvested ears. Kernels were selected from either an insect-damaged row or the adjacent, undamaged row. The surfaces of kernels were sterilized with 2 percent sodium hypochlorite for 2 minutes, rinsed twice with sterile water, placed on ME agar (malt extract, 3 percent; agar, 1.5 percent) in petri plates, incubated at 28°C, and examined under a microscope after 6 days.

Ear (Fig. 1)	Kernel row	Kernels examined (No.)	Kernels infected (No.)			Sterile kernels (No.)
			A. flavus	*Fusarium*	*Penicillium*	
B	Insect-damaged	25	2	25	6	0
B	Undamaged	25	0	18	2	7
C	Insect-damaged	17	11	14	0	3
C	Undamaged	17	0	0	0	17
D and E	Insect-damaged	24	13	9	0	4
D and E	Undamaged	22	0	1	0	21
F	Insect-damaged	23	23	2	0	0
F	Undamaged	20	0	16	4	0

greenish-yellow spore masses was definite evidence for the presence of *A. flavus*, the question remained of the extent of concealed mycelial proliferation throughout the ear. This problem was examined by determining the incidence of the fungus in selected kernels; a row of seed exhibiting insect damage was compared with undamaged kernels from the adjacent row. In addition, other predominant internal fungi of kernels sterilized on the surface were enumerated after 6 days of incubation on malt extract agar (*7*) at 28°C.

Since the four test ears depicted in Fig. 1 were considered representative of all those containing *A. flavus*, kernels from these ears were examined for fungi (Table 1). The most striking observation in this test was the exclusive association of the fungus that produces aflatoxin with kernels damaged by insects. Corn from ear 1B had a low incidence of *A. flavus* in kernels from the damaged row and a high occurrence of *Fusarium* in both rows. *Aspergillus flavus* on this ear was restricted to the ear tip with widespread *Fusarium* infection. *Fusarium moniliforme* Sheldon was the predominant *Fusarium* species on all ears (*11*). Seed from ear 1C exhibited a high incidence of *A. flavus* and *Fusarium* in the kernels damaged by insects, with no fungi in the adjacent row. Insect-damaged kernels of ear 1D-1E from the middle region of the ear exhibited considerable *A. flavus* and *Fusarium* infection, but seed in the adjacent row was essentially free of fungus. Ear 1F contained *A. flavus* in all damaged kernels; *Fusarium* was limited in damaged seed but its incidence was higher in the adjacent row.

Penicillium oxalicum Currie and Thom was observed on numerous ears in the field and in a limited number of test kernels (Table 1). A few kernels were infected with *Aspergillus clavatus* Desm. However, the three predominant fungi observed on the ears damaged by insects were *A. flavus*, *F. moniliforme*, and *P. oxalicum*.

Eleven test ears were shelled, and seed from individual ears was ground, extracted, and assayed for aflatoxin (*12*). Corn from all of the ears contained aflatoxin B_1, ranging in concentration from 1 part per billion to 1560 parts per billion, with a mean of 430 parts per billion. The reason for the great variation in toxin levels is not known, but it might be the result of differences in aflatoxin production capabilities of strains of *A. flavus*. Fifteen isolates of the fungus from ear 1F were transferred to the APA (aflatoxin-producing ability) medium (*13*); this qualitative test showed that five of the 15 produced aflatoxin.

Our observations of Iowa corn provide conclusive evidence for the infection of field corn by *A. flavus* in the Midwest and the production of aflatoxin in the seed before harvest. Infection by the fungus appeared to be exclusively associated with injury resulting from feeding of larvae of the second generation European corn borer.

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Elevated Plasma Zinc: A Heritable Anomaly

Abstract. *An extremely high concentration of zinc in the plasma (hyperzincemia) was found in five out of seven members of one family and in two out of three second-generation individuals, an indication that the condition is heritable. The excess zinc in the plasma appears to be bound to serum proteins, with no apparent clinical symptoms or abnormalities.*

Numerous acquired disease conditions have been demonstrated to result in lowered concentrations of zinc in the plasma (or serum) (*1*, *2*). In addition, achrodermatitis enteropathica, a hereditary disease in which the zinc metabolism is altered, dramatically responds to oral zinc therapy (*3*). In contrast, few if any clinical conditions causing high concentrations of zinc in the blood have been reported. Although the effect of zinc toxicity on concentrations in the blood in humans has not been studied under controlled conditions, one report indicates that the increase in serum zinc was minimal even when toxic quantities of zinc were ingested (*4*). Zinc sulfate therapy can result in increased serum zinc concentrations, but this increase is usually less than twice baseline values and is transitory (*5*). With the above exceptions, we have found no clinical conditions that are characteristically accompanied by a sustained elevated zinc concentration.

During the course of screening patients for zinc in the plasma we noted that in a 28-year-old black male the concentration was extremely high (> 300 μg/100 ml). Initially we suspected that the high concentration in the plasma might be the result of toxic ingestion. However, subsequent blood samples from this subject over a 20-week period, as well as a study of his siblings, established that the increased concentration cannot be attributed to toxic ingestion (*6*).

The zinc concentration in the plasma for the fasting subject ranged from 270 to 360 μg/100 ml, with a mean and standard deviation of 315 ± 17 for 11 samples taken over a 5-month period. The normal values for our laboratory are 81 ± 13 (mean ± S.D.) μg/100 ml, by atomic absorption spectrophotometry.

The concentrations of zinc in the plasma of the propositus and several members of the family are shown in Fig. 1. We studied six of his siblings; three brothers and one sister exhibit hyperzincemia. The siblings live in five different households located in three states. The parents of the subject are deceased. We studied the 4-year-old son of the propositus as well as the two children of the 41-year-old brother with hyperzincemia. The plasma zinc concentration of the son was elevated (285 μg/100 ml); in the brother's daughters, the concentration of plasma zinc in the 14-year-old was 435 μg/100 ml (elevated), whereas that in the 18-year-old was 70 μg/100 ml (not elevated). Thus, the fact that the hyperzincemia is present in the second generation seems to indicate that it is heritable. The overall health of the affected subjects does not appear to be impaired. The only identified heritable disease in this family is sickle cell anemia, but this is not correlated with the distribution of hyperzincemia.

The propositus was admitted to our hospital in June 1975, suffering from acute alcoholic hepatitis and was assigned to the Veterans Administration Narcotic and Alcoholic Treatment Association. Although he had been consuming excessive alcohol for approximately 10 years, evidence of cirrhosis or other debilitating disease was not found. Gross hematuria, present intermittently, was attributed to sickle cell trait and occurred over a brief period of our study. Except for occasional elevated values of lactate dehydrogenase and serum glutamate oxaloacetate transferase, there was no evidence of active liver disease during the study of zinc metabolism. During the period of hospitalization (June through December) the subject exhibited no symptoms that could be attributed directly to hyperzincemia.

For the propositus, 24-hour urinary zinc excretion was 567 ± 77 μg (mean ± S.D.) during 11 consecutive collection periods. An 8-day balance study indicated a daily balance of +5.1 mg, representing 38 percent retention of dietary zinc.

A dietary zinc load was administered to the subject by way of an oyster meal that contained 75 mg of zinc. After the meal was consumed, the plasma zinc rose from a baseline level of 400 to 490 μg per 100 ml at 3½ hours. For comparison, one of us (J.C.S.) consumed the same oyster meal, but ingested smaller quantities (59 mg) with the plasma zinc rising from a baseline of 75 to 150 μg/100 ml after 2½ hours. The oysters provided 99 percent of the total dietary zinc in both cases. Although the meal supplied five times the U.S. recommended daily allowance and the plasma concentration exceeded three times that of the control, the subject showed no ill effects.

Table 1. Pertinent clinical data on the propositus. Results are expressed as means ± the standard deviation; the parentheses indicate the number of analyses on separate samples.

Item	Mean ± S.D.	Normal range
Serum protein concentration		
Total protein (grams per 100 ml of serum)	7.1 ± 0.3 (6)	6.0 to 8.0
Albumin (grams per 100 ml of serum)	5.0 ± 0.2 (6)	3.5 to 5.0
Globulins (grams per 100 ml of serum)	2.1 ± 0.4 (6)	1.7 to 4.5
α_2-Macroglobulin (milligrams per 100 ml of serum)	299 ± 5 (2)	150 to 350
Tissue zinc concentration		
Erythrocyte (micrograms per milliliter)	10.0 ± 1.1 (4)	11.8 ± 1.7 (51)
Hair (micrograms per gram, dry)	189 (1)	179 ± 37 (14)
Bone (micrograms per gram, dry)	191 (1)	140 ± 25 (18)

Pertinent clinical data and zinc concentrations in three different tissues are presented in Table 1. Albumin, α_2-macroglobulin, and transferrin—zinc binding proteins—were within the normal range, as well as the total protein and globulin concentrations. Likewise, serum iron, the total iron binding capacity, and ceruloplasmin were normal. Dialysis of serum against deionized water revealed very little (approximately 1 percent) dialyzable zinc. Zinc concentration of the erythrocytes was within normal range.

The concentration of zinc in the hair for the subject was 189 μg/g (dry weight), which is within our normal range (176 ± 37 μg/g, mean ± S.D., for 14 adult controls). The concentration of zinc in the fingernails was 133 μg/g compared to 173 μg/g for a control subject. A bone sample, obtained during alveoplasty, showed a zinc concentration of 191 μg/g (dry weight). In comparison, bone from 18 other subjects undergoing alveoplasty had a zinc concentration of 140 ± 25 (mean ± S.D.) μg/g (dry weight), the range being 112 to 187 μg/g. The plasma zinc concentration for these other patients was 71 ± 7 μg/100 ml.

On the basis of dialysis experiments, the zinc in the circulating plasma for the subject appears to be bound. In addition, the concentration in the tissues without a major blood supply (hair and fingernails) appears to be normal. Although the bone zinc may be considered to be increased, the concentration (191 μg/g) is only slightly greater than 2 standard deviations above the mean for the control patients, 140 ± 25 (mean ± S.D.). It is possible that this increase in concentration is due to the high zinc concentration of blood within the alveolar bone. The significance of the bone zinc concentration is unclear since one patient having a plasma level within normal range exhibited a bone zinc level nearly as high as that of the subject, 187 μg/g. At present, it appears that tissues other than plasma commonly used to assess zinc status have not accumulated zinc to any appreciable extent. The urinary excretion of 567 ± 77 μg per 24 hours is within normal

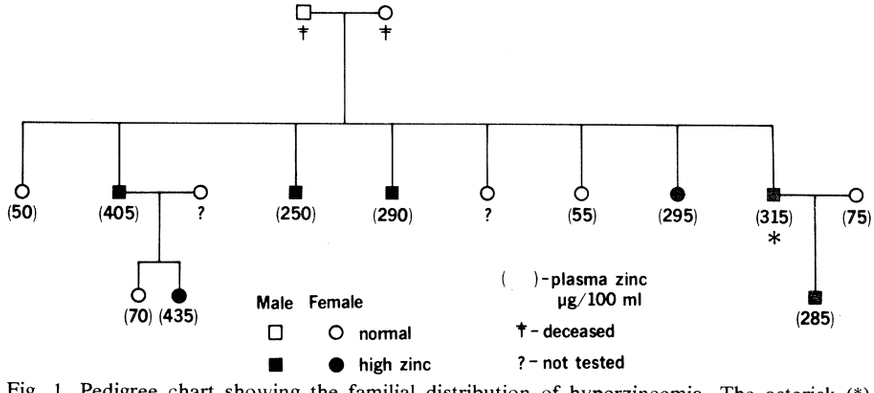

Fig. 1. Pedigree chart showing the familial distribution of hyperzincemia. The asterisk (*) indicates the propositus whose mean plasma zinc concentration was 315 ± 17 μg/100 ml (mean ± S.D.) for 11 samples taken over a 5-month period. Five out of six siblings and two out of three second-generation subjects show hyperzincemia, including the son of the propositus. (Normal concentration of zinc in the plasma in our laboratory is 81 ± 13 μg/100 ml.)

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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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.

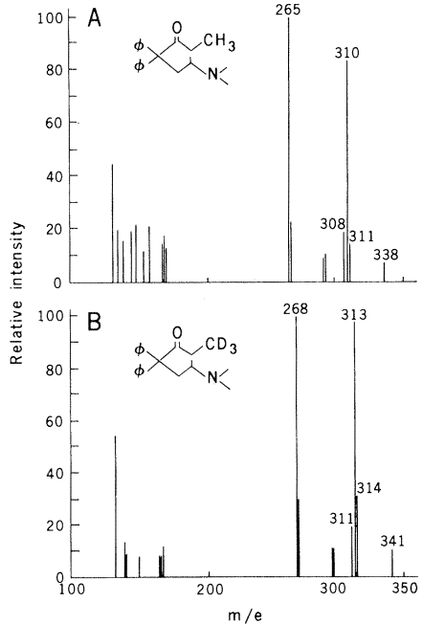

Fig. 1. Chemical ionization mass spectrum of (A) methadone and (B) methadone-d_3. The data were recorded on a Biospect mass spectrometer; methane was used as the ionization gas via the direct probe mode; the probe temperature was 250°C. The quasi-molecular ions (M + 1) of methadone and methadone-d_3 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-