

sleep onset (5 minutes). The deep sleep period (5½ hours) was longer and was characterized by fewer response bursts than it was for both normal and drugged sleep conditions.

The control record showed a 23-minute sleep latency (similar to unconditioned responding) but an immediate sleep onset (0 minutes). Thus, conditioned responding did not show the gradual sleep onset characteristic of unconditioned responding. Note also that the initial conditioned response rate during the latency period (base line) was lower than the unconditioned rate, showing inaccurate recall. The deep sleep period was longer (5¾ hours), and the rate of response during deep sleep was lower for conditioned responding than for unconditioned responding.

The records of body movements did not show the sleep-latency or sleep-onset differences for the conditions of deprivation and sedation that were shown by operant responding. Fewer movements were made during deep sleep than during the later waking state for all conditions, however, and therefore the method could show that deprivation and drugs increase the duration of deep sleep. This effect has been reported previously (5).

The subjects' reports of the number of times they recalled awakening were not related to the number of response bursts in the sleep records. Neither subject reported ill effects of the experiment, and both felt rested after the sessions.

These observations show that unconditioned operant responding to turn off an aversive stimulus during sleep is more sensitive to intermediate sleep levels and to deprivation and drug effects than is responding supported by verbal instructions with previous conditioning or the recording of body movements. This sensitive and widely applicable method should enable scientists to study sleep behavior more effectively. It can be used to investigate the effects of drugs, neurosurgery, deprivation, and awakening stimuli on the sleep of lower animals as well as on that of human beings. Records of operant responding during sleep and hypnosis should be compared with electroencephalographic records in normal and abnormal subjects.

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#### References and Notes

1. W. K. Estes and B. F. Skinner, *J. Exptl. Psychol.* 29, 390 (1941).
2. O. R. Lindsley and B. F. Skinner, *Am. Psychologist* 9, 419 (1954); O. R. Lindsley, *Psychiat. Research Repts.* 5, 118, 147 (1956).
3. This investigation was supported by a research grant (M-977) from the National Institute of Mental Health, National Institutes of Health,

U.S. Public Health Service. The assistance of Douglas Bowden is gratefully acknowledged.

4. The rate analyzer is commercially available from Grason-Stadler Co., West Concord, Mass.
5. N. Kleitman, N. R. Cooperman, F. J. Mullin, *Am. J. Physiol.* 105, 574 (1933).

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## Surface Ionization of Silver; Silver in Meteorites

Isotopic dilution followed by mass spectrometric analysis has been applied to many of the naturally occurring elements (1). Concentrations may be accurately determined at the level of one part per million (ppm) and even lower. Surface ionization of a solid sample is the usual source of ions for the heavier elements. A recent study (2) of microgram amounts of lead, uranium, and thorium from typical rock minerals emphasizes the importance of these sensitive techniques to geochemistry. The experiments described in this report (3) demonstrate that silver may be similarly analyzed.

These techniques may lead to a better understanding of the distribution of silver in the earth and in meteorites. The analysis of small amounts in meteorites is especially important for the following reasons.

1) A recent estimate (4) places the cosmic abundance of silver at 0.26 atom per million atoms of silicon. This value is based on a reasonable interpolation from elements whose abundances are better established. A typical earlier estimate (5) is an order of magnitude higher. In this case, the ratio of 2.7 atoms of Ag per million of Si is based on analyses of meteoritic phases by the Noddacks. There are several indications that their concentration may be too large (6).

2) The isotopic composition of silver may reveal part of the early history of the solar system. If processes leading to the formation of the planets occurred shortly after nucleogenesis, variations in the relative amounts of Ag<sup>107</sup> and Ag<sup>109</sup> may exist as a result of the decay of Pd<sup>107</sup>. The half-life of this extinct nuclide is reported (7) as  $7.5 \times 10^{-6}$  years. Even with very favorable fractionation of Ag relative to Pd, the detection of such an effect would imply that the period between nucleogenesis and the formation of the earth was considerably shorter than the minimum time estimated on the basis of the  $17.2 \times 10^{-6}$ -year half-life of I<sup>129</sup> (8).

Although positive results from a search for isotopic differences in silver appear to be unlikely, a sample of troilite has been investigated. This material was selected initially because of its relatively high concentration of Ag. It was hoped

that the troilite either might have scavenged Ag<sup>107</sup> from the surrounding palladium-rich (9) metallic phase or might have remained isolated from an early stage and thus retained primeval silver.

Troilite from the Xiquipilco (Toluca) iron meteorite, a medium octahedrite, was received in the form of slabs several millimeters thick and several centimeters in breadth (10). The surface was ground off with an Al<sub>2</sub>O<sub>3</sub> refractory wheel (dental size). The pieces were rinsed several times with 2N H<sub>2</sub>SO<sub>4</sub> (11) and quadruply distilled water.

The dried sample, weighing 18.40 g, was dissolved, except for a black residue, in two stages requiring nearly 20 ml of concentrated H<sub>2</sub>SO<sub>4</sub> in about 250 ml of water. The residue was centrifuged away from most of the iron and nickel, washed, and digested in quartz-distilled concentrated HNO<sub>3</sub>. This solution of about 30 ml was mixed with 30 ml of a 20 percent solution of purified ammonium citrate. The pH was adjusted to slightly greater than 1 with NH<sub>4</sub>OH (carefully prepared from gaseous NH<sub>3</sub> and distilled water). Successive 2- to 3-ml portions of a dithizone solution (12) were briefly shaken with the acid solution until they became violet after shaking instead of yellow (silver dithizonate). The CHCl<sub>3</sub> was evaporated, and the residue was taken to dryness several times with concentrated HNO<sub>3</sub>. The residue was taken up with a few drops of concentrated HNO<sub>3</sub>, diluted to about 25 ml, and the pH was adjusted to between 1 and 1.5 with NH<sub>4</sub>OH. Extraction with standard dithizone solution in 2-ml portions required 20 ml and was equivalent to 30 µg of silver. This is only an upper limit for the amount of Ag, for Hg is also extracted under these conditions. The dithizonate was again converted to nitrate (13).

A chunk of Canyon Diablo iron meteorite, free of visually obvious troilite inclusions, was rinsed four times with 6N H<sub>2</sub>SO<sub>4</sub> and washed with distilled water after each acid rinse. The dried weight of this piece was 102.6 g. It was almost completely dissolved in 150 ml of concentrated H<sub>2</sub>SO<sub>4</sub> and 550 ml of distilled water. The odor of H<sub>2</sub>S was present during the reaction. Further treatment of the residue required approximately an additional 40 ml of concentrated H<sub>2</sub>SO<sub>4</sub> and roughly 700 ml more water. Gaseous H<sub>2</sub>S was also added shortly before separation of most of the iron and nickel in the supernatant from the residue. The final residue consisted of sulfur, black particles, and a small amount of heavier, metallic slivers about 1 mm long.

The solids were digested with about 150 ml of concentrated HNO<sub>3</sub>. The resulting 25 ml of solution were mixed with 25 ml of the ammonium citrate solution, adjusted to a pH of 1.5, and extracted

Table 1. Surface ionization of microgram amounts of silver.

Run	Source of sample	Filament type	Ag <sup>107</sup> /Ag <sup>109</sup> average atomic ratio	Number of spectra	Mean deviation (±)
1	Terrestrial	Triple	1.083	122	0.020
2	Terrestrial	Single	1.079	104	0.015
3	Terrestrial	Single	1.083	296	0.021
4	Terrestrial	Single	1.084	97	0.015
5	Meteoritic	Single	1.067	16	0.028

in brief shakes with 2-ml portions of dithizone solution. The first 12 ml were violet after shaking. Later additions turned only pale violet. The CHCl<sub>3</sub> solution was back-extracted with 30 ml of NH<sub>4</sub>OH solution at a pH of 8 to 9. The residue, after evaporation of the CHCl<sub>3</sub> and digestion with concentrated HNO<sub>3</sub>, was again extracted at a pH somewhat greater than 1 with the standard dithizone solution. The only color which appeared was a violet during the addition of the first 10 ml, following which the color was only slightly changed from that of the original solution. No yellow color characteristic of silver dithizonate was observed. The minimum amount that could be detected was 4 μg. Unoxidized silver (14) as scattered atoms may not have attached itself to centrifugable solids and may have been lost in the supernatant. Other experiments showed that most of the Ag<sub>2</sub>S formed in 25-μg and even in 50-μg Ag solutions could not be centrifuged down, at least not in the absence of a carrier. However, if even a yield as low as 25 percent is assumed, the concentration of Ag in this iron meteorite is less than 0.2 ppm (15).

The silver extracted from the troilite indicates a concentration of no more than 1.6 ppm (assuming 100-percent yield). If the yield is only 50 percent, the concentration is no more than 3 ppm. These figures do not contradict the estimate of about 5 ppm made with the emission spectrograph of Oiva Joensuu of the department of geology, University of Chicago. Joensuu also found that the concentration of silver in the metal phase of the Canyon Diablo meteorite is no more than 1 ppm. Lovering (16) has found less than 0.5 ppm of Ag in three troilites, including Toluca. The values indicated by these experiments are lower by roughly an order of magnitude than the averages previously accepted for meteoritic iron and troilite (5), but they agree with the estimate of Suess and Urey (4) for the cosmic abundance of silver.

The mass spectrometer used to compare the isotopic ratio of the meteoritic silver to that of terrestrial silver was a single-focusing instrument with a 12-in. radius of curvature and a 60° deflection. The output of the electron multiplier was measured with a vibrating-reed elec-

trometer. Filaments were tungsten ribbon, 30 mil by 1 mil. Ion intensities were recorded on a strip chart. The amounts of silver on the filaments ranged from a few micrograms to around 15 μg. The smallest amounts previously used for mass spectrometric analysis appear to be 3 milligrams (17).

The sensitivity of multiple filaments (18) was first thought to be an advantage. Of two trials with Ag<sub>2</sub>S on the side filament, one produced completely reasonable data (Table 1, run 1). Other masses were present with low intensity, but in this case there was no evidence for impurity peaks at masses 107 and 109. In general, though, multiple-filament experiments (19) were characterized by spurious, unstable, and unreliable peaks (mass 107 often too high) and by little control of ion beam intensity by varying the temperature of the side filament.

When AgNO<sub>3</sub> and borax were placed on either oxidized or unoxidized single filaments, some emission of Ag was observed, but spurious ions were too numerous to be tolerable (20). To reduce the extraneous signals from cations from borax, a small drop of saturated boric acid was added to the silver compound on the filament instead of borax. After careful evaporation to dryness, the current warming the filament was increased to bring it to a dull glow for a few minutes. Filaments prepared in this way with about 10 μg of Ag and mounted in the mass spectrometer displayed hours of steady emission of Ag<sup>+</sup> with high intensity. The particular silver compound placed on the filament before boric acid is added evidently is not important as long as it is reasonably pure, for in run 2 (see Table 1) boric acid was added to Ag<sub>2</sub>S on the filament and in runs 3 and 4, to AgNO<sub>3</sub>.

The meteoritic silver (run 5) was placed on the filament as a strongly basic solution of Ag(NH<sub>3</sub>)<sub>2</sub><sup>+</sup>. Then boric acid was added. This run was of very low intensity and suggested that the amount of silver which had been placed on the filament was no more than 1 microgram and probably less. Possibly the metal extracted from the troilite was mostly mercury rather than silver. Some of the 107 peaks were corrected for 0.5 to 5 percent of K<sub>2</sub>BO<sup>+</sup>, which was pres-

ent as an impurity. This correction was not necessary for the high-intensity runs 2 to 4. Here extraneous masses were not usually observed, although occasionally mass 108 was discernible at less than 0.5 percent of the intensity of the main peaks. Only in run 2 were ratios (obtained with lower intensity and not included in the average) observed that agreed closely with the average of run 5. However, within the error of the latter, there is no difference in isotopic abundances in the Ag samples (21). In the present experiment the ratio of Ag<sup>107</sup> to Ag<sup>109</sup> is 2.5 percent higher than the value of 1.055 ± 0.003 reported by White and Cameron (22). The chemical atomic weight calculated from our ratio with recent values of isotopic masses (7) is 107.870, while that calculated from White and Cameron's data is 107.883. This is in good agreement with the observed value of 107.880. Presumably our discrepancy is due to discrimination of the mass spectrometer, although no source magnet was used. All ratios have been corrected for mass discrimination of the electron multiplier (23).

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## References and Notes

- M. G. Inghram, *Ann. Rev. Nuclear Sci.* **4**, 81 (1954).
- G. R. Tilton *et al.*, *Bull. Geol. Soc. Am.* **66**, 1131 (1955).
- This work was supported in part by the U.S. Atomic Energy Commission and in part by a grant from the National Research Council.
- H. E. Suess and H. C. Urey, *Revs. Modern Phys.* **28**, 53 (1956).
- H. Brown, *ibid.* **21**, 625 (1949).
- For example, the concentration reported by the Noddacks for lead in meteorites is at least 10 times too high [C. Patterson *et al.*, *Phys. Rev.* **92**, 1234 (1953)].
- W. H. Sullivan, *Trilinear Chart of Nuclides* (Oak Ridge Natl. Lab., U.S. Government Printing Office, Washington, D.C., 1957).
- S. Katcoff *et al.*, *Phys. Rev.* **82**, 688 (1951).
- E. Goldberg *et al.*, *Geochim. et Cosmochim. Acta* **2**, 1 (1951).
- The material was received from H. E. Suess, now at Scripps Institution of Oceanography, La Jolla, Calif. Suess has subsequently commented that no precautions were taken to avoid contamination in the preparation of the slices. This may account for the fact that the isotopic composition of the lead in this material is almost as radiogenic as modern average crustal lead. Suess obtained the meteorite from H. Rose of the Hamburg Mineralogical Institute, Hamburg, Germany.
- Distilled in Pyrex from concentrated acid which meets ACS specifications.
- The solution consisted of 2 mg of diphenylthiocarbazon in 500 ml of H<sub>2</sub>SO<sub>4</sub>-extracted, spectrophotometer grade CHCl<sub>3</sub>.
- Comparable volumes of the reagents (nearly 20 ml of concentrated H<sub>2</sub>SO<sub>4</sub>, 100 ml of concentrated HNO<sub>3</sub>, and approximately 150 ml of distilled water) were mixed with 40 ml of the ammonium citrate solution. This aqueous phase (about 500 ml after adjustment to a pH of 1.5) was extracted with excess dithizone in 75 ml of CHCl<sub>3</sub>. The nonaqueous phase was then back-extracted three times with NH<sub>4</sub>OH (pH 7.5 to 8) to remove ex-

cess dithizone. The  $\text{CHCl}_3$  was evaporated, and the residue was taken to dryness six times with concentrated  $\text{HNO}_3$ . The residue was taken up in  $\text{HNO}_3$  and extracted at a pH of 1 with 1 ml of standard dithizone solution. No color change was noted. Contamination appears to be much less than 1.5  $\mu\text{g}$ .

14. The values of  $\Delta F^0$  [Natl. Bur. Standards (U.S.) Circ. No. 500 (1952)] for sulfides indicate that Ag should not occur as  $\text{Ag}_2\text{S}$  in meteorites.
15. The assistance of Sibyl Epling in these procedures is gratefully acknowledged.
16. J. F. Lovering, *Geochim et Cosmochim. Acta* 12, 253 (1957).
17. W. Paul, *Naturwissenschaften* 31, 419 (1943).
18. M. G. Inghram and W. A. Chupka, *Rev. Sci. Instr.* 24, 518 (1953).
19. Experiments by this technique with  $\text{Ag}_2\text{SO}_4$  and  $\text{Ag}^+$  in borax were not successful. A little success was had in one run with  $\text{AgNO}_3$  placed on the side filament and also with  $\text{AgNO}_3$  treated with phosphoric acid on the filament.
20. Attempts to examine the isotopic composition of silver in  $\text{Ag}_2\text{S}$ ,  $\text{AgNO}_3$ , or  $\text{Ag}_2\text{SO}_4$  with single-filament sources were not successful. Silver peaks were observed when phosphoric acid was used with the silver sample, but the 109 peak was often as high as the 107 peak and sometimes higher. With phosphorous acid and a single filament, the results were no better; the ratio of  $\text{Ag}^{107}$  to  $\text{Ag}^{109}$  varied from less than 1 to more than 1.
21. We are especially indebted to M. G. Inghram, Argonne National Laboratory and physics department, University of Chicago, who not only kindly provided the mass spectrometer but also contributed his efforts and interest to these experiments.
22. J. R. White and A. E. Cameron, *Phys. Rev.* 74, 991 (1948).
23. M. G. Inghram et al., *Natl. Bur. Standards (U.S.) Circ. No. 522* (1953), p. 257.

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### Adrenal Lipid Response in Chinese Hamsters Infected with *Trichinella spiralis*

The unique paucity of histochemically demonstrable lipid in the adrenal of the golden hamster (1), together with its atypical response to stress situations (2), suggests the possibility that the Chinese hamster may present a similar picture. Leatham and Stauber (3), using the intracellular protozoan parasite *Leishmania donovani*, showed that the adrenal cortex of the golden hamster responded to the stress of infection with a progressive accumulation of sudanophilic substance:

The present study (4) was carried out with 14 Chinese hamsters, each of which had been fed approximately 100 *Trichinella spiralis* larvae from a donor animal. Adrenals were removed from infected animals (under Nembutal anesthesia) on days 3, 5, 14, and 26 and from normal controls at the beginning of the experiment. They were fixed in 10 percent formalin and embedded in gelatin, and sections were stained with Sudan black B (5).

The adrenals of normal Chinese hamsters, unlike those of the golden hamster, were shown to have uniformly distributed lipid material in the cortex (Fig. 1A). Infected animals sacrificed on the third day of parasitization exhibited some loss of lipid in the outer zone of the

cortex (Fig. 1B). On the fifth day, cortical lipid was absent (Fig. 1C). On the 14th day of infection, adrenals exhibited a renewal of sudanophilic substance. The latter was most marked in the inner zone of the cortex (Fig. 1D). By the 26th day of infection, the normal diffuse pattern of lipid was again evident (Fig. 1E).

The results reported here indicate that the adrenal of the normal Chinese hamster resembles that of other mammals more closely than does that of the golden hamster. Similarly, the adrenal of the Chinese hamster responds to the stress

of infection in a more conventional way. The stress, in this case, is a result of the intestinal response to the adult worm of *T. spiralis*. Insofar as adrenal lipid is a measure of stress in this host-parasite complex, we see a rapid loss of the indicator material during the early development of worms in the intestine, followed by recovery at a time (the 14th to 26th day) which corresponds to the secondary insult of migrating larvae. Adult female worms with larvae fully developed at six days of infection have been observed, and larvae have been iso-

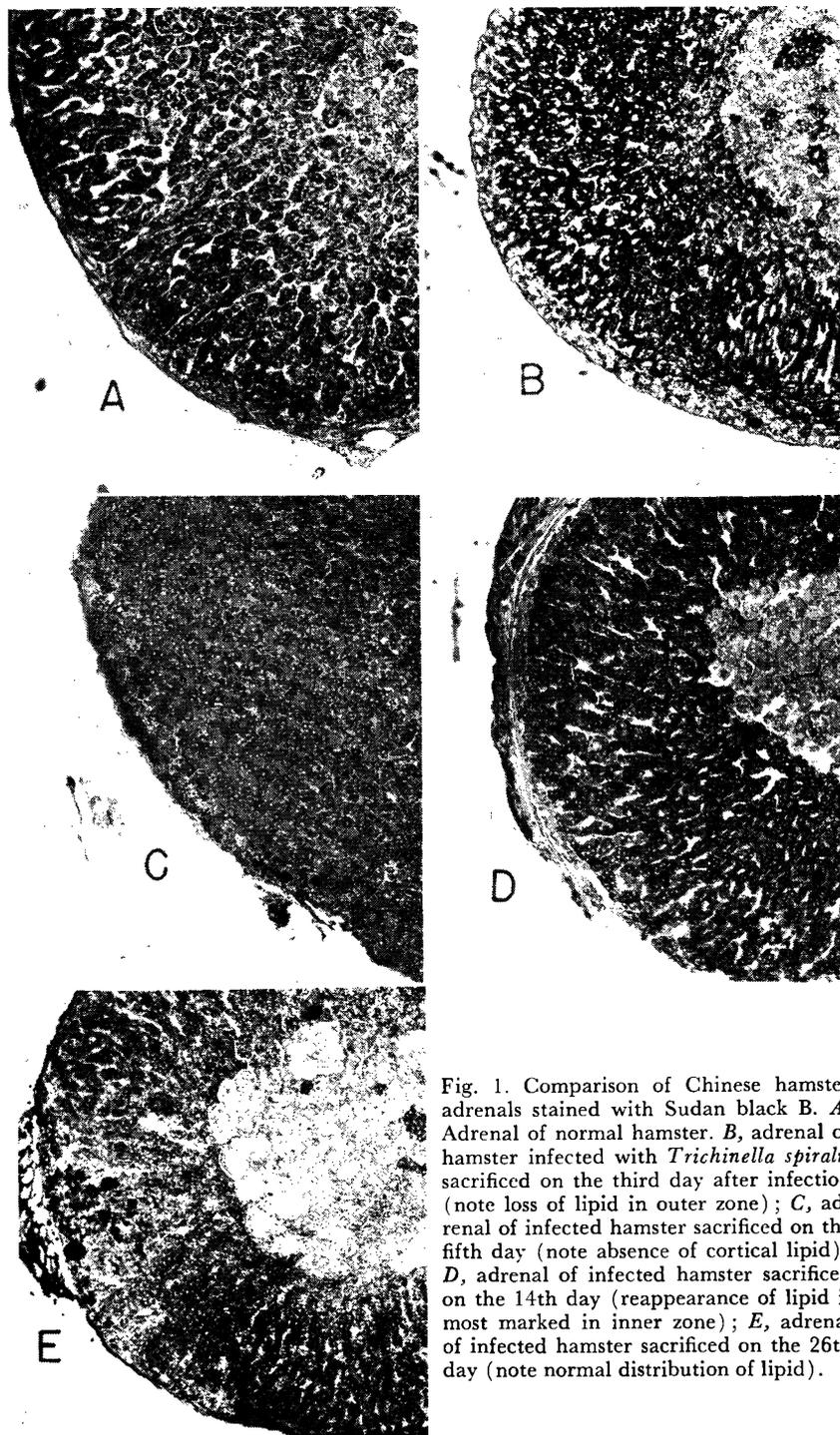


Fig. 1. Comparison of Chinese hamster adrenals stained with Sudan black B. A, Adrenal of normal hamster. B, adrenal of hamster infected with *Trichinella spiralis* sacrificed on the third day after infection (note loss of lipid in outer zone); C, adrenal of infected hamster sacrificed on the fifth day (note absence of cortical lipid); D, adrenal of infected hamster sacrificed on the 14th day (reappearance of lipid is most marked in inner zone); E, adrenal of infected hamster sacrificed on the 26th day (note normal distribution of lipid).