Identification of the autoinhibitory substance and the specific metabolic reactions against which it is active may provide a tool for determining more selectively the sequence of biochemical reactions resulting in germination. Effort is being devoted at present to determine more completely the properties of the autoinhibitory substance, including its effects on specific metabolic processes.

R. L. STEDMAN, E. KRAVITZ,
M. ANMUTH, J. HARDING
Philadelphia Naval Shipyard, Naval Base, Philadelphia, Pennsylvania

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18 June 1956

Comparison of Suggestibility during "Light Sleep" and Hypnosis

Some recent experiments (*1*) indicate that "hypnosis" may involve a state of "light sleep." Attempting to determine directly the relationship between these "states," I recently completed a study (*2*) of 22 subjects who volunteered "to be given psychological tests at night while they were asleep." I approached the subjects—in the middle of the night while they were sleeping in their own rooms—and whispered to them: "Clasp your hands together."

After a subject clasped his hands together—all subjects did so within 10 seconds—I repeated over and over for 1 minute: "Your hands are hard . . . solid . . . completely interlocked . . . it is impossible to unclasp those hands. Try it." In this way I gave seven standard tests of suggestibility, including the following: "you cannot unclasp your hands," "you cannot open your clenched fist," "your fingers are rising," "your hand is dead and dull and numb and cannot feel anything at all," "you are becoming very thirsty and will wake up in exactly 5 minutes and drink lots of water," and "you cannot remember anything that I said . . . you cannot remember anything at all."

When tested in this way, three of the subjects "woke up" and seven either moved or opened their eyes for a moment and later stated that they were "drowsy" during the experiment. However, the remaining 12 subjects seemed to be in a stage of "light sleep," since they did not move, did not open their eyes, responded reluctantly to requests like "clasp your hands together," continued breathing slowly and easily, and later stated that they either had been in "some stage of sleep" or had complete amnesia for the experiment.

These 12 subjects, who seemed to be "lightly sleeping," responded to the seven tests of suggestibility as if they were in some stage of "hypnosis" as measured by the Davis and Husband scale of hypnotic depth (*3*). Some responded as if they were in the second stage of "hypnosis"—for instance, they were completely unable to unclasp their hands. The majority of the "lightly sleeping" subjects responded as if they were in at least the third stage of "hypnosis," since they had complete amnesia for the experiment or followed the "postsleep" suggestion to wake up in 5 minutes and drink water. (No tests were given that could have differentiated the fourth, or "deepest," stage of "hypnosis.")

In a second experiment, the same seven tests of suggestibility were given in the same way immediately after a standard hypnotic-induction procedure. In a third (control) experiment, the same tests were given in the same way to the subjects when they were normally awake.

As Table 1 indicates, there were no significant differences between the subject's responses on the seven tests of suggestibility when the subjects were "lightly sleeping" and after they had been subjected to hypnotic induction. From Table 1, it can also be seen that the subjects were more suggestible on all seven tests during the first experiment—when they were "lightly sleeping"—than they were during the control experiment when they were normally awake, and that they were significantly more suggestible on five of the seven tests.

Table 1. Mean scores on the seven tests of suggestibility when the subjects were "lightly sleeping," after hypnotic induction, and when the subjects were normally awake.

Seven tests of suggestibility	Experiment			Critical ratio of difference	
	"Sleep"	Hypnosis	Control	"Sleep" and hypnosis*	"Sleep" and control
Hand-clasp test	44.4	37	7.3	0.75	5.3†
Finger-rigidity test	40.8	35	8.5	0.54	3.7†
Finger-levitation test	4.2	2.8	1.4	0.36	1.0
Anesthesia test	1.7	1.8	0.08	0.2	4.8†
Thirst test	9.0	4.7	1.25	1.8	4.5†
Five-minute-waking test	3.3	3.0	2.0	0.2	0.9
Amnesia test	3.3	2.5	0.0	0.55	5.7†

* Not significant at the 0.05 level. † Significant at the 0.001 level.

The correlations between the subject's scores on the seven tests during the first and second experiment—when "lightly sleeping" and after hypnotic induction—were in all cases above + 0.61 and in nine out of the 12 cases above + 0.92.

I concluded from this study that the subjects of this experiment were as suggestible when they were "lightly sleeping"—that is, when they appeared to be asleep and later stated that they had been in "some stage of sleep"—as they were after hypnotic induction.

THEODORE XENOPHON BARBER*
Department of Psychology, American University, Washington, D.C.

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* At present a postdoctoral research fellow, National Institute of Mental Health. Present address: Psychological Clinic, Harvard University, Cambridge, Mass.

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Strontium Content of Human Bones

The refinements of analytic techniques for trace elements, together with a growing interest in the distribution of trace elements in human tissues, has resulted in a continued addition of information on these elements to the biochemical literature. The present paper (*1*) reports strontium analyses on 277 human bones from a world-wide sampling.

Hodges *et al.* (*2*), investigating the strontium content of human bones by an emission-spectrographic technique, found for a limited sampling an average of 220 ppm for bone ash. Tipton (*3*) has found a lower value for bones (120 ppm), also using an emission-spectrographic technique.

The present paper reports results indicating that there are marked regional differences. It is possible that the aforementioned discrepancy may be explained on this basis. It is also possible that regional effects may be confounded with systematic errors in the analytic data. The investigation reported here is an attempt to resolve these uncertainties and to explore the significance of the data as related to biochemistry and geochemistry.

The bone samples were analyzed (*4*) as ashes that had been prepared at 800°C for 12 to 24 hours. The individual bone samples were received from the contributors in various forms of preparation or in different stages of desiccation; hence, there can be no significance attached to the loss on ashing. It was felt that the most significant number that could be determined was the ratio of strontium to calcium, inasmuch as this would avoid any of the ambiguities of varying histories of preparation or variations owing to density of bone mineral.

For values above 0.35(% Sr/% Ca × 10^3) the coefficient of variation is about 9. The specimens were run in duplicate, giving a standard error of ±6.5 percent. On the 95-percent confidence level, this means an error of ± 13 percent for values of 0.35 (% Sr/% Ca × 10^3) and higher. Values below 0.35 (% Sr/% Ca × 10^3) have a higher error.

The study reported here (*5*) indicates agreement with Hodges, *et al*, (*2*), in that no effect of bone type on the ratio of strontium to calcium was observed. In addition, there is no correlation with age or sex. The main source of variation appears to be related to regional influences, because, as Table 1 indicates, there are marked differences in the means of several geographic areas. From Table 1 it can also be seen that the standard deviation varies for different regional samples. Where large variances occur, they may either be attributed to a more mobile population or to regions with diverse geologic, and consequently geochemical, environments. In some areas the ethnic diet may play an important role.

It has been established by Turekian and Kulp (*6*) that there are marked regional differences in the strontium content of almost all types of rocks composing the geologic realm. Waters draining from these rocks and plants growing in these areas would take on the strontium complexion of the locale. This has been demonstrated for a large number of elements and is a useful tool of geochemical prospecting. These regional differences are the most likely reasons for the variations in the strontium content of human bones from one area to the next.

It may be possible to check this conclusion by examining human bone tissue from a known high Sr/Ca area. The Waukesha (Wisconsin) water supply (*7*) has been found to have 30 to 50 ppm strontium and 50 to 90 ppm calcium. Hence, the region should have a very high Sr/Ca ratio. It would be interesting to check bones from such a locale.

A histogram of the strontium content of the 277 human bone specimens analyzed (Fig. 1) approximates a normal distribution. If the 277 observations can be considered a large enough sampling of the world, then it is possible to arrive at an average ratio of strontium to calcium in human bones. This average is 0.60 (% Sr/% Ca × 10^3). If it is converted to parts per million strontium on the assumption of pure calcium phosphate ash, this value is equivalent to 234 ppm. This compares closely with the value of Hodges *et al.* (*2*), but, considering the marked regional variations, it may only be fortuitous.

Turekian and Kulp (*6*) have demonstrated that the average (% Sr/% Ca × 10^3) ratio in igneous rocks ranges from 6.5 to 23.0 and in sedimentary rocks from 1.5 to 6.0; hence, it is evident that the human body markedly discriminates against strontium relative to calcium when compared to the natural environment. This has been documented by Alexander *et al.* (*8*) in the case of several small laboratory animal specimens. The mineral apatite occurring in nature, which compares in part to some portions of the skeleton, is reported by Noll (*9*) to have from 0.1 to 4.5 (% Sr/% Ca × 10^3). Hence, the vital effect of the organism is seen operative in the differentiation of strontium and calcium in human bones. Schulert (*10*) of the Lamont Observatory has demonstrated the existence of such differentiations using Sr^{85} and Ca^{45}.

It is conceivable, considering the foregoing information, that human bones that have started in the direction of fossilization should possibly show a time-dependent increase in the strontium content upon burial. Hence, it is possible that this may be used in a manner similar to the fluorine method for relative chronologies. This aspect of the study awaits further exploration.

Table 1. Summary of data on regional variations in the strontium content of human bones.

Region	No. of specimens	Average (%Sr/%Ca) × 10^3	Standard deviation	Range
Colorado	28	0.61	0.27	0.34–1.35
Texas	12	0.54	0.16	0.25–0.80
England	3	0.67	0.55	0.30–1.30
Switzerland	7	0.35	0.15	0.11–0.55
Cologne	21	0.36	0.11	0.21–0.68
Bonn	15	0.35	0.12	0.17–0.52
Denmark	2	0.89	0.23	0.73–1.05
Italy	9	0.71	0.14	0.49–0.88
Venezuela	47	0.60	0.24	0.31–1.15
Chile	47	0.62	0.16	0.20–0.94
Brazil	6	1.33	0.33	1.09–1.98
Puerto Rico	5	0.62	0.30	0.37–1.05
Vancouver	21	0.50	0.15	0.33–0.75
China	19	0.67	0.16	0.42–1.00
Japan	5	0.70	0.14	0.58–0.93
India	29	0.69	0.28	0.34–1.50
Liberia	1	1.25		

Karl K. Turekian
J. Laurence Kulp
Lamont Geological Observatory, Columbia University, Palisades, New York

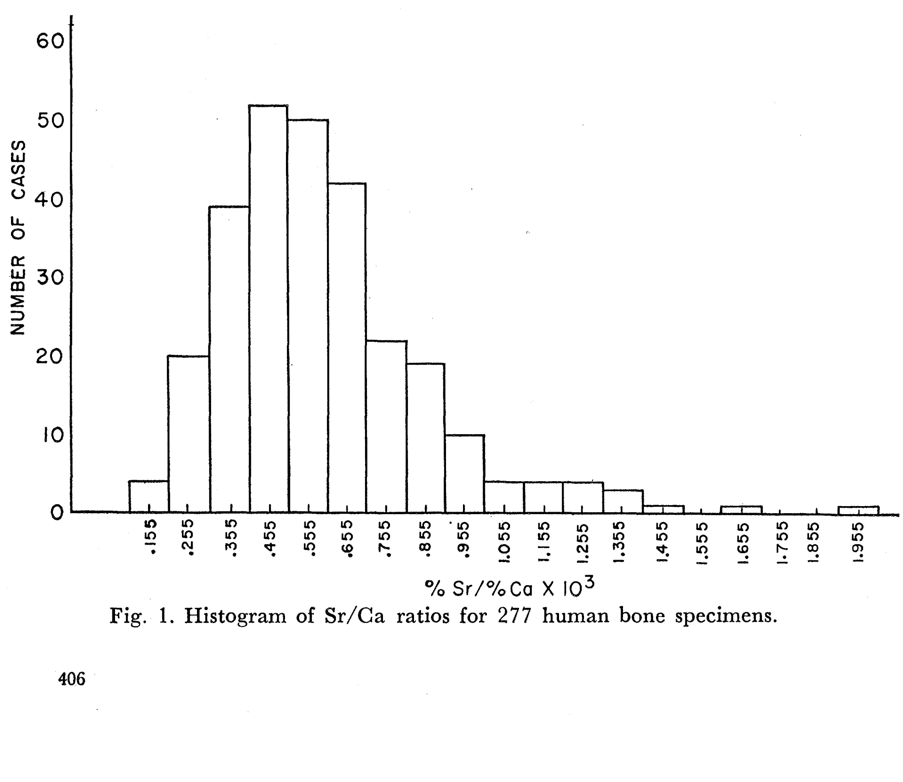

Fig. 1. Histogram of Sr/Ca ratios for 277 human bone specimens.

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4. Strontium-free calcium phosphate was prepared by ion-exchange separation. Standards for the spectrographic analyses were then made by mixing this strontium-free calcium phosphate and calcium phosphate with a known strontium content. All measurements were made using the Jarrell-Ash Ebert grating spectograph and the Jarrell-Ash 2100 micro-

photometer. The critical constants are as follows: approximately 5 mg of bone ash was weighed into necked deep crated 3/16-in. electrodes (National Carbon Co. No. 4000) and arced at 16.5 amp to completion. A glass filter, to absorb second-order interference lines, was placed before the 30-μ slit. A rotating sector allowed the transmission of 35 percent of the light. Kodak SA No. 1 film was used and D-19 developer at 3 min and 20°C. The Sr 4607 line was used as the analysis line and Ca 4579 was the monitor line.
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24 April 1956

Chemically Altered "Permanent" Behavior Patterns in Fish and Their Cure by Reserpine

In the course of enzymatic studies on the mode of action of a variety of drugs, we had occasion to use the guppy (*Lebistes reticulatus* as a test organism. It soon became apparent that this organism provided an excellent tool for the study of the effects of chemical substances on behavior, primarily because any abnormal alterations in behavior were easy to observe. We found that the guppy responded to exposure to small quantities of LSD (lysergic acid diethylamide) in a characteristic manner.

When the fish (size or sex seemed not to influence the results) were placed in a solution containing LSD (4 μg/ml at *p*H 6.5 to 7.5 and 25° to 28° C) for 1 hour and then transferred to water, they responded with a characteristic vibrating behavior. This consisted of a rapid swimming until the wall of the container was reached, at which point the fish continued to swim apparently unaware that they were not making any progress. We found that, although many behavoral changes may be observed with various substances during exposure, possibly owing to local irritation, and so on, the changes that may be observed after the fish have been returned to water are more characteristic of true behavioral change.

A good system for observing such changes consists of keeping the treated subjects in a 500-ml beaker containing 300 to 350 ml of water and held in a quiet and undisturbed environment. The action of LSD observed under these circumstances was so characteristic that it served as a method of studying the action of this substance on behavior. The effect is not so characteristic as that observed with snails, but the behavior pattern of the fish is more complex and has the advantage of being more susceptible to observable modification. Other organisms were studied: *Betta splendens* (*1*), cave-fish, goldfish, and so on. Each of these responds in a somewhat different manner, but the differing behavior patterns are all distorted aspects of normal behavior. The goldfish, for example, tends to swim backward; the cave-fish does not move at all. However, the guppy appeared to be as good a tool as the other organisms, so that it was studied more intensively.

All the kinds of fish responded to other hallucinogenic drugs (such as mescaline, yohimbine), but in a manner distinctly different from that observed with LSD and frequently with less characteristic behavior. The proper choice of experimental organism might well serve to distinguish between an array of these substances. However, this use is complicated by the fact that the response to mixtures of hallucinogenic agents produces a mixture of behavior patterns that are frequently difficult to separate. We have so far been unable to devise a quantitative method for study of the behavior pattern and are therefore limited at the moment to descriptions of its nature.

We have studied a variety of substances for their effect on the behavior pattern of the guppy without finding any that produce a pattern as characteristic as that produced by LSD. One incidental finding is of interest. The antihistaminic drugs are exceedingly toxic to the guppy and cannot be used in concentrations higher than 10^{-5} or $10^{-6}M$.

Because of the antagonism between LSD and serotonin, we attempted to antagonize the LSD effect in the guppy by previous or simultaneous exposure to serotonin. This treatment had no observable effect on the LSD response. We therefore studied other indoles in attempts to antagonize LSD. We found that 1 hour's exposure to indole or tryptamine ($10^{-4}M$, $10^{-3}M$ is toxic) followed by exposure for 1 hour to LSD (4 μg/ml), far from antagonizing the effect of LSD, tended to prolong it markedly.

In a variable number of the specimens treated with indole plus LSD (ranging from 10 to 60 percent of the fish exposed) abnormal behavior usually remained for as long as a week after treatment. In some cases these abnormalities lasted for months. The aberrant behavior consisted of periods of normal behavior interspersed at intervals (perhaps every 10 to 20 minutes) with periods of LSD-type behavior. Throughout these periods the courtship pattern of the guppy was not disturbed.

So far as our present experience goes, the permanent induction of the characteristic abnormal behavior can be accomplished by pretreatment with indole or tryptamine followed by LSD under the conditions specified here but is not induced by a variety of other substances, including an array of indoles, for example, serotonin. Indole or tryptamine alone (at $10^{-4}M$) has very little effect on repeated exposure, and the LSD symptom may be induced by repeated treatments without becoming permanently established. It appeared, therefore, that, by treatment with indole and LSD, we had influenced the behavior pattern of a living creature in a "permanent" fashion and that one could, by chemical means, set up within a living creature a behavior pattern which remained long after the substances causing it had been removed from the environment. The behavior pattern consisted of an aspect of normal behavior conducted in an exaggerated and abnormal manner.

We have had many fish whose abnormalities lasted for many weeks. If such fish were treated with reserpine (20 μg/ml for 3 days) their behavior pattern returned to normal and remained normal subsequently. The cycle of chemical induction of abnormal behavior and reversion to normal with reserpine may be repeated, but we have the impression that the fish are more sensitive to a second treatment. Chlorpromazine proved to be very toxic to the fish; hence, the lack of effect in normalizing behavior may be owing to the low concentrations that it was necessary to use.

It appears, therefore, that one can cure this chemically induced abnormal behavior by a further chemical treatment. The fact that the substances employed have an action in human behavior (*2*), which may well be a counterpart of their action in the fish, would seem to be more than fortuitous and would suggest that fish might be an important tool for the study of these effects.

Doris L. Keller
Wayne W. Umbreit
Merck Institute for Therapeutic Research, Rahway, New Jersey

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13 June 1956