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BACTERIAL MUTATION STUDY (U)

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EXECUTIVE SUMMARY (U)

The experiment presented in this document was a conceptual replication of reported work in the parapsychological literature, claiming positive statistical evidence for psychoenergetic interactions with biological systems. Both the energetic and informational aspects of human interaction with bacteriological systems were examined, with the ultimate objective of determining, to first order, whether biological systems can be employed as psychoenergetic "intrusion detectors."

(U) There were two principal experimental hypotheses under consideration. The first, which will be referred to as the Intuitive Data Sorting (IDS) hypothesis, posits that individuals are able to identify or "sort out" locally-deviant subsequences contained within a larger random sequence using psychoenergetic means. In our experiment, an IDS hypothesis predicted that individuals would be able to identify psychically--from a set of test tubes with a normal statistical spread of mutation rate--subsets of test tubes either with slightly higher or slightly lower average mutation rates than the overall mutation rate for the entire set. Because an IDS mechanism appears to be predicated on an individual's ability to gain information about a system psychoenergetically, it is thought to involve *informational processes* primarily.

(U) The second experimental hypothesis, which will be referred to as the Remote Action (RA) or IDS Unfavorable (IDSU) hypothesis, postulates that certain individuals are able to effect either a predetermined increase or decrease in a given samples's mutation rate, by somehow "mentally" causing physical (e.g., genetic) changes in the bacteria. Because an RA mechanism appears to be predicated on an individual's ability to effect physical changes in a system psychoenergetically, it is thought to involve *causal or energetic processes* primarily.

A total of seven subjects contributed six sessions each: three sessions were designed to test the IDS hypothesis, and three were designed to test the RA hypothesis. In all sessions, the subject was confronted with nine test tubes, which were visible inside a locked, environmentally-stable ice chest. The tubes contained dilute solutions of the bacterium *Salmonella typhimurium*. The bacteriological preparations were carried out by SRI's Microbial

Genetics Department, which routinely uses the Ames *Salmonella* assay that was adapted for use in this study.

(U) In the IDS sessions, the subjects were able to choose three test tubes in which they wished to promote the mutation rates psychoenergetically (high aim), three tubes in which they wished to inhibit mutation rates (low aim), and three that they wished to leave "uninfluenced" as controls (no aim). In all of the RA sessions, Tubes 1, 2, and 3 were predetermined as the low-aim tubes (the subject would attempt to inhibit mutation rates); Tubes 4, 5, and 6 were the no aim controls; and Tubes 7, 8, and 9 were the high-aim tubes (the subject would attempt to promote mutation rates). The basic premise in comparing the IDS and RA conditions is that the subjects were given the opportunity to select high-versus-low mutation rates from a natural spread of nine in the IDS sessions. Given the predetermined tubes of the RA sessions, however, the subjects were required to cause physical changes in the bacteria, in order to achieve the desired high-versus-low mutation rates.

(U) The overall result of the experiment showed weak statistical evidence that individuals are able to sort bacteriological samples according to mutation rate--that is, a $p \leq 0.05$ was obtained overall in the IDS sessions for the mutation rates of the low-aim test tubes being lower than the no-aim controls. Statistical significance was not achieved in any of the other IDS conditions (i.e., for no-aim mutation rates being less than high aim or for low aim being less than high aim). There were no significant differences for various aims observed in the RA condition. It must be concluded, therefore, that while there was some evidence that subjects are able to gain information psychoenergetically about the mutation rates of *Salmonella*, there was no compelling evidence that subjects are able to cause physical perturbations in these bacteria.

According to criteria set forth in the beginning of this study, a physical system will not be considered a candidate intrusion detector unless there is clear evidence that it is registering energetic effects (i.e., physical perturbations) concomitantly with psychoenergetic intent. To first order, therefore, it must be concluded on the basis of this one experiment that the *Salmonella* bacterium does not appear to be a promising intrusion detector.

Because this is the only known experiment of its kind using *Salmonella* bacteria as the target biological system, replication is strongly recommended--both to verify the

robustness of the IDS capability, and to evaluate definitively the efficacy of using *Salmonella* as an intrusion detector.

I OBJECTIVE (U)

The objective of this subtask was to determine the veracity of the claims in the parapsychological literature regarding psychoenergetic interactions with biological systems. A conceptual replication of the most promising of these earlier claims was undertaken, as a means to examine whether biological systems register physical effects concomitantly with psychoenergetic "intent" by an observer. This initial experimental effort was an attempt to determine, to first order, whether biological systems can eventually be employed as psychoenergetic "intrusion detectors."

II INTRODUCTION (U)

One of the ultimate applications goals of psychoenergetic phenomena is the determination of whether psychoenergetic intrusion can be detected, and whether countermeasures exist against such intrusion. From a phenomenological perspective, the term *psychoenergetic intrusion* can entail what appears to be either energetic or informational processes, or both, as indicated by the following set of operative definitions:

- The direct perturbation of physical systems that appear to be well shielded against, or otherwise inaccessible to, human influence (energetic).
- The psychoenergetic acquisition of information thought to be secure against access (informational).
- The perturbation of a physical system that occurs indirectly as a result of an individual's attempts to acquire information through psychoenergetic means (energetic and informational).

Only those intrusions that entail causal interactions with physical systems are likely to be detected. A physical system will not be considered a candidate intrusion detector, therefore, unless it registers energetic effects directly (as a result of intentional perturbation), or indirectly (as a result of concomitant acquisition of information).

In the parapsychological literature, the energetic manifestations of psychoenergetic intrusion are variously referred to as remote action (RA), remote perturbation (RP), psychokinesis (PK), telekinesis (TK), and so forth; informational processes are most often referred to as remote viewing (RV), clairvoyance, precognition, and the like. The term *countermeasures* may be defined as the shielding or jamming of psychoenergetic intrusion by either physical or mental processes.

Before the higher-order problem of countermeasures can be addressed, experimental verification of the existence of psychoenergetic intrusion must first be obtained. Detection of the putative energetic aspects of psychoenergetic intrusion can be accomplished most directly by designing experiments in which an individual's primary task is to actively attempt to cause perturbations in various types of physical systems. Numerous RA

experiments of this type, using a wide variety of physical systems, have been cited in the parapsychological literature.

(U) One category of candidate target physical systems is biological systems; the precedent for using these in RA experiments has been well established. Of particular interest (because of its similarity to the experiment detailed in this document) is Carroll B. Nash's experiment involving the psychokinetic control of bacterial mutation.* The published abstract of the Nash experiment is provided here:

> *Three experimenters each tested 20 subjects not known to be psychically gifted. Because of procedural errors, results were obtained for only 52 subjects. Each subject was tested in a single run with a separate set of nine tubes of a mixed culture of lac-negative and lac-positive strains of Escherichia coli. Mutation of lac-negative to lac-positive was mentally promoted in three of the tubes, mentally inhibited in three, and three of the tubes served as controls. The mutant ratio of lac-positive to total bacteria was greater in the promoted than in the inhibited tubes, with two-tailed $p < 0.005$; less in the inhibited tubes than in the controls, with two-tailed $p < 0.02$; and greater in the promoted tubes than in the controls, although not significantly so. The results are interpreted to suggest that the rate of bacterial mutation was psychokinetically affected.*

The experiment described in this report also undertook to investigate psychokinetic influence on bacterial mutagenicity, but it differs significantly from the Nash experiment in certain of its experimental protocols and underlying theoretical assumptions. The overall objective was also different than that of the Nash experiment in that the SRI study is concerned with providing a "first order" examination of the existence of psycho-energetic intrusion detection with biological systems.

*(U) Nash, C. B., "Psychokinetic Control of Bacterial Growth," *Journal of the Society for Psychical Research*, Vol. 51, pp. 217–226 (1982).

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with medium containing histidine), and selective minimal glucose plates (i.e., plates with medium lacking histidine) to allow for the growth and appearance of bacterial colonies.

2. (U) Procedural Terms

The following are the most common procedural terms:

- **Subject**--One of seven volunteers who undertook to psychoenergetically influence the mutagenicity of the bacterial samples.
- **Monitor**--The individual recording the events that transpired during an experimental session, and supervised the subject's activities.
- **Technician**--The microbiologist who was responsible for all aspects of the pre- and post-session preparation of the biological samples.
- **Session**--A single sitting in which the subject attempted to (1) increase the mutation rate of bacteria placed in three test tubes, (2) decrease the mutation rate of those placed in three different test tubes, and (3) leave yet a different group of three uninfluenced as "controls." Each of the seven subjects contributed six such sessions.
- **Trial**--An attempt by a subject to psychoenergetically influence (or not influence, as in the case of control test tubes) the bacterial culture in a single test tube. There were nine such trials in each experimental session.
- **Controls**--Two types of controls were employed in this experiment: intrasession and extrasession. Intrasession control test tubes consisted of three bacterial test tubes, which the subject was instructed **not** to attempt to actively influence, from among the set of nine session test tubes. Extra-session controls consisted of two tubes per session that were prepared by the technician in exactly the same manner as the session test tubes, but were not used as part of the experimental session set of nine tubes. The extrasession controls remained at all times in the Microbial Genetics Laboratory, and provided the requisite data for establishing an independent measure of mutation rate.
- **Feedback**--A drawing presented to the subject that indicated his/her performance on a given session. Feedback for a given session was typically administered prior to the start of the subject's next session.

B. (U) Biological Background

In this section, we will give a general overview of the Ames *Salmonella* assay that is used routinely by SRI's Microbial Genetics Department, and that was adapted for use in this experiment to study psychoenergetic effects on mutation frequency.

(U) The heritable material of living organisms is contained in the DNA (RNA in some viruses), a large molecule so constructed that it can replicate itself in a most exact fashion one cell generation after another. This is the basis of biological continuity and unity. It is also, however, the basis for biological diversity, which occurs through mutations. Each mutation alters the action of a specific gene, which is a genetic entity with its own specific end product, or protein. Genes are very stable structures, but each has its own spontaneous mutation frequency. The probability that a spontaneous mutant cell will be obtained every time a cell divides is constant, provided the environmental conditions are unchanged. Changes in the environment are known to influence the mutation frequency. Such changes include the presence or absence of certain trace elements (e.g., selenium), plus the presence of physical or chemical agents (mutagens).

Bacteria provide a convenient way to study mutations because millions of cells can be grown in a very short period of time. Over the past few years, several bacterial assays have been developed to screen chemicals for their ability to induce mutation. Because there is a close correlation between mutagenesis and carcinogenesis, such mutagenicity assays are very often used together with the *in vitro* tests that employ single microbial and/or mammalian cells, as well as *in vivo* tests that employ multicell organisms from insects (fruit fly) to mammals (rodents). One of the best known bacterial mutagenesis assays is the *Salmonella*/ mammalian microsome histidine reverse mutation assay developed by Dr. Bruce Ames at the University of California in Berkeley. The Microbial Genetics Department at SRI International is using this assay system on a daily basis for Government agencies and commercial clients to determine the mutagenic potential of chemicals; they have performed such testing over a period of more than 10 years.

(U) The *Salmonella* assay employs several tester strains of *Salmonella typhimurium*, each with a unique specificity for detecting chemical mutagens. The *Salmonella* strains, under optimum conditions, have a generation time of less than 30 minutes. The bacterial strains are unable to grow in the absence of the essential amino acid histidine because of a mutation in one of the genes that is needed for histidine synthesis. When these bacteria are plated on defined selective medium having little or no histidine, little or no growth occurs except those few bacteria that spontaneously mutate back to histidine independence (ability to grow in the absence of histidine). In this case, a nonfunctional gene product is reverted back to a functional one. This event allows the mutant cells to grow and divide. Because a mutation is stably inherited, all progeny of the mutated cells retain the ability to grow in the absence of

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histidine. Distinct individual colonies will appear on the solid selective growth medium, with each colony containing billions of progeny of the spontaneously mutated cells. Exposure of the bacteria to a chemical mutagen will result in an increased number of colonies appearing on the solid selective growth medium, due to an increase in mutation induction.

(U) In the Ames *Salmonella* assay, a small amount of histidine is added to the growth medium to allow for a few cell divisions of all the plated histidine requiring mutants ($\sim 10^{*}$). Such growth is often necessary for chemical mutagenesis to occur. The results of the *Salmonella* assay are usually expressed in terms of the number of revertant colonies per amount of chemical added to the selective growth medium, which is usually delivered to the plate in 25-ml volumes. Because of the presence of limited histidine in the selective medium, the results of the Ames *Salmonella* are considered "semiquantitative," since residual growth on all plates (control as well as chemical treated) does not allow for quantitative survival determination. A quantitative mutation frequency, however, can be determined. It is more labor intensive than the standard Ames assay, because survival determination requires diluting of the cell cultures, and a different growth medium is needed for determining (1) the mutant fraction and survivors for each of the controls, and (2) the different exposure concentrations of the test chemical. The mutation frequency is defined in terms of number of mutants per given number of surviving cells, usually per 10^6 cells.

Because of its simplicity and the rapid response time of about two days, the Ames *Salmonella* assay can readily be adapted to study the effect of RA on the mutation frequency. Such an adaptation was established by SRI's Microbial Genetics Department for use in this experiment; a detailed discussion of the specific biological procedures that were followed can be found in Section D, *Protocols*.

C. (U) Experimental Design

1. (U) Conceptual Replication

The experiment undertaken in this study represents a conceptual replication of the Nash experiment described in our Introduction chapter. The replication presented here is termed **conceptual**, because several of the experimental details of the Nash experiment have been changed and improved. First, two potential mechanisms have been postulated that could account for the acquisition of a statistically significant effect--that is, an IDS hypothesis has been advanced, in addition to the more established RA hypothesis. Second, *Salmonella*

typhimurium rather than *Escherichia coli* were used as the target bacterial cultures. Because this particular species of *Salmonella* is used most frequently by SRI's Microbial Genetics Department in toxicity studies, its behavior is particularly well understood in terms of assay conditions and experimental protocols. Finally, the Nash analysis was extended to include multiple analyses of variance.

As in the Nash experiment, nine test tubes filled with dilute bacterial culture were used per session. Mutation from histidine dependence to histidine independence was mentally promoted by the subject in three of the tubes, mentally inhibited in three, and the remaining three tubes served as controls. For the purposes of obtaining baseline data, two additional control test tubes (for a total of eleven altogether per session) were prepared in the same manner as the session test tubes, but were kept in the Microbial Genetics Laboratory.

2. (U) Model Testing Criteria

a. (U) The IDS Model

(U) As mentioned previously, there were two primary models under investigation in this experiment. A pivotal concept to the first, or IDS favorable model, is **freedom of choice**: namely, that by using some type of psi-mediated informational processes, subjects have the opportunity to select out locally-deviant subsequences from a larger random sequence. For example, in half of the sessions, the subjects were allowed to select the three test tubes in which they wished to promote mutation, and the three test tubes in which they wished to inhibit mutation. A statistically significant deviation from mean chance expectation (MCE) in this condition, therefore, could be interpreted theoretically in two ways: (1) the subjects somehow mentally "forced" genetic changes to occur in the bacteria in accordance with their desires to either promote or inhibit mutation rates (the RA hypothesis); or (2) given the natural spread of mutation rates in a biological system, the subject was able to psycho-energetically **sort** those test tubes containing bacteria with high mutation rates from those tubes containing bacteria with low mutation rates (a session-by-session IDS hypothesis).

b. (U) The RA or IDSU Model

(U) The second model under investigation has been termed the Remote Action (RA) or Intuitive Data Sorting Unfavorable (IDSU) model. In this condition, the conduits by which either the subject or experimenter are able to **select** test tubes are rendered

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the seven had been participants in previous psychoenergetic experiments--i.e., all four had demonstrated some ability in remote viewing. One of these four subjects also scored significantly in an earlier SRI random number generator PK experiment, and another had demonstrated some ability previously in Computer-Assisted Search (CAS) tasks. All of the participants were SRI employees: one was a statistician, two were secretaries, and the remainder were research professionals in either physics or computer science.

b. (U) Experiment Site Locations

(U) For reasons stated in Section C.2.a, it was determined that the biological technician should be kept entirely blind as to all facets of the experiment, and that, in order to facilitate this situation, the psychoenergetic testing should occur in a location that was different from the one used for the biological preparations. The Microbial Genetics Laboratory, therefore, was used for all aspects of preparation of the biological cultures, and a room in another building at SRI was used for the psychoenergetic sessions.

c. (U) Hardware Construction

(U) Once it had been determined that two separate facilities were necessary for conducting the experiment, a container had to be constructed that was suitable for transporting the biological samples from the Microbial Genetics Laboratory to the psychoenergetics facility. There were three primary factors that dictated the design of the container: (1) the biological samples had to be protected from extreme variations in temperature; (2) the samples had to be protected from sunlight; and (3) the container had to be lockable.

(U) To control against extreme variations in temperature, which can greatly affect the mutagenicity of *Salmonella*, a Coleman® ice chest was chosen as the transport container. Triple-paned insulated glass windows were specially installed in the top and front side of the ice chest to allow an unobstructed view of the experiment test tubes. Because sunlight also affects the mutagenicity of the bacteria, a tarpaulin was used to completely cover the cooler during transport between the biological laboratory and the psychoenergetics facility. A lock was installed; the key was retained exclusively by the biological technician, to preclude the possibility of tampering with the biological samples once they had been removed from the Microbial Genetics Laboratory.

2. (U) Presession Protocols

(U) A series of activities took place prior to the start of every experimental session. First, the experiment monitor identified the session type from the fixed session sequence, "aababb," (cf. Section C.2.b), to determine whether the session would be an IDS favorable or an IDS unfavorable (RA or IDSU) condition.

Second, the technician in the Microbial Genetics Laboratory prepared the bacterial cultures for the session (see Appendix). Eleven numerically-labelled, sterile, 16-×-150-mm test tubes were aseptically filled with 2.5 ml of glucose minimal broth. Fifty µl of a 37°C overnight culture of strain TA100 of *Salmonella typhimurium* was then added to each tube.* In a standardized manner, the first nine tubes were arranged in a test-tube rack, which was placed in the specially designed ice chest, and then locked. The remaining two control cultures were shielded from visible light by a covering of aluminum foil, and were maintained at room temperature in the Microbial Genetics Department laboratory.

(U) The ice chest, with its enclosed cultures, was placed on a cart and covered with a tarpaulin to ensure that the mutation rate of the cultures was not affected by sunlight during transportation from the laboratory to the experimental facility.

(U) Third, the experiment monitor transported the covered ice chest on the cart from the biological laboratory to another facility at SRI, where the psychoenergetic portion of the experiment was performed. Prior to the arrival of the subject, the monitor wheeled the ice chest into a room equipped with a table and two chairs. The ice chest was then uncovered and positioned in such a way that a seated subject could readily view the nine numbered test tubes through the glass.

3. (U) Session Protocols

(U) For other than the first session for each subject, a session usually commenced with feedback to the subject of the previous session's results (to be discussed in "Postsession

*(U) It should be noted that there was no visible evidence of "cloudiness" caused by the bacterial culture in any of the prepared test tube solutions. The appearance of the liquid was uniformly that of clear tap water. Thus, there were no visual cues available to the subject, as to which test tubes might contain greater amounts of the bacterial culture.

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the appropriate test-tube-selection numbers. The order of selection and an estimate of the duration of effort per each test tube were noted in the "Comments" section. Upon debriefing the subject at the end of a session, the monitor also recorded any comments the subject wished to make regarding possible strategies employed in the performance of the task, and any personal statements the subjects wished to volunteer pertaining to their state-of-mind, health, and so forth.

4. (U) Postsession Protocols

(U) At the conclusion of the session, and after the departure of the subject from the psychoenergetics facility, the monitor once again covered the ice chest, then transported it on the cart back to the Microbial Genetics Laboratory.

(U) The microbiologist removed the nine bacterial cultures from the ice chest and placed them, together with the additional two *extrasession* control cultures, in an incubator (G24 Environmental Incubator Shaker, New Brunswick Scientific Company, Inc., Edison, New Jersey). The bacterial cultures were shielded from visible light by aluminum foil, and grown with gentle shaking (100 rpm) for about 24 hours.

(U) Following the incubation period, testing of the eleven bacterial cultures to determine the extent of mutation induction was initiated. The testing was divided into two parts:

- Quantitation of number of cells plated, which measures the number of plated cells that are able to form colonies (CFU) on medium containing histidine (yeast complete medium).
- Quantitation of mutant cells, which measures the number of cells that are able to grow in the absence of histidine.

(U) The quantitation of CFU was accomplished according to a standardized set of laboratory procedures. First, each of the eleven bacterial cultures (i.e., the cultures contained in the nine-session test tubes plus the two controls) was serially diluted by combining 0.20 ml of the culture with 1.80 ml of sterile saline until an overall million-fold dilution was obtained (10^{-6}). Complete medium plates were then divided into three sections with a marking pen, and a 10-µl aliquot of the 10^{-4}, 10^{-5}, and 10^{-6} dilutions were then delivered in triplicate to the appropriate sections on the plate. The 10-µl spots were allowed to dry on the surface of the solid medium in the plates. The plates were then incubated at 37°C for up to 24 hours

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There was no compelling evidence, however, that subjects are able to interact causally with this particular biological system.

(U) Tables 2 through 8 display the data contained in Table 1 in a subject-by-subject format. One subject (310) produced strong effects in the IDS condition: his low-aim condition was significantly lower than his high-aim condition ($p \leq 0.003$), and his low-aim condition was also significantly lower than the no-aim condition ($p \leq 0.018$).

(U) In addition to the various t-tests, a multiway analysis of variance (ANOVA) was conducted as a second form of analysis (post hoc). Aim (low, no, and high) and condition (IDS and RA) were used as the two "main effects" for the analysis. When the ANOVA examines one "main effect," it sums all the data in the other "main effects." For example, to examine the IDS and RA condition, the ANOVA sums across all aims. Likewise, to examine an aim effect, the ANOVA sums across the IDS and RA conditions. No significance was anticipated in these two dimensions, and none was observed. Significance was observed, however, in the interaction term between the IDS and the RA condition ($p \leq 0.05$), which may indicate that there is some difference between the IDS and RA conditions when examined as a function of aim. This does not imply that IDS or RA is "more significant." It should be noted that the interpretation of the ANOVA interaction term has been traditionally difficult, and Rosenthal has suggested that ANOVA with more than one "main effect" should not be used in the social/psychological sciences.* The analysis has been included here merely for the sake of completeness.

In summary, this experiment has produced a relatively weak, but statistically significant effect, which most readily supports the conclusion that subjects are able to acquire information, psychoenergetically, about the mutation rates of *Salmonella*, but are unable to cause physical perturbations in these bacteria. To reiterate the criteria set forth in the *Introduction*, a physical system will not be considered a candidate intrusion detector unless it registers energetic effects directly (as a result of intentional perturbation), or indirectly (as a result of concomitant acquisition of information). To first order, therefore, it must be concluded on the basis of this one experiment, that the *Salmonella* bacterium does not appear to be a promising intrusion detector.

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*(U) R. Rosenthal and R. Rosnow, *Essentials of Behavioral Research*, p. 254 (McGraw Hill Book Co., New York, 1984).

Table 2

(U) NORMALIZED MUTATION RATES $\times 10^{-6}$
(Subject 164)

Condition	Aim		
	Low	No	High
Remote Action (RA)	3.00	2.76	3.54
Remote Action (RA)	3.69	2.90	2.59
Remote Action (RA)	3.06	3.09	3.09
Mean	3.25	2.92	3.07
Statistics*	t (Low < No) = −1.430 n.s.		
	t (No < High) = 0.484 n.s.		
	t (Low < High) = −0.475 n.s.		

Condition	Aim		
	Low	No	High
Intuitive Data Sorting (IDS)	2.99	2.89	3.26
Intuitive Data Sorting (IDS)	2.79	3.54	2.93
Intuitive Data Sorting (IDS)	3.57	2.68	2.83
Mean	3.11	3.04	3.01
Statistics*	t (Low < No) = −0.310 n.s.		
	t (No < High) = −0.112 n.s.		
	t (Low < High) = −0.475 n.s.		

* Degrees of freedom = 16.

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Given the weak statistical nature of the effect and the potential importance of intrusion detection, replication is recommended for a variety of compelling reasons. First, there are a number of proposed methodological changes to this experiment (as discussed in Chapter V) that would in all likelihood enhance the robustness of the effect. From this perspective, the experiment might legitimately be considered a pilot study. Second, this is the first experiment of its kind that has used *Salmonella* as the target biological system;