Etiology of Romero-Fulci Disease: The Case for Prions

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A recent flurry of interest in Romero-Fulci disease (RFD) among the general public has resulted in the premature promulgation of the *Solanum* hypothesis as the RFD etiology generally accepted among experts. A brief review of the pertinent facts concerning *Solanum* and RFD is conducted, with special consideration paid to the importance of rigorous determination of cause under the Koch-Smith postulates. The problems with the *Solanum* hypothesis are exposed, and a novel etiology involving an unknown fast-acting prion is proposed. Lastly, some considerations regarding the future of the field are elucidated.

INTRODUCTION

The recent popular treatment by Brooks (1) has done much to enervate the nascent field of zombie biology. Besides serving as a cogent and timely review of the scattered ethnological and anthropological literature, Brooks' volume addresses the problem of nomenclature, dispels many myths and misconceptions about the symptoms whereby RFD presents, and lays out an epidemiological framework for the rational treatment of the zombie outbreak as a public health issue. But, perhaps more importantly than any of these, the book heralds a sea-change in public perceptions of the zombie phenomenon, a dredging-up of the problem from the mire of superstitious dread which has long subsumed it (and inhibited real progress toward its solution). This cultural change could not be better reflected than by this journal's forthcoming [Ed: With this very issue!] change of name from "Journal of Zombie Studies" to "Journal of Zombie *Science.*" Even the most dogged of the social-*gestalt* theorists have come to acknowledge that, whatever the broader implications of RFD may be, the individual zombie is still a physical system governed by the same scientific laws of cause and effect that organize the rest of the known universe.

However, like most popularizations, Brooks' treatment has been a mixed blessing to professionals in the field. Although he has contributed immeasurably to the legitimacy of the discipline in the minds of the tax-paying public, Brooks is guilty of the popularizer's sins of painting *too* clear a picture of the growing body of expert knowledge, of oversimplifying to the point of error, and of resurrecting ghouls which, among the initiated, were considered dead and safely buried years ago. As an example of the latter, consider Brooks' dismissive treatment of the terminal-i/terminal-e question. Instead of promulgating the established convention (2) of using *zombi* to refer to the psychosocial construct created by deliberate tetrodotoxin poisoning in certain West Indian cultures (3) whilst reserving *zombie* for victims of Romero-Fulci disease, Brooks adopts the cumbersome and politically inappropriate "voodoo zombies" to refer to *zombis* after Davis and simply "zombies" to refer to RFD sufferers. Given the sheer quantity of ink splattered in the literature over the past two decades in settling this issue, Brooks' conflation of the two terms (which is bound to generate even more confusion) is well nigh unpardonable.

Contrariwise, Brooks treats as *faits accompli* several scientific questions which are actually far from settled. Chief among these is the identification of the virus *Solanum vanderhaven* as the sole causal agent in RFD, in spite of the absence of conclusive data to that effect. There is, in fact, a growing body of evidence to suggest that the primary disease-causing agent in RFD is actually not a virus at all, but a prion (proteinaceous infectious ion) similar to those which cause bovine spongiform encephalothapy (BSE or "mad cow disease"), Creutzfeldt-Jacob disease (CJD), and *kuru.* Experts should already be familiar with the salient issues of the debate, but in light of the recent publication of Brooks' work, a review of the known facts is in order.

REVIEW

RFD is highly transmissible by contact between the blood of an uninfected person and RFD-contaminated tissue, blood, mucus, or saliva. Like rabies, the usual mode of transmission is by bite. Onset of symptoms is extremely rapid, beginning with fever, aches and pains, and vomiting, and progressing through dementia, loss of motor coordination, paralysis, coma, and clinical death within 24 hours. Reanimation usually occurs within three hours of cardiac arrest, but has been known to happen as little as one minute thereafter. Scattered reports exist of subjects reanimating with

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essentially zero refractory period (4). Thereafter, reports of the syndrome's details vary widely, except in one respect: A zombie has an insatiable desire to cannibalize uninfected persons, which tends to have the effect of creating more zombies. Those who are bitten and escape eventually succumb to RFD and become zombies themselves; those who do not escape are often entirely consumed. Sometimes, the focus of the feeding frenzy is reported as particularly brain matter, with zombies rejecting the softer tissues in favor of a direct biting attack upon the skull (5). Accounts of retained cognitive function range from none (Brooks et. al.), to slight (6), to full (7). Likewise with retained motor function: The popular perception is of zombies as shambling, clumsy creatures, but the last two decades have seen an increasing number of reports of zombies running (8), climbing ladders, firing weapons (Romero 1984), and performing other relatively complex tasks.

An infectious isolate from RFD-contaminated tissue was first identified by the Swedish physician Jan Vanderhaven in 1913. Working out of a field laboratory in Paramaribo, Surinam, investigating what was believed to be an anomalous leprosy, Vanderhaven demonstrated conclusively that injections of filtered alcoholic extracts of zombie blood or saliva caused a fatal infection (*sans* reanimation) in *Cavia porcellus*. Brooks produces a passable translation of the now-famous passage from Vanderhaven's notebook:

> Experiments with alcohol, formalin, and heating tissue to 90 degrees centigrade have erased the possibility of bacteria...I must therefore deduce that the agent can only be contagious living fluid...dubbed "Solanum." (p. 217)

The understanding of *contagium vivum fluidum* as a distinct non-bacteriological pathogen was relatively novel at the time, and the contemporary distinctions we acknowledge between viruses, virions, viroids, and prions were decades in the future. Although a novel virus *per se* was subsequently identified in Vanderhaven's original tissue samples by Luria in 1947 and assigned *Solanum*, to date only one of the four Koch-Smith postulates have been satisfied in attributing RFD to this organism, and that not at all conclusively. For the unfamiliar, the Koch-Smith postulates are the accepted criteria for the reliable assignation of cause of a particular disease to a particular pathogen. Briefly, they are

i. The agent must be present in every case of the disease.
ii. The agent must be isolated from the host and grown *in vitro*.
iii. The disease must be reproduced when a pure culture of the agent is inoculated into a healthy susceptible host.
iv. The same agent must be recovered once again from the experimentally-infected host.

Of *Solanum*, it is today possible reasonably to claim only that postulate (i) has been satisfied with respect to RFD: The agent has been identified in tissue samples from every well-studied case of the disease. These, however, can be counted on the fingers of both hands. Experts are all too familiar with the difficulties of obtaining RFD-positive tissue samples for laboratory study. Besides the logistical challenge and physical danger associated with collecting wild-type specimens, the political pressures against the preservation of any potentially-infectious material in the case of even a Class I outbreak are usually insurmountable. Even if in the future, however, a larger data set eventually proves conclusively that *Solanum* is always present in RFD-contaminated tissue, the positive assignation of *Solanum* as the cause of RFD will still await satisfaction of Koch-Smith postulates (ii), (iii), and (iv). Without these, the consistent association of the virus with the disease might just as readily demonstrate that the disease causes the virus (perhaps as an opportunistic infection , e.g.) as the converse.

Numerous attempts (Brooks 2-3) to produce *in vitro* cultures of *Solanum* have failed. As with human parvovirus B19, no convenient cell culture technique has been forthcoming, and although more sophisticated contemporary methods such as synchronized culture may hold some promise (9), their implementation awaits the availability of viable wild-type specimens.

Laboratory studies are further complicated by the lack of suitable animal models. While it has been known since Vanderhaven that direct inoculation of animals with RFD-contaminated blood, sputum, or saliva produces fatal infection with symptoms similar to those of RFD, the characteristic symptom, viz. reanimation, is absent in every studied animal system. Even more damning is the fact, which Brooks himself acknowledges, that both pre- and post-mortem infected animal specimens are not themselves infectious. No live *Solanum* virus has ever been recovered from an exposed animal. Indeed, it is entirely reasonable to argue that the term "infection" is incorrectly applied to the fatal syndrome caused by animal inoculation with RFD-positive tissue. "Poisoning" is perhaps more

accurate, and because all animal tests have been conducted directly from tissue or from fresh extracts of tissue, the hypothesis is just as plausible that death results not from *Solanum* but from some unidentified "cofactor" present in the tissue samples.

THE CASE FOR PRIONS

Figure 1 shows a cortical cross-section, magnified 400X, taken from the brain of a 26-year old white male killed by iatrogenic Creutzfeldt-Jacob disease (CJD) (10). Figure 2 shows an analogous cross-section from the brain of a 25-year old white male who died of Romero-Fulci disease (RFD) during the London outbreak of 2004 (11).

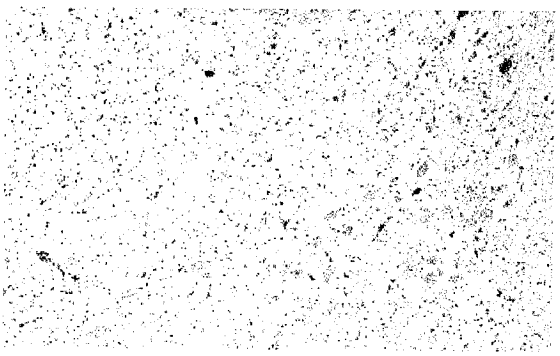

Figure 1. Neuronal vacuolization in iatrogenic CJD .

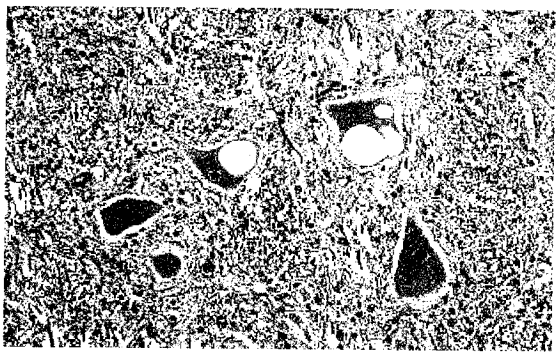

Figure 2. Neuronal vacuolization in RFD.

Examination of the two images shows a neurodegeneration in RFD which is in its character, if not in its degree, very much like the vacuolization demonstrated by the classic spongiform encephalothapies, which are without exception diseases caused by prions.

For the uninitiated, a prion is simply a misfolded protein which, by some mechanism as yet unknown to science, is capable of reproducing itself by reconfiguring susceptible host proteins in its own image. Prions were first hypothesized as a possible cause of scrapie in sheep by Stanley Prusner in 1982 (12), and first isolated and cloned by Oesch et. al. in 1985 (13). Interest in prions and prion diseases skyrocketed in 1996 with an official proclamation in the United Kingdom that, in the absence of a more likely explanation, the probable cause of a statistically-anomalous outbreak of new-variant Creutzfeldt-Jacob disease (vCJD) among Britons was exposure to bovine spongiform encephalothapy (BSE or "mad cow disease"), which had been identified in English cattle as early as 1986. To date no conclusive link between vCJD and BSE exposure has been established, but compelling epidemiological evidence and public outcry have resulted in stringent new regulations to control BSE in the beef industry, as well as the destruction of more than half a million infected or potentially-infected cattle. CJD is one of four known transmissible spongiform encephalothapies (TSEs) in humans. Besides CJD and its variants, these are familiar fatal insomnia, Gerstmann-Sträussler-Scheinker syndrome, and *kuru*—all of which exhibit a general progression characterized by slow cerebral neurodegeneration culminating in death and diagnosed by vacuolization of brain matter as exhibited in Figure 1.

Besides the evident histological similarities, there are some striking parallels between the modes of transmission and symptomology of TSEs and those of Romero-Fulci disease. Here perhaps the most compelling example is *kuru*, an endogenous disorder unique to the Fore-speaking peoples of the eastern highlands of Papua New Guinea. *Kuru*, which means "trembling with fear" in the Fore tongue, reached epidemic proportions in Papua New Guinea prior to 1971, when the practice of ritual funerary endocannibalism responsible for its transmission was abolished by law. Traditionally among the Fore people the cooking and consumption of the corpse—particularly the brain—of a recently-deceased loved one was considered a gesture of respect for the departed and an integral part of the mourning process. Because *kuru's* incubation period can be as long as three decades, some older Fore who participated in cannibalistic rites are still dying of the disease. However, no young person has exhibited symptoms of *kuru* since the practice of endocannibalism was discontinued (14). Although the incubation period of RFD is radically shorter, the similarities between it and *kuru* are difficult to ignore: Both are fatal neurodegenerative diseases transmitted orally by an act of cannibalism focused particularly upon the brain matter. *Kuru* is known to be caused by prions; it therefore seems not unreasonable to propose—especially in light of the striking histological similarities exhibited in

Figure 1 and Figure 2—that the causal agent in RFD is also a prion, albeit of a hitherto unknown fast-acting variety.

THE FUTURE

Futures which include a human understanding of the etiology of RFD can be divided into two sets: Those in which *Solanum vanderhaven* is determined as the sole cause of the disease, and those in which it is not. If the former scenario is to come to pass, the agenda for present science is defined, essentially, by the Koch-Smith postulates:

1) A larger data set must verify that *Solanum* is present in every known case of RFD.
2) A workable culture technique must be developed to allow *Solanum* to be grown *in vitro*.
3) A viable animal model must be determined to demonstrate the laboratory transmission of full-fledged RFD, including reanimation, under controlled conditions.
4) Living *Solanum* must be isolated and cultured according to [1] from subjects infected according to [3].

The greatest hope for defenders of the *Solanum* hypothesis rests in the development of a working culture protocol. Only when and if a domesticated strain of the virus becomes readily available to experimenters can the question of animal models be addressed properly. Until that time, the ethical, political, physical, and scientific difficulties associated with the use of wild-type specimens will likely impede rational analysis of the question indefinitely. Even if culture succeeds, a significant possibility exists that no suitable animal model will be found. In that event, an alternative means of demonstrating controlled transmission will be necessary, which question raises a specter that has haunted zombie biologists since the inception of the discipline—that of human experimentation. The usual persistent rumors of Japanese biowarfare experiments (Brooks 220) and other secret government programs aside, however, it is unlikely in the extreme that any healthy human subject ever has been or ever will be deliberately exposed to RFD against his or her will. Although at least one prominent authority (15) has made public his willingness to volunteer for inoculation at the time of his natural death, the scientific questions which might be answered by such heroic measures may well be resolved before the legal ones which might allow them.

Among those etiologies which do *not* implicate *Solanum* as the sole cause of RFD, there are those in which the virus is determined to be irrelevant or incidental to the condition, and there are those in which it is determined to be a necessary (but not sufficient) part of a complex pathology involving one or more as-yet-unknown cofactors. The prion hypothesis is a strong contender, either as an alternative first cause or as a cofactor. If the prion hypothesis is eventually confirmed, it would be a boon not only to zombie science but to the broader field of prion biology, which is presently hampered by the long period (often several years) between agent exposure and exhibition of symptoms in a subject, which tends to make systematic laboratory study extremely tedious and expensive. The existence of a fast-acting prion agent as hypothesized here for RFD might remove this impediment and hasten the progress of ongoing research by orders of magnitude, provided a suitable animal model can be found.

Other possible causes or cofactors exist. Several reports—including George F. Romero's original observations (16)—implicate ionizing radiation in the origins of RFD outbreaks. In Romero's study, radioactive contamination of a recently-recovered space probe was proposed as a culprit, and it has long been suspected that the U.S. Air Force's research into enhanced-radiation weapons (especially neutron bombs) was halted in the mid-80s as a result of concerns over classified data suggesting that the use of such weapons might cause widespread outbreaks of RFD in combat areas (17). This idea is not incompatible with the prion hypothesis. The initial degeneration of healthy protein to the infectious prion isoform may well be caused by a molecular ionization event induced by very specific frequencies of high-energy electromagnetic radiation. Thereafter, the misfolded protein could replicate itself and infect other hosts according to the accepted model of prion transmission.

Another possibility is presented in the form of some hitherto unknown, acutely toxic chemical species. As the first draft of this paper was in review, an account was published in Russia (18) describing experiments with a synthetic compound which is reported to have produced an RFD-type syndrome at very low doses in pigeons and dogs. An English translation of the paper is forthcoming. [Ed: See next month's issue.] The compound (which the author rather dramatically christens "Death-1"), is 2,4,5-Trichloro-*m*-trioxin under the IUPAC system (Figure 3).

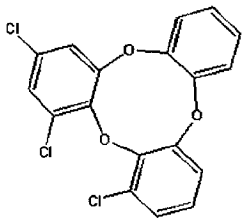

Figure 3. 2,4,5-Trichloro-*m*-trioxin (2,4,5-Trioxin).

Although it bears some structural resemblance to a class of selective herbicides developed under U.S. government contract during the mid-1960s (19), 2,4,5-trioxin appears to be an entirely novel compound. Its structure presents considerable synthetic challenges, but these are by no means insuperable. Work to verify its activity is currently underway.

Whether one, some, none, or all of these factors are eventually identified as causal agents in RFD, significant progress in the field is unlikely to occur in the absence of an adequate supply of active tissue specimens, and securing such a supply should be the first priority the integrated science of zombie biology. Fortunately, a long-running initiative in the expert community (20) to organize a coordinated government-academic effort to capture and safely preserve a stable of wild-type specimens—including recommendations for the establishment of a novel Biohazard Safety Level 5—has lately met with some success. Recently-renewed interest in RFD by the American Center for Disease Control is probably attributable to the popular success of Brooks' treatment, and whatever the flaws in his scientific understanding may be, the community will remain indebted to him for the political impact of his work.

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The Ways and Nature of the Zombi

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This article presents a review of zombiism and our personal investigations on the hitherto little-known spirit zombi. The Haitian zombi is of African origin. Numerous references to zombis or zombi-like entities are found in Equatorial and Central Africa and in the Caribbean. There are two types of zombis, the zombi of the body, or living dead, and the zombi of the soul. Both are closely related to the Haitian concept of a dual soul, which is also of African origin. Properties of the spirit zombi are described. Zombi stories or sightings may be explained by the observation of vagrant or exploited mentally ill. The various "zombi powders" so far studied seem to belong to the domain of sympathetic magic, and their pharmacological effectiveness remains to be proved.

INTRODUCTION

Zombis, or zombies, the living dead, have always created much morbid interest. Recent media events have brought the topic to the forefront again. A captivating if sensationalist and problem-