

Serendipity and New Drugs for Infectious Disease

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Abstract

Serendipity, in various shades of semantic legitimacy, is abundantly evident in the history of the chemotherapy of infectious disease. We may be on the threshold of a new era of rational drug design, but most medications for infectious diseases have arisen, and continue to arise, from chance observation, clinical experience, and the empirical search for substances active against pathogens. Chance does not produce drugs; but where chance has played a pivotal role in drug discovery, the event may be considered serendipitous to a greater or lesser degree. In a deliberate search for new drugs, it is often difficult to assess the degree to which any resulting discovery is serendipitous, and the usefulness of the term becomes debatable. Many therapeutic advances emerge from research involving animals, and a triggering “happy accident” may reside in the most basic aspects of animal care or in the most arcane knowledge of animals. The examples discussed in this article deal mostly with parasitic disease and the use of animal models in the discovery of antiparasitic agents. In this area, as in others, chance has laid the groundwork for scientific advancement and practical benefit. Although the applicability of the word serendipity to drug discovery may often be uncertain, the role played by chance should be recognized and welcomed.

Key Words: anthelmintic; antiparasitic; cedarwood; drug discovery; empiricism; ivermectin; levamisole; serendipity

Throughout history, the treatment of disease has benefited from—indeed, depended on—serendipity. In prospect, we may envision new medications arising from entirely rational design, but in retrospect, we see that they have emerged from discoveries that were, to a greater or lesser extent, serendipitous. In ancient times, presumably all of them were found as a result of chance encounters with substances, mostly of plant origin, that resulted in pharmacological effects. Those effects in turn prompted the follow-up observations and actions that were needed to result in

their acceptance as drugs. (Many, of course, were used without such confirmation, but those are not relevant here.) Over time, as folklore was slowly and incompletely overtaken by science, serendipity remained a “necessary but not sufficient” source of drug discovery.

Thus, drug discovery has historically been empirical—based on trial and error or on particular clinical observation. In modern times, the new chemical and biological sciences have given rise to rational and semirational approaches to the discovery of drugs. Their impact on the treatment of infectious disease has not yet matched that of the traditional approaches, and the focus of this essay is on empirical discovery.

Side Effects Are Sometimes Good

One aspect of empirical drug discovery involves the discovery of valuable therapeutic agents as a result of testing a candidate drug for an expected pharmacological effect and finding a quite different effect. In such a case, the test substance is then developed as a practical means of achieving the second effect. An example is seen in the history of piperazine. At the end of the 19th century, piperazine (hexahydropyrazine) was introduced as a treatment for gout because it forms a very soluble salt with uric acid. In the early 20th century this treatment was abandoned as unsatisfactory; but a pharmacist in France had noticed that although treated patients may not have lost their pain, they often had lost their intestinal worms. Prompted by this chance observation, the Wellcome organization in Britain developed piperazine citrate into one of the most widely used anthelmintics of the 20th century (Goodwin 1980). Sildenafil citrate (Viagra) provides a modern, and more famous, example of drug discovery arising from the exploitation of a side effect. In this case, the test compound was being given to patients for the treatment of heart disease, and its development for the treatment of sexual dysfunction arose from the observation that the treated patients exhibited an unexpected elevation in erectile function. de Stevens (1986) cites other examples of drug side effects that have led to scientific advances.

Although the cases described above involved human clinical trials, the same principle is applicable to drug testing in laboratory animals. One example is of special interest because of its origin in an alert and informed observation of altered appearance in a laboratory animal. In the 1930s, Otto Schaumann studied an antispasmodic compound in rats.

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The tails of treated rats promptly assumed an S-shaped curvature that had been reported previously for rats given narcotic analgesics such as morphine. Although the test compound did not appear to be structurally similar to morphine, it proved to be a narcotic analgesic (Sneader 2006). Later, as the structural determinants of morphine and its analogs became better understood, it could be seen that the test compound, now known as meperidine, does have certain structural features in common with morphine (Le Couteur and Bureson 2003). Ultimately, meperidine was found to be less potent than morphine; it acted faster, wore off sooner, and caused less nausea. This drug is widely prescribed under such trade names as Demerol.

The meperidine case is instructive because the abrupt appearance of a sigmoid tail in treated rats is something that might well be observed by an animal caretaker or technician. The observer might not have the knowledge or insight to associate the wayward tail with the known pharmacology of morphine, but could report the observation to someone who could make that connection. It is important for a given institutional culture or laboratory milieu to encourage alertness and reporting.

Sifting and Winnowing

Although drug side effects have pointed the way to some important drugs, a more productive aspect of empirical drug discovery is the use of “screening” techniques to separate kernels of therapeutic value from the accompanying inert chaff. In this approach, synthetic chemicals or microbial metabolites are examined, often more or less randomly, for a desired pharmacological effect. The element of chance is thus built deliberately into the system, and exploitation of chance is the declared objective. Three episodes that deal with the search for antiparasitic drugs are presented below to illustrate various features of this method. All three episodes involve laboratory animals, and each represents a different kind of serendipity.

Trematode Worms and Mouse Management

The first example involves a substance that is antiparasitic in its effect but has never been developed for practical use. It was exceptional in that the key to its discovery lay in the routine aspects of animal care in a research laboratory.

The project in which this event occurred, and in which I was involved, was designed to find an orally effective treatment for human schistosomiasis. Having devised a protocol for assessing drug efficacy in a mouse model, I exposed mice weekly to the skin-penetrating larvae of the trematode *Schistosoma mansoni*. After 8 wk of a specific treatment regimen, the mice were necropsied and the status of the infection was assessed. On one such occasion, all of the mice were observed to be totally free of infection. Thinking that the infective larvae (cercariae) had been mis-

handled in some way, I was not particularly concerned. That situation changed when the same thing happened on the following week, and successively on each of several additional weeks. At some point, considerably more than 8 wk in the past, something had gone wrong.

Thoroughly baffled and concerned, I made inquiries. Mr. Joseph Pietrowski, the supervisor of the animal attendants, was confident that the procedures had not been changed in any significant way. Wood shavings had been used for years as bedding for the mice, and were still being used. It was true that a switch had been made from pine shavings to cedar shavings, but that was the extent of change. I was desperate enough to grasp at any straw (this was my first project as a professional researcher), so I proceeded to investigate the cedarwood factor.

To my surprise I found that cedarwood oil is an outstanding topical prophylactic for schistosomiasis. Apparently the tails of the mice had acquired a coating of the oil from the shavings, and it was through the tail that I had exposed the mice to suspensions of infective cercariae. Applications of purified oil in various solvents were fully protective and quite resistant to washing. Other plant oils were much less effective. In reporting these findings, I acknowledged the help provided by Mr. Pietrowski (Campbell and Cuckler 1961). A few other workers took up the subject (Maples 1990; Mors et al. 1967; Pellegrino 1967), but none of us saw a way to turn our results to practical advantage in the prevention of human schistosomiasis.

The relevance of this episode to the use of animals in research is clear. The choice of bedding material for mice might be considered a humdrum matter, and in this case neither the investigator nor the animal supervisor thought it necessary to consult each other regarding a change of material. Had they consulted, the result would have been no different! In the 1950s, animal care arrangements were more informal than they are currently. The change (pine to cedar) would certainly have seemed trivial, and at that time there was no reason to suspect that contact with any kind of wood shavings would interfere with the infection process. The principle, nevertheless, is evident: close communication between investigator and animal care personnel is a good thing at any time—and especially when something goes wrong!

Levamisole: Was the “Wrong” Model the Right Model?

Many helminths (parasitic worms) of clinical and commercial importance are nematodes (roundworms). They parasitize humans and other mammals, so it is hardly surprising that candidate anthelmintic drugs are usually tested in small laboratory mammals. Scientists at the Janssen company in Belgium, however, “screened” compounds for their potential anthelmintic activity against worms in chickens (Raeymaekers et al. 1966). They found a chemical compound (thiazothienol) that was active against the worms, but when they put it into worm-infected rats and mice, it was inactive.

Because roundworms of mammals were the primary target, the investigators might have been expected to ignore this lead. Instead, they performed experiments that showed that the feces of the treated chickens contained a substance that was active against worms in rats and mice. Interestingly, it was not the original substance but was instead its metabolic product! That excreted substance (thiazothielite) was chemically modified to improve its efficacy and was developed as the veterinary drug tetramisole. The levo-isomer of tetramisole, named levamisole, was later found to have an improved safety margin, and was developed into an enormously successful anthelmintic agent for use in livestock and, to a lesser extent, in humans. (Levamisole subsequently found an adjunctive role in cancer therapy, but that is another story.)

Here we have the “happy accident” so characteristic of serendipity. The compound that led to the discovery of levamisole was not synthesized by the scientists; it was synthesized by the chickens! That result was totally unexpected. It was the scientists, however, who had the insight to recognize, from the “failed” rodent tests, that the secret to the previously observed anthelmintic efficacy lay not in what went into the chickens but in what came out! From this insight, an important drug was developed. That development was not a matter of chance; it was instead the rational exploitation of chance, which is the essence of serendipity. This episode differs from the foregoing example in the finding of what had been sought. Nevertheless, it fits the broad definition of serendipity despite the fact that Walpole, who coined the word, would not have admitted it to the ranks of serendipitous discoveries (Campbell 2005).

Oddly, the original compound thiazothienol is metabolized to the active form in sheep as it is in chickens. So the element of surprise in finding this new class of drugs was not only a question of the metabolism of birds versus that of mammals, but also the fact that the mammals usually considered appropriate for screening (rats and mice) would have been the wrong choice for making this particular discovery.

Obviously, the lesson to be drawn from this episode is not that we should deliberately select the wrong animal model. If there is a lesson to be learned, surely it is two-fold: we should be ever alert to the unexpected, and there is no such thing as a “failed” experiment. Failure in having our findings carry over from one animal model to another should not result in failure to wonder why.

Ivermectin and Elusive Serendipity

The use of an animal model was a key factor also in the more recent discovery of the antiparasitic agent ivermectin. In this case, microbial fermentation broths rather than synthetic chemicals were screened for antiparasitic (anthelmintic) activity. Many cultures were tested before a promising one was found, and this fact alone raises the specter of serendipity. A brief summary of the discovery process may

provide a basis for examining the ways in which it was, or was not, serendipitous.

In the early 1970s, scientists at Merck & Co., Inc., developed an assay in which mice were infected with the nematode *Nematospiroides dubius*. Each mouse was then fed a diet containing a dried microbial broth, and was monitored for persistence of the worm infection. The rationale and protocol for this assay, and the emergence of ivermectin from the program, have been reviewed elsewhere (Campbell 1992; Stapley and Woodruff 1982). Antiparasitic activity was shown by the broth of a filamentous bacterium that was described as a new species, *Streptomyces avermilitis*, and that produced a substance with unprecedented potency in a variety of nematode infections (Burg et al. 1979; Egerton et al. 1979). The microorganism had been isolated from soil by Satoshi Omura and his colleagues at the Kitasato Institute in Japan, and was included in a batch of microbial cultures sent, by formal agreement, to Merck & Co., Inc., to be screened for pharmacodynamic activity in the Company’s research programs. The active substance (existing in several structural forms) was shown to be a macrocyclic lactone and was named avermectin. A program of structural modification resulted in the selection of a hydrogenated derivative, ivermectin, as the preferred drug candidate (Albers-Schonberg et al 1981; Chabala et al 1980). Extensive studies showed that ivermectin had a wide margin of safety in mammals, and exceptional potency and breadth of spectrum against roundworms, and against arthropod parasites, in livestock and pets (Campbell et al. 1983). It proved also to be effective in humans against *Onchocerca volvulus*, the agent of river blindness (Aziz et al. 1982).

Only a broad definition of serendipity will cover the discovery of ivermectin by means of empirical screening, and even then some qualification is warranted. Screens are designed to find a drug of a particular kind, and the original coiner stipulated that the term *serendipity* is not applicable in cases where the discoverer had been trying to find the thing that was found. In screening operations, the investigators know, in broad terms, what they are looking for. The key to successful screening, however, is having an input of test substances that is as random and as comprehensive as possible. An input of selected chemical structures is appropriate for “second-generation” compounds within a given chemical or pharmacological class. In the hunt for truly novel drugs, selection on the basis of known structure-activity relationships is counterproductive. It does not follow that success is entirely a matter of chance.

In the case of ivermectin, as in so many others, discovery was a mixture of chance and choice. It is not easy to pick out with certainty the “happy accident” that certifies a bona fide case of serendipity.

- Was it the scooping up of that specific piece of Japanese soil? Perhaps!—because the microorganism that was isolated from it has never been found elsewhere. Yet it

was scooped up for the purpose of finding a useful microbial metabolite.

- Was it the testing of the isolated microbe in the anti-parasitic assay as opposed to some other assay? Not really!—because the *N. dubius* assay had been set up to enable us to test crude fermentation broths from any available source.
- Was it the observation of efficacy in the single mouse to which that broth was fed? Perhaps so, because an unrelated microbial product in the broth had made that mouse ill, and it might well have died before the anthelmintic effect could be observed (and a retest might not have been feasible). Yet the observation was made by people trained to make that sort of observation.
- Was it the efficacy itself, rather than the observation, that was fortuitous? Well, yes—but our objective was to find efficacy, not create it.

So we can have our happy accident or not, according to taste; but clearly chance played a part, and clearly chance did not produce the end result.

There remains one aspect of the ivermectin story that could fit the strict definition of serendipity. We were looking for a broad-spectrum anthelmintic, and we found one. However, we also found a broad-spectrum agent for the control of ectoparasitic insects and mites. This finding proved so important in commerce that ivermectin and its second-generation progeny are widely known as endectocides (a popular neologism despite its overtone of killing both the inside and the outside). One could argue that encountering a mite-infected rabbit in our animal colony was a happy accident. However, the drug was administered to that animal specifically to learn whether it had a therapeutic effect, and we soon had dramatic before-and-after pictures to show that it did. It was simply a matter of rational opportunism. Perhaps the most pure serendipity in this segment of the program came from the unexpected discovery that a horse had a stomach-bot infection. The horse had been treated with ivermectin, and the abrupt exit of bots in its feces signaled both the diagnosis and the therapeutic effect—an effect that would be confirmed and widely exploited in equine medicine.

Conclusion

In the more than two centuries that the word serendipity has existed, it has grown in popularity and expanded in meaning. On the basis of the original definition, empirical drug discovery could be excluded from the realm of serendipity; after all, it represents the finding of something sought. In current usage, the “unsought” provision is not rigorously applied, yet there is good reason to use “serendipity” sparingly, even within the more expansive modern rubric. The scientific elements that pervade every aspect of the screening process cannot be separated from the elements of chance. At most, we can say that discovery depends on the

chance that something scientifically interesting lies hidden in the materials that constitute the input to the screen. It may be necessary to devise a suitable screen, and all suitable screens may not be equally suitable. Nor is it obvious what fodder should be fed into a particular screen, or what significance should be attached to a particular “activity” in the output. In short, an interesting substance may, by chance, lurk in some hidden nook or cranny of the material universe; but finding it takes more than chance.

The “rational” approach to drug discovery hinges largely on the identification of a biochemical pathway or event that occurs in the parasite but not in the host (or that operates in a different way or at different magnitude). The objective then is to find a substance that will disrupt that event, bringing misfortune to the parasite and benefit to the host. The search may take the form of a biochemical “screen” in which substances are tested for the desired biochemical action in vitro after which “actives” are tested for antiparasitic efficacy in vivo. The project thus enters an empirical phase, and the element of chance becomes evident. Alternatively, the goal may be to design and construct a molecule that will thwart the chosen biochemical operation in the parasite without harming the host or otherwise being impracticable. In that case, the project will be intellectually rewarding and may create the illusion of escape from the realm of chance. As I have argued elsewhere (Campbell 1983), chance does not disappear—largely because the putative safe and effective drug may chance to interfere with biochemical events (known and unknown) other than those that have been the target. Those old arguments may cease to apply as current molecular insights and genomic ingenuity gradually shift the balance of probability in the search for new drugs. The record of success of empiricism remains unsurpassed, but we should not rely solely on it. In whatever approach is taken to the discovery of new drugs, chance will continue to provide opportunities for rational exploitation. The components of serendipity are luck and thinking, and it would be a mistake to underestimate the importance of either one.

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