Erin Welsh	"I remember the astonishment when the first cases of pneumococcal and streptococcal septicemia were treated in Boston in 1937. The phenomenon was almost beyond belief. Here were moribund patients who would surely have died without treatment, improving in their appearance within a matter of hours of being given the medicine and feeling entirely well within the next day or so. The professionals most deeply affected by these extraordinary events were I think the interns, the older physicians were equally surprised but took the news in stride. For an intern it was the opening of a whole new world. We had been raised to be ready for one kind of profession and we sensed that the profession itself had changed at the moment of our entry. We knew that other molecular variations of sulfanilamide were on their way from industry and we heard about the possibility of penicillin and other antibiotics. We became convinced overnight that nothing lay beyond reach for the future. Medicine was off and running."
TPWKY	(This Podcast Will Kill You intro theme)
Erin Allmann Updyke	Oh I loved that!
Erin Welsh	Right? I got chills reading it.
Erin Allmann Updyke	Oh that's very cool.
Erin Welsh	I searched high and low to try to find... Maybe my search terms we off but I wanted to read a whole collection of doctors who were using penicillin and sulfanilamide and other antibiotics for the very first time cause that was such a revolution.
Erin Allmann Updyke	Right? Oh my gosh.
Erin Welsh	Hi, I'm Erin Welsh.
Erin Allmann Updyke	And I'm Erin Allmann Updyke.
Erin Welsh	And this is This Podcast Will Kill You.
Erin Allmann Updyke	We are thrilled about today's episode.
Erin Welsh	Absolutely thrilled.
Erin Allmann Updyke	Today we're talking about antibiotics.
Erin Welsh	Yes. It's a huge topic.
Erin Allmann Updyke	Oh it's too massive.
Erin Welsh	It's too massive and so just like we did with the vaccines episodes, we're splitting this into two separate episodes. And so this week we are talking about antibiotics themselves, how they work, how they were developed, and sort of what the current status is of antibiotics today. And we are very excited to bring on a very super cool researcher who does super cool work on antibiotics.
Erin Allmann Updyke	Yeah, that's very true. Super, super cool.

Erin Welsh	So stay tuned for that. And then next episode we're gonna talk about antibiotic resistance.
Erin Allmann Updyke	Right.
Erin Welsh	And how resistance works, the history of resistance, etc, and then hopefully another super cool guest.
Erin Allmann Updyke	Yeah, it's such a huge topic and everyone wants to know what's going on with antibiotic resistance cause that's in the headlines, it's a big deal. But in order to understand antibiotic resistance you have to understand antibiotics and so that's what this episode is about. Plus antibiotics are cool as heck.
Erin Welsh	They are so cool. I am legit very thrilled for this episode cause I know the history obviously from reading about it but there was only little glimmers of insight into how they worked in the history books.
Erin Allmann Updyke	It's really cool, I can't wait to go over it.
Erin Welsh	Well first of all before we can fully express our excitement about antibiotics we have some business to take care of.
Erin Allmann Updyke	Of course.
Erin Welsh	First of all it's quarantini time.
Erin Allmann Updyke	Of it's quarantini time.
Erin Welsh	We are drinking this week, very appropriately, the drink called Penicillin. So we didn't come up with this recipe.
Erin Allmann Updyke	We're not reinventing the wheel here.
Erin Welsh	Because we don't need to because this is such an excellent cocktail on its own. So what is in the Penicillin? It is scotch which we haven't done a scotch quarantini for a very long time.
Erin Allmann Updyke	Since Season 1 I think actually.
Erin Welsh	I think so. And I like scotch, like I don't know why we've avoided it so much. It's not intentional. And then honey ginger syrup and lemon juice. Super simple. And we will post the recipe for that quarantini and the nonalcoholic placeborita on our website thispodcastwillkillyou.com as well as posting it on social media channels. And then we have one more little bit of business and that is related to our last normal season episode on eastern equine encephalitis. And one of the things that we sort of discussed was why horses experience such a higher mortality rate. And first of all Erin, we really need to get a vet on here, that was our bad.
Erin Allmann Updyke	Yeah, yeah. 100%.
Erin Welsh	In the future we promise. So a veterinarian reached out to us to kind of shed some light on our question about how horses have higher mortality rates. And so they reached out to other equine specialists in the vet community and got a general consensus for that answer.

Erin Allmann Updyke	Ooh.
Erin Welsh	Are you ready to hear it?
Erin Allmann Updyke	I'm so ready.
Erin Welsh	It's pretty interesting. Okay so this is a quote from the email: "It's not well known whether there is a difference in the immune response between species but most likely the increased mortality in horses is related to the difficulty in providing nursing care to a 1000-1500 pound recumbent animal and the issues that occur from the horse being down. So like pneumonia, pressure sores, self-trauma. Horses are also dangerous to work around when they are neurologic and having seizures. And even if the horse recovers, most people cannot afford to support an animal that has continued neurological deficits and will never be ridden again."
Erin Allmann Updyke	Right.
Erin Welsh	Yeah. So because Triple E causes these neurological deficits and so it seems to be that euthanasia is often sort of what must be done for some of these cases.
Erin Allmann Updyke	Right, that makes sense.
Erin Welsh	So there you go.
Erin Allmann Updyke	Awesome!
Erin Welsh	So yeah, thank you so much for reaching out and sending us that information.
Erin Allmann Updyke	Yeah.
Erin Welsh	It's very interesting. All right.
Erin Allmann Updyke	Now that that's out of the way-
Erin Welsh	Let's talk antibiotics please.
Erin Allmann Updyke	I can't wait. We'll take one quick break first.
TPWKY	(transition theme)
Erin Allmann Updyke	Obviously we've already said this is a massive topic so here's how we're gonna break it down when we talk about the biology of antibiotics. First we have to talk about bacteria, right?
Erin Welsh	Yeah of course.
Erin Allmann Updyke	We have to understand what bacteria are, how we classify and identify them, and then we can kind of start to understand how we target those bacteria using antibiotics, right.
Erin Welsh	I'm very excited, I love bacteria.

Erin Allmann Updyke	Me too! And what's really fun about this is that all of our other episodes where we've covered a bacterial pathogen I kind of gloss over a lot of this part of it. So this is kind of like you'll understand more about many of our past episodes now.
Erin Welsh	Ooh.
Erin Allmann Updyke	Yeah, anyways. Okay, let's get into it. All right. What is a bacterium?
Erin Welsh	It's a single-celled prokaryotic something.
Erin Allmann Updyke	Okay, yep. Yes. So what's a prokaryote then? That's the first question I guess we have to answer. The term 'prokaryote', it's not a great term but we still use it because it's still useful. It's basically how we separate bacteria and archaea from eukaryotes which is plants and animals and fungi. All right? So let's talk about some of the differences between the cells of prokaryotes and eukaryotes. So our cells for example are basically bags of water, right. And these little bags of water are surrounded by a membrane. This membrane is made of lipids which are fats and they have some proteins interspersed in there, okay? Inside of our cells, so human cells, animal cells, plant cells, fungus cells, we have things called organelles which are also bound by membranes, so they have little lipid membranes around them, and those carry out all of the functions of our cell. Okay? And that includes in our cells a nucleus which is where all of our DNA is, that's our genetic material.
	Bacterial cells are a little bit more simple. So there's still a bag of water, okay, they're still surrounded by a lipid membrane but here's one of the first differences. Inside of that bag of water there's so other organelles, okay.
Erin Welsh	Right.
Erin Allmann Updyke	There's just a little round piece of DNA and then a bunch of ribosomes which are essentially RNA and protein mixed together. Okay?
Erin Welsh	Yep.
Erin Allmann Updyke	And then the other big difference between bacterial cells and animal cells is that on the outside of their plasma membrane, that lipid membrane, they have a cell wall, okay. That's a big, huge difference. And it's really important because anywhere that there is a difference between bacterial cells and human cells for example, that means we can use that as a target to kill those bacteria. So now let's talk about the ways that we classify these bacteria. We're not gonna get into evolutionary relationships cause that's way beyond my capacity. What we do wanna talk about though is the way that we can classify bacteria in order to identify them so that we can choose the right antibiotics to use to treat them. Okay?
Erin Welsh	Right.
Erin Allmann Updyke	All right. So one of the very first ways, and this if you've ever heard one of our podcast episodes you've heard me say these words. We can first classify a bacteria by is it gram-positive or gram-negative, right. And normally I just say that means it's pink or purple under the microscope.
Erin Welsh	Yep. (laughs) Actually means a whole lot more than that.

Erin Allmann Updyke	Yes it does. So let's actually define what that means, okay. So Gram staining, when we say the term 'Gram stain' this is a tool that we use to visualize bacteria under the microscope. And basically what you do is you mix two different dyes, you put a purple dye over a bunch of bacteria and then you wash that purple dye off and then you put a pink dye on, okay. And what happens is that purple dye gets stuck on the cell walls of these bacteria. Remember I said bacteria have cell walls around their membranes. So if bacteria take up that purple stain in their cell wall, then they're what we call gram-positive. Okay?
Erin Welsh	Yep.
Erin Allmann Updyke	If they don't take up that purple stain, then they'll look pink under the microscope because they'll take up that pink stain and those are what we call gram-negative, all right, because they don't pick up that Gram stain. So what is the difference between these gram-positive and these gram-negative bacteria?
Erin Welsh	Their cell walls!
Erin Allmann Updyke	Their cell walls. So they both have cell walls that are made of the same basic stuff and that is generally a substance called peptidoglycan. This is something that our cells don't have.
Erin Welsh	Right.
Erin Allmann Updyke	So it's very different than eukaryotic cells. But gram-positive bacteria have a really thick layer of this peptidoglycan and it sits right outside their plasma membrane and then that's the end, there's nothing else, okay. Gram-negative bacteria have a thinner layer of this peptidoglycan and then on the outside of that cell wall, they have another membrane. And this membrane is made usually of something called lipopolysaccharide, LPS. That's not as important. But it's basically another barrier between the inside of that bacterial cell and the outside world, okay.
Erin Welsh	Right.
Erin Allmann Updyke	So that's a really huge and important difference because these cell walls, they act as kind of an exoskeleton almost, right, they give the bacteria its shape and structure. But they also in the case of gram-positive bacteria, they're fairly permeable to small molecules, okay. So a lot can still get in and out of gram-positive bacteria that just have this peptidoglycan cell wall.
Erin Welsh	Yeah.
Erin Allmann Updyke	Gram-negative bacteria on the other hand have an extra membrane on the outside so it's harder for things to get in and out. The way things get in and out of those cells is by little proteins that are along the surface called porins. Wanna guess what they do?
Erin Welsh	They're pores! They're channels.
Erin Allmann Updyke	They make a pore. We're really creative here in naming. Okay so those are gram-positive vs gram-negative bacteria. Importantly some bacteria have entirely different cell walls so they might not take up either of those pink or purple stains, right. So that's something like microbacterium like tuberculosis that we've talked about a lot, right.
Erin Welsh	Right.

Erin Allmann Updyke	All right, Erin. Other ways that we classify bacteria that we've talked about a lot. We can look at their shape, are they round, are they rod-shaped, are they little spirals? Okay. This is helpful when we are trying to identify specific bacteria. Again because the antibiotics that we're going to use we wanna make sure target the right bacteria so that they're actually effective. And then the other thing that we can look at is how and where do these bacteria live? Are they aerobic meaning they need oxygen in order to survive? Or are they anaerobic meaning they can't live in the presence of oxygen like Clostridium botulinum that we talked about recently? So those features of bacteria are common across pretty much all bacteria. So one thing that's important to keep in mind with antibiotics is that depending on the antibiotics you use, they might be killing a lot more than just the pathogen that you're targeting, right.
Erin Welsh	Right. And so I think when we say bacteria or think of bacteria I think a lot of us are used to thinking of pathogenic bacteria.
Erin Allmann Updyke	Right.
Erin Welsh	And so instead of saying oh well the pathogenic bacteria, we say bacteria and assume it's going to lead to an infection or death. But as we have increasingly become more aware in the past 30-40 years or so, humans, animals, plants have a microbiome. And so these are bacteria that may themselves not be pathogenic or they may be opportunistically pathogenic but they are also an unintended target of antibiotics and that could lead to issues.
Erin Allmann Updyke	Right, yeah. The vast, vast, vast majority of bacteria are not pathogenic. It's a very small subset of bacteria that are actually able to colonize, infect, and cause illness in animals, plants, etc. And it's kind of a whole separate topic like what those specific mechanisms are that allow for bacteria to make us sick. And we kind of touch on those when we talk about specific bacteria, right.
Erin Welsh	Right.
Erin Allmann Updyke	All right. So those are bacteria. So hopefully you understand now, listeners, how they are different from our cells because those differences are what we are going to exploit to be able to use antibiotics to kill those pathogenic bacteria. All right so what is an antibiotic? We don't have to do the etymology of this, right? Like anti-bio is anti-life, okay.
Erin Welsh	Pretty easy, pretty easy.
Erin Allmann Updyke	Pretty straightforward. So antibiotics are generally small, low molecular weight, so really small molecules that have the action of either killing or halting the growth of bacteria.
Erin Welsh	Originally the term was specifically for compounds that are produced by other living organisms was the intention but now it's been more widely expanded as synthetic antibiotics have been developed. Isn't that interesting though?
Erin Allmann Updyke	Ooh. Interesting. All right so I've said this a couple times already but when we're thinking about antibiotics we have to make sure that they're specifically targeting bacterial cells and hopefully not causing too much damage to animal cells, our cells. And so we tend to target things that are specific to these bacteria. And then the other thing is that understanding the mechanisms, the specific mechanisms of action of these antibiotics can tell us not only what groups of bacteria they're likely to work against but also how antibiotic resistance can eventually evolve. All right. So in general there are four broad picture ways that we target bacteria in order to kill them with antibiotics. These four are: we can target their cell wall synthesis, right.

Erin Welsh	Right. You can't build a wall, you can't exist.
Erin Allmann Updyke	Yeah. It's not true for humans but true for bacteria. We can target their DNA replication and you might say, 'But Erin, don't human cells also replicate their DNA?' And you'd be right if that's what you said but we'll talk about some differences. We can target their protein synthesis which again our cells of course make protein but there are some differences in the way that bacteria make protein and the way that we make protein. And then we can also target some specific elements if bacterial metabolism that are very different from animal and plant metabolism.
Erin Welsh	Right.
Erin Allmann Updyke	So those are the four major targets. Let's get into the nitty gritty, shall we?
Erin Welsh	We shall.
Erin Allmann Updyke	So cell wall synthesis. I said this, most bacterial cell walls are made of peptidoglycan and this is a substance that animal cells don't produce. The way that bacteria make these cell walls is they have to make chains of peptidoglycan and then they have to cross link those chains together in a specific way in order to build a strong wall. It kind of is similar to the way that we make collagen in our bodies if you remember the scurvy episode, right.
Erin Welsh	Yeah.
Erin Allmann Updyke	So if you can mess up the way that that cell wall cross links then you basically make an ineffective cell wall.
Erin Welsh	Like you're peeling apart the Pull N Peel Twizzler?
Erin Allmann Updyke	Yeah, exactly! Just like that. So it turns out that the beta lactam antibiotics which include penicillins, the most famous, I can't wait for you to tell us the story of penicillin, Erin.
Erin Welsh	It's a good one.
Erin Allmann Updyke	It is. Penicillins, cephalosporins which you've probably heard of, carbapenems, and monobactams. All of these are beta lactam antibiotics. They target a very specific part of this cell wall synthesis. They block the enzyme that catalyzes that cross-linking reaction, okay. So it's really similar to the scurvy situation where if you don't have vitamin C you can't cross link collagen to make your good collagen. If you block this enzyme, you can't cross link peptidoglycan, you can't make a cell wall. Okay?
Erin Welsh	Right.
Erin Allmann Updyke	So it turns out that we named this enzyme a penicillin-binding protein. There's a bunch of different penicillin-binding proteins, a lot of different versions of these enzymes and they all do the different steps of peptidoglycan synthesis and cross-linking in slightly different ways. So we have a variety of different beta lactam antibiotics that can target the different processes. Does that make sense?
Erin Welsh	Mm-hmm.

Erin Allmann Updyke	So knowing that beta lactams block cell wall synthesis, what types of bacteria are they most likely going to be able to be really, really effective against?
Erin Welsh	Gram-pos.
Erin Allmann Updyke	Right because gram-positives have that cell wall right there and it's really, really important to them. So beta lactams historically are really, really good at treating gram-positive infections like strep, like staph, etc. okay? Gram-negative bacteria on the other hand still do have a cell wall so you still can target them with beta lactam antibiotics but because that cell wall is surrounded by another membrane, it's a little bit harder to get in and to get those beta lactams through.
Erin Welsh	Right.
Erin Allmann Updyke	Right. So that's why we develop better and better, they are called second and third and fourth and fifth generation beta lactams that have slightly different structures that can do a better job of getting in to treat those gram-negative infections.
Erin Welsh	Okay.
Erin Allmann Updyke	Cool?
Erin Welsh	Cool.
Erin Allmann Updyke	Now there are other bacteria that don't have peptidoglycan cell walls like microbacterium tuberculosis. Their cell wall is made of a different substance called mycolic acid. So obviously beta lactam antibiotics are gonna be entirely useless against those types of bacteria but we do have other antibiotics that are specific to mycobacterial cell walls, so they do the same thing, just on a different substance. Cool?
Erin Welsh	Cool.
Erin Allmann Updyke	So that's cell wall synthesis inhibitors. Ta-da!
Erin Welsh	Bam. Got it.
Erin Allmann Updyke	I think that's so fun. And it's such a beautiful target because we don't have peptidoglycan, right.
Erin Welsh	Right.
Erin Allmann Updyke	It's very cool. So the next thing that we could target. How about proteins? We've talked a lot on this podcast about how cells' basic function is to make proteins, proteins are how we do all the things that cells do. So if we could target bacterial protein synthesis, then we could stop bacteria from making proteins that'll eventually make the cells die because they won't be able to do their job. The only problem is that our cells also make proteins. But the good news is that it turns out that the specific ribosomes that bacteria use to make protein are different in their shape and structure and function than human ones are. So we can target those.
Erin Welsh	That's great news.

Erin Allmann Updyke	It's really good news. So there's two steps that we can target in terms of protein synthesis. First we can target the process of transcription. This is where we take DNA and turn it into RNA which serves as the template for making proteins. This is what the rifamycins do. This targets bacterial RNA polymerase, so it basically blocks the ability of bacterial cells to make RNA. So it totally is going to kill them.
Erin Welsh	That's great.
Erin Allmann Updyke	But that's just sort of one class. The vast majority of antibiotics that we have that target protein synthesis block the ribosomes which are integral in making protein.
Erin Welsh	How exactly do they block the ribosomes?
Erin Allmann Updyke	So there's a bunch of different ways. Ribosomes have two parts, they have a small subunit and large subunit. So depending on the antibiotic class, they're either going to bind to the small subunit or the large subunit and basically inactivate them.
Erin Welsh	Okay.
Erin Allmann Updyke	So they bind to those ribosomes and they block those ribosomes from acting on the RNA to make it into protein, if that makes sense.
Erin Welsh	Right, that makes sense, yeah.
Erin Allmann Updyke	And there's a lot, a lot of different antibiotics that do this whether they target the large or the small subunit. So those are the aminoglycosides like streptomycin, gentamicin, tobramycin; the tetracyclines like doxycycline; also the macrolides which are like erythromycin, azithromycin, okay. So in the case of these, whether it's rifampin that's blocking RNA synthesis or any of these classes that are blocking the ribosomes and blocking protein synthesis, what types of bacteria do you think that we could target with these antibiotics?
Erin Welsh	Lots of them.
Erin Allmann Updyke	Lots of them. Pretty much any of them. We use these antibiotics, aminoglycosides, tetracyclines, macrolides for so many different infections, gram-positives and gram-negatives.
Erin Welsh	Which also means that they do a very good job of wiping out microbiome stuff. We should do an episode on the microbiome.
Erin Allmann Updyke	It's such a separate topic, yeah.
Erin Welsh	It's just hard not to think about it every time I think about an antibiotic.
Erin Allmann Updyke	Oh yeah, absolutely.
Erin Welsh	So anyway.
Erin Allmann Updyke	You should think about it every time you think about an antibiotic though cause it's absolutely a consideration.

Erin Welsh	Yeah. It's pretty interesting. Okay.
Erin Allmann Updyke	All right. So that's blocking protein synthesis. If we take a step back from protein synthesis we have DNA, right. So we could block DNA replication itself so long as bacterial DNA replication is different from eukaryotic DNA replication. It is mostly the same but there are a couple of enzymes that are different. So we can target those specific enzymes, all right. And it turns out that we have antibiotics that do exactly that. Fluoroquinolones which are a synthetic group of antibiotics, they target a bacterial enzyme called DNA gyrase that eukaryotic cells don't have, so that's great. And so they can block that enzyme and thereby inhibit all DNA replication. If you can't replicate DNA, you can't make a new cell. Boom.
Erin Welsh	Boom.
Erin Allmann Updyke	Boom, over. That one is short. We don't have a lot of those, mostly fluoroquinolones. All right the last big way that we can target, and this one's really fun even though there's very few antibiotics that we have that do this, is we could block some of the metabolism of bacteria, specifically folate synthesis.
Erin Welsh	Okay.
Erin Allmann Updyke	So most people have probably heard of folate, right, because you've heard of folic acid that you need, if you're pregnant you have to make sure that you're getting enough folic acid, that's the context that most people have heard of it.
Erin Welsh	Right.
Erin Allmann Updyke	Okay so folic acid is vitamin B9. This in both bacteria and eukaryotes is very heavily involved in the actual synthesis of DNA building blocks, okay. So you don't use folic acid itself but it's necessary to make the building blocks of DNA.
Erin Welsh	Right.
Erin Allmann Updyke	So if you don't have enough folic acid, you can't make DNA. We have to get folic acid from our diet, we have to eat it, we can't make it ourselves. Bacteria as it turns out make their own folic acid.
Erin Welsh	That's very cool.
Erin Allmann Updyke	It's very cool. And so if we can block bacteria's ability to make folic acid, they can't uptake it from their environment so then they're going to die because they can't make DNA, so they can't replicate. All right so turns out we have antibiotics that inactivate enzymes in the folic acid synthesis pathway in bacteria. So those are the sulfonamides and an antibiotic called trimethoprim. And so the sulfonamides and trimethoprim target two different steps in the synthesis of folic acid. So we actually often use them together in combination. You've probably heard trimethoprim sulfamethoxicide.
Erin Welsh	I don't think I've heard of that but sure.
Erin Allmann Updyke	Oh you haven't? Have you heard of Bactrim?
Erin Welsh	Yes!

Erin Allmann Updyke	There you go, then you have heard of it. Ta-da!
Erin Welsh	Which group is chloramphenicol?
Erin Allmann Updyke	Chloramphenicol is a protein synthesis inhibitor.
Erin Welsh	Okay thank you, just curious.
Erin Allmann Updyke	It's not in one of those big groups that I talked about but it is its own protein synthesis inhibitor.
Erin Welsh	Right. Okay.
Erin Allmann Updyke	So yeah, that's kind of the big, broad strokes of the different classifications of antibiotics and how they work. Antibiotics can be either bacteriostatic or bactericidal. So bactericidal like pesticide means that they kill the bacteria, okay, whereas bacteriostatic means they just stop the growth of bacteria and then we rely on our immune system to come in and finish the job.
Erin Welsh	Right.
Erin Allmann Updyke	Okay. It's a little bit more nuanced than that because some antibiotics are bacteriostatic against some organisms and bactericidal against others.
Erin Welsh	Interesting.
Erin Allmann Updyke	Yeah, it's a little bit complicated but it's partially because of how we define bactericidal which is basically you have to kill 99.9% of bacteria within 24 hours. So you might be bactericidal but it actually takes longer than 24 hours so you're not technically bactericidal.
Erin Welsh	Gotcha. Okay.
Erin Allmann Updyke	So that's basically antibiotics in a nutshell. That was like you pharmacology course and your microbiology course. But overall what I think is kind of the most important takeaway is that there aren't any good antibiotics or bad antibiotics. You might hear people say 'these are big gun antibiotics', that's a terrible term actually even though people use it all the time. There are the right antibiotics and there are the wrong antibiotics for any given infection. There are antibiotics that are going to work and there are ones that aren't going to work. And so we can see based on these mechanisms of action that some of these antibiotics will work against a large number of pathogens and we call those broad spectrum antibiotics whereas others work against a more narrow spectrum of pathogens, right. And then on top of that some antibiotics are more potent so they might need less of a concentration in your system in order to kill the bacteria. But some of those that are more potent might also be more indiscriminate in killing maybe our own cells, right, like they might have adverse side effects on our own cells.
Erin Welsh	Right.
Erin Allmann Updyke	And of course pretty much without a doubt antibiotics end up killing our own microbiome as well as the pathogenic bacteria that they're supposed to be targeting. So when we're thinking about what antibiotics we use in certain situations, it depends on both the severity of the infection, what that infectious organism is or is likely to be, and what the overall antibiotic resistance looks like in that area.

Erin Welsh	Yeah.
Erin Allmann Updyke	And that's how we have to decide what antibiotics we use for specific pathogens.
Erin Welsh	I'm sure we're gonna talk about this much, much more, like there are so many questions that I wanted to ask about antibiotic resistance.
Erin Allmann Updyke	I know. This is really hard to not talk about.
Erin Welsh	Just save it, save it. And so I'm sure we're gonna talk about this more in that resistance episode but I think it really also comes down to if somebody is very ill from what seems to be a bacterial infection, then oftentimes it's not possible to sort of choose this antibiotic is going to be the best one.
Erin Allmann Updyke	Exactly. So then what we do is called treating them empirically, right. So you look at okay what type of infection is this, where do we think this infection likely came from? Because then we can start to narrow it down. Do we think that this is a gram-positive or a gram-negative infection or are we note sure? Do we think we're trying to treat aerobic bacteria or anaerobic bacteria, right? What was the source of these bacteria? Where are they growing? And then we also yeah, we have to look at how sick the person is because if someone is really, really sick then we might accept using an antibiotic that has greater range of side effects if it's going to be more effective at killing those bacteria. Right?
Erin Welsh	Right, right.
Erin Allmann Updyke	And then the other thing too is that in general you want to use the most narrow spectrum antibiotic that you can in this situation. So sometimes you might start out when you're not sure what the infection is with one antibiotic and then once you have a clearer picture you can switch to another antibiotic. But so for example there are some antibiotics that we talked about already that are really, really effective and they're effective against a really wide range of pathogens, for example rifampin. This targets RNA polymerase so it's effective against tons and tons of bacteria and it also actually has relatively few side effects, it's a pretty good drug. But it's such a good drug that we don't wanna use it against just anything.
Erin Welsh	Right.
Erin Allmann Updyke	So we save that for use in very severe infections, we generally use it just to treat tuberculosis and meningitis as well.
Erin Welsh	Yeah. If we had discovered that one first instead of penicillin, I mean we wouldn't be using it at all because of resistance.
Erin Allmann Updyke	Yeah because resistance also develops really rapidly to rifampin as well.
Erin Welsh	Yes.
Erin Allmann Updyke	And so it's also often used in combination with other antibiotics.
Erin Welsh	Right, right.

Erin Allmann Updyke	So yeah, it's a complicated but really fun topic, I think.
Erin Welsh	It's really interesting and I also wish I had read more about sort of the history of microbiological developments because I feel like we're gonna talk about this later on in the episode but like how the development of new antibiotics has really slowed down and I wonder if part of that is because our knowledge of the differences between... Like we've already kind of taken out all the low-hanging and middle-hanging fruit when it comes to differences between bacterial cells and eukaryotic cells.
Erin Allmann Updyke	Totally.
Erin Welsh	And so now it's like what targets do we even have left to identify?
Erin Allmann Updyke	Absolutely, yes.
Erin Welsh	It's interesting, it's interesting.
Erin Allmann Updyke	So Erin, how did we come up with these? Where do antibiotics come from? Tell me everything about them.
Erin Welsh	Okay, I can't wait. We'll take a quick break first.
TPWKY	(transition theme)
Erin Welsh	From defense to offense, from art to science. This is the story of antibiotics. (laughs) I couldn't resist.
Erin Allmann Updyke	Fabulous.
Erin Welsh	It's hard to know exactly where to begin this story. Do we for instance start with Lister and the use of carbolic acid to disinfect wounds? Or do we start with Fleming and penicillin? Or do we go back in time before germ theory to when people used moldy bread to treat infected cuts? Yeah, that happened.
Erin Allmann Updyke	What? That happened? Did not know that.
Erin Welsh	But like you talked about, the word 'antibiotic' simply means against life. An antiseptic like Lister's carbolic acid may effectively kill bacteria making it antibacterial but it also kills human and animal cells, so it's not really an antibiotic.
Erin Allmann Updyke	Right.
Erin Welsh	And also it shouldn't be injected into your body which unfortunately does need to be said considering some recent statements made by someone.
Erin Allmann Updyke	Oh the world that we live in.
Erin Welsh	Plus I would also really love to tell the story of Lister and antiseptics in surgical practices one day, so let's put a pin in that and come back to it in another episode.

Erin Allmann Updyke	Perfect.
Erin Welsh	Instead I would like to go back to the earliest known use of mold to treat infections. And it's surprisingly widespread, that practice.
Erin Allmann Updyke	That's so weird.
Erin Welsh	So there are descriptions of using moldy bread for various ailments found in Ancient Egypt, China, Serbia, Greece, Rome, Central America. It's amazing. And some of these are topical treatments like rub moldy bread crumbs on a pustular scalp infection. Yep. And others actually instruct you to make a moldy bread mixture to eat to quote "soothe the pipes" if you have bladder or urinary tract inflammation.
Erin Allmann Updyke	Oh I thought it meant like pharyngitis.
Erin Welsh	Maybe that too, any kind of pipe. (laughs) But for whatever reason, these uses of mold to treat infections kind of fell out of style or were forgotten about for a few thousand years. And before going into the modern history of antibiotics I want to paint a little picture of what the pre-antibiotic era was like.
Erin Allmann Updyke	Oh gosh, that sounds depressing.
Erin Welsh	It is but it also shows how far we've come. So before antibiotics, 3 out of 10 pneumonia patients died, 9 out of every 1000 births led to the mother's death, untreated ear infections and strep throat led to hearing impairments and heart failure, and as you can imagine war was an absolute feast for pathogenic bacteria. In the American Civil War more soldiers died from typhoid fever and dysentery than directly from combat and similarly in WWI, more people died of dysentery and typhus than the fighting itself. The modern history of antibiotics starts or at least I'm gonna start it with a lecture given by Paul Ehrlich in 1907. And this name may sound familiar to you for any number of reasons, one of those reasons may be this now infamous lecture because in that lecture he used the term 'magic bullet' to describe this ideal drug that could be used to kill bacterial infections and at this time it was still a hypothetical concept.
	Up to this point, early 1900s, germ theory had been well established and discoveries were still pouring in. The understanding of how infectious diseases worked had really grown over the past 50 years and technology for carrying out microbiological studies had also advanced a ton and so that really helped. So microscopes and lab equipment made it much easier to find and identify bacterial cells as well as create vaccines for both bacteria and viruses. But even though many horrible diseases could now be prevented, there hadn't been much progress in terms of treatment for those diseases. And so this had led to a really interesting shift that I hadn't really thought about before in the philosophy or attitude of many physicians. So before germ theory, let's say like the late 1700s and the mid 1800s, the predominant strategy for medicine in much of Europe and the US was called 'heroic medicine'. And so what this consisted of was essentially trying to shock the body back into balance. So we're talking excessive bloodletting, purging, sweating, you name it. Basically extreme, extreme intervention was the mode.
Erin Allmann Updyke	Okay, yeah.

Erin Welsh	And as you can probably imagine, most of the time the recipients of this heroic medicine were treated to death. But then in the mid 1800s germ theory came around and with it came this recognition that for these infectious diseases there was often no amount of intervention that could stop their progress. Could you prevent these through vaccines? Sometimes, that was great when that could happen. And you could also lay out a timeline of what an infected person would experience at each excruciating stage of an infectious disease. But you couldn't do anything most of the time to stop fate. And so doctors went from this extreme interventionist, heroic medicine to what has been called therapeutic nihilism or fatalism. So it's like well we can't do anything, like it's just so hopeless.
Erin Allmann Updyke	Right, yeah.
Erin Welsh	So on the one hand that meant fewer people being bled to death but on the other if your patient developed a fatal infectious disease, there was nothing you could do but ease their passing. But fortunately researchers and physicians weren't satisfied by that. Enter Paul Ehrlich. Ehrlich had spent his dissertation exploring the use of dyes for staining various tissues and cell types and one of his important discoveries was that you could selectively stain certain types of bacteria. Side note, Ehrlich's fascination with dyes made him a really recognizable figure in all the places that he worked because his fingers were always stained and his clothes were always stained. And if you watch the show Charité which we both have watched and loved, they depict him as such. He has stains all over his lab coat and stuff.
Erin Allmann Updyke	That reminds me of my high school French teacher who always had chalk dust on his fingers and the edges of his pockets.
Erin Welsh	One of my favorite things in middle school and high school is when a teacher would have a chalk dust handprint that they would put in some very dorky place and I loved it. (laughs) Wow. Wild times, high school.
Erin Allmann Updyke	Chalkboards, you know.
Erin Welsh	But his discovery that you could selectively dye certain types of bacteria had huge implications for medicine because basically if you could get a dye to recognize those specific bacteria, maybe you could get a magic bullet then to target and destroy them. So Ehrlich went to work with Robert Koch in Berlin in 1891 to get started on this. And there he collaborated with Behring, if you remember Behring from our diphtheria episode, to work on this diphtheria antiserum among other things. But Ehrlich also had recognized the limitations in antiserums in terms of safety and scaling up production and also because not all bacteria produced toxins for antiserum development. And so it's kind of not a rich field for treatment.
Erin Allmann Updyke	Right.

Erin Welsh	And so he partnered up with a chemical dye company and went in search of a chemical compound that could deliver the same targeted blow as antiserums. And he began looking at treatments for trypanosomiasis, African sleeping sickness, which is caused by a protozoan parasite. And he looked at this because these protozoan parasites were a bit easier to see and identify under the scope. And so he and his collaborator Sahachiro Hata tested out many, many different compounds and versions of compounds and eventually they found success with compound 606. And that is not as it is often told the 606th compound to be developed but it was the 6th version of the 6th compound tested. But anyway they found it worked to cure sleeping sickness and this alone was fantastic. But then Ehrlich made a fortuitous mistake. He thought that syphilis was also caused by trypanosomes. It's not as we know from our syphilis episode, it's caused by the bacterium Treponema pallidum. My pet theory is that he just got the names confused, I can't remember when syphilis was called Treponema but trypanosome and Treponema, they sound similar. If that was the case, pretty cool.
	Anyway. But he tested it out, he tested out 606 on syphilis and this compound 606 which would later be called salvarsan or nowadays arsphenamine was hailed as a wonder drug because it worked. This was the first real synthetic chemotherapeutic drug and it was widely prescribed all over the world. And it wasn't great as you may remember from our syphilis episode but it was adequate and neosalvarsan was a slight improvement when it was developed a few years later. And this would be followed by other synthetic drugs that targeted malaria for instance. And this incremental progress continued through the early 20th century. But these drugs were super specific and sometimes had really, really nasty side effects. And the hunt was still on for something that could be more widely used to kill bacteria but success was really hard to come by. So for example a physician named Iago Galdston wrote that: "By 1930 it was the universal opinion of physicians that nothing could be discovered which would be effective against the ordinary diseases produced by bacteria." 1931.
Erin Allmann Updyke	(laughs) Nothing. Nothing.
Erin Welsh	Nothing, nothing. We have no hope! He was maybe a bit of a pessimist.
Erin Allmann Updyke	Yeah.
Erin Welsh	But so a year later in 1931 a team of researchers headed by Gerhard Domagk at Bayer, check out our aspirin episode for more on that company, he was working on a super lethal strain of strep and he found that if he combined an azo compound which was a type of synthetic dye with sulfanilamide, an organic sulfur compound, they had a drug on their hands that could wipe out this super strep in the mice that they had tested. And it also proved effective in humans to treat strep and non-strep infections like spinal meningitis and gonorrhea. But maybe most impressively, Prontosil which is the commercial name that this drug got, was found to be effective in treating Strep. pyogenes which is the cause of puerperal or childbed fever which was horrible and we'll do an episode on it at some point. With this drug mortality from childbed fever fell from around 20-30% to just 4.7%.
Erin Allmann Updyke	Whoa.
Erin Welsh	Yeah. Can you imagine?
Erin Allmann Updyke	Wow.
Erin Welsh	Yeah. We owe it all to chemistry.
Erin Allmann Updyke	Our whole lives are chemistry, you know what I mean?

Erin Welsh	They are, they are. And for this Domagk got the Nobel Prize. Okay anyway, it turns out that the azo dye in Prontosil has absolutely no antibacterial effect, it doesn't do anything. It's just the sulfanilamide.
Erin Allmann Updyke	(laughs) That's hilarious.
Erin Welsh	It's good news for the world but it was bad news for Bayer because they couldn't copyright or patent this drug anymore because sulfanilamide had been in the public domain for a few decades because it had been identified and published in a doctoral thesis back in the early 1900s. Yeah. So this meant that these sulfa drugs could be made pretty easily around the world and that satisfied demand much more effectively than if Bayer alone had gotten their patent like they had wanted. And these drugs were viewed as a miracle, rightly so. No one had ever seen such incredible and rapid improvement of patients who seemed on death's door and dreaded strep infection were no longer this death sentence that they had previously been. But there's a dark side to the widespread production of sulfa drugs and that is the regulation or more accurately the lack of regulation. One company in the US combined sulfa, raspberry flavoring, saccharine, and diethylene glycol to create a sweet little sulfa syrup.
Erin Allmann Updyke	Oh no!
Erin Welsh	Yeah, your face was very telling. (laughs)
Erin Allmann Updyke	You don't wanna eat ethylene glycol!
Erin Welsh	No, no. So diethylene glycol is a compound found in brake fluid, coolants, resins.
Erin Allmann Updyke	Antifreeze.
Erin Welsh	And when it's ingested by animals it produces dizziness, intoxication, nausea, elevated heart rate, muscle spasms, and ultimately kidney failure.
Erin Allmann Updyke	And death.
Erin Welsh	So this however didn't stop the launch of the drug, this sulfa syrup, in October of 1937. And almost immediately deaths were reported. And the FDA launched into action and tracked down almost the entirety of that initial shipment.
Erin Allmann Updyke	Oh thank gracious.
Erin Welsh	So I mean this company was brought to trial over this but they were not brought to trial because their drug had killed a bunch of people, the only thing that they could be fined or sued for is because they had mislabeled their drug as an elixir and it wasn't an elixir because by law elixirs were required to contain alcohol and this did not.
Erin Allmann Updyke	I can't.
Erin Welsh	I know.
Erin Allmann Updyke	I don't even have any words right now, quite honestly.

Erin Welsh	So they were fined, that's about it.
Erin Allmann Updyke	This was 1937.
Erin Welsh	Yeah.
Erin Allmann Updyke	Okay. Cool, cool, cool, cool, cool.
Erin Welsh	And one good thing did come out of this and that was the 1938 federal Food, Drug, and Cosmetic Act that introduced some much, much needed regulation and oversight into the manufacture and sales of medicines.
Erin Allmann Updyke	Whew.
Erin Welsh	Yeah.
Erin Allmann Updyke	It's about dang time.
Erin Welsh	Sulfa drugs continued to be widely used and were a key component in fighting infections during WWII and so it might not be that surprising that these were the first antibacterial drugs that we see antibiotic resistance towards. For example in the late 1930s, 90% of soldiers treated for gonorrhea with this drug were cured but by 1942 that had fallen to 75% and would continue to drop in just a few years.
Erin Allmann Updyke	Whoa.
Erin Welsh	But this concerning development was somewhat overshadowed by the introduction of an entire suite of antibiotics.
Erin Allmann Updyke	Snaps.
Erin Welsh	Here we go.
Erin Allmann Updyke	Into it.
Erin Welsh	I feel like this is where most people probably expected the story to begin so I apologize.
Erin Allmann Updyke	Oh 100%. Yeah I think the whole story of sulfa drugs is not as well known as penicillin for sure. Ooh, spoilers.
Erin Welsh	Oh yeah, whoopsie. (laughs) Yeah and there's a whole book about it that I have to confess I haven't read but I wanted to if I had more time called the demon under the microscope and that's about sulfa drugs.
Erin Allmann Updyke	Cool.
Erin Welsh	But anyway. Okay so yes, I think most people are at least somewhat familiar with the story of Fleming's "accidental" quote unquote discovery of penicillin. But just in case I'll take us through it cause there's also some fun things that I learned.

Erin Allmann Updyke	Of course Erin, there always are.
Erin Welsh	There always are. All right so in 1928 Alexander Fleming was a researcher in a lab in St. Mary's Hospital in London. And by this time his work during WWI on the role of anaerobic bacteria in battle wounds and the harm that antiseptics in wound treatment could cause as well as his discovery of the digestive enzyme lysozyme, these things had established him as an intelligent and insightful scientist. He also claimed to have discovered lysozyme when a drip of snot from his nose accidentally fell on a plate of bacteria he was culturing.
Erin Allmann Updyke	And what happened?
Erin Welsh	And they died. And so he's like, 'Oh there must be something in my snot.' And so he discovered lysozyme.
Erin Allmann Updyke	Okay, that's pretty cool.
Erin Welsh	Yeah.
Erin Allmann Updyke	So he just admitted to the world that he had a snotty nose that he just let drip everywhere.
Erin Welsh	Yep, yeah. I mean he was known by his coworkers to be pretty messy.
Erin Allmann Updyke	Oh yeah, well.
Erin Welsh	Not that having a snotty nose makes you messy but this is just I think in addition. So in August of 1928 as the story goes he left for a vacation in Scotland, leaving petri dishes of staph cultures just out on the bench and a window open. When he came back a couple of weeks later he found spots of fungal contamination on one of his plates and the fungus probably he assumed blew in through the open window. And around the spots of fungus, technically mold actually, was a clearing, so a ring where all the staph cultures on these plates had died. Fleming recognized that this mold represented some amazing possibilities in terms of killing bacteria and so he set to work trying to cultivate it.
	And he later discovered, actually was told my another colleague, that it was Penicillium notatum, now Penicillium chrysogenum. I think that's how you pronounce it, I'm not sure. And he reasoned that there was something which he referred to as 'mold juice' produced by this mold that inhibited the growth of bacteria and he called that penicillin. And he published this finding in March of 1929 in an article called 'On the antibacterial action of cultures of a Penicillium with special reference to their use in the isolation of B influenzae.' What an amazing story of accidental brilliance and insight. Or was it?
Erin Allmann Updyke	I will say what's impressive is like you pay that much attention to the plates that you supposedly just forgot and left lying around all open and empty. Right?
Erin Welsh	Right.
Erin Allmann Updyke	Like you come back and you're like, 'Let me inspect this. Ooh, I see a fungal growth and a small clearing around it.' That's pretty good.

Erin Welsh	Yeah. I mean I am by no means saying that this is not impressive. I just think, and this is not a thought unique to me, this is definitely something that I picked from these books that I read. This probably wasn't as accidental of a discovery as he claimed it to be. The insight was still brilliant and amazing. But let's just go through some of the points of the story that don't make a lot of sense. First of all that window that just happened to be open? According to other people in the lab it was never opened, so it would be kind of strange that he would leave it open for a bunch of weeks at a time.
Erin Allmann Updyke	Yeah.
Erin Welsh	And then the timeline itself is a bit fuzzy. So first Fleming said he was gone for at least 5 weeks and then it was no more than 2. And that could just be not remembering well if you're recalling this years into the future but the biggest plot hole lies in the biology of this Penicillium species. So although Fleming wouldn't have known this, the staph on a plate would have killed the mold before it would produce penicillin. If the plates already had those cultures there like they did before he left and then the Penicillium blew in through the window, that staph would have killed the mold before it could produce the compound penicillin. And so those rings weren't possible. So what actually was going on? Well Fleming was a rather inventive guy who liked to play games. He would paint pictures of the Union Jack or the logo of St. Mary's using different bacterial species and this was in the 1920s when you would've had to have encyclopedic knowledge of bacteria to be able to pull that off.
Erin Allmann Updyke	Yeah.
Erin Welsh	That's pretty cool.
Erin Allmann Updyke	Yeah.
Erin Welsh	And he was also painfully shy and hated discussing his methods or results with anyone. So the best guess from the author of the book that I read about this is that he invented the story so as not to have to describe his process of discovery. And he may have actually been looking at more sources for lysozyme and thinking maybe it's found in mold or Penicillium as well.
Erin Allmann Updyke	Cause basically they're saying he would have had to basically plate the mold before he plated the Staph. aureus to be able to actually kill staph with that mold?
Erin Welsh	Right, exactly. But regardless of how he arrived at this discovery, he still discovered it and connected those dots.
Erin Allmann Updyke	Right, yeah.
Erin Welsh	That's pretty cool.
Erin Allmann Updyke	They're impressive dots to connect, that's for sure.
Erin Welsh	Super impressive. I just don't know why he would've chalked it up to serendipity. I don't know, maybe it's like more fun to have a lightbulb moment than the incremental progress and years of hard work and insight. I don't know, I don't know.
Erin Allmann Updyke	Yeah. I don't have a single idea.

Erin Welsh	Yeah. (laughs) But if there's one name that we associate with penicillin it's Fleming, right? And if there's one era, it's in the first half of the 20th century. Right? However-
Erin Allmann Updyke	Ooh, always the however.
Erin Welsh	It turns out that the bacteria-killing quality of Penicillium molds had been observed before as early as the 1800s when Sir John Scott Burdon-Sanderson, Joseph Lister, and John Tindall all observed separately that bacteria would not grow in media that had been contaminated by mold. And Lister and Tindall went as far as to describe the mold as a Penicillium species. And there are other instances of people recognizing the power of mold, so what made Fleming's discovery a breakthrough while the others remained simply observations?
Erin Allmann Updyke	Right.
Erin Welsh	So one reason is that Fleming saw the implications that this could have for treating infections and also because he set out trying to isolate his mold juice compound, basically turning his lab into this penicillin farm.
Erin Allmann Updyke	Okay.
Erin Welsh	And when they - 'they' meaning Fleming and his assistant Stuart Craddock - finally had enough mold juice to test it out, they realized that it killed not just staphylococci bacteria but also streptococci and a bunch of other groups of bacteria, gram-positive bacteria. They also realized that some bacteria were immune to penicillin, gram-negative, among others. And the final really important realization was that it was not harmful to non bacterial cells.
Erin Allmann Updyke	Yeah.
Erin Welsh	That was the big one.
Erin Allmann Updyke	Right.
Erin Welsh	This was finally like, was this the magic bullet? Seemed to fit. But all of these observations were made through bench work alone, Fleming never tested out penicillin on an animal. Maybe he never thought of it, maybe it was just too difficult to isolate the compound. And finally the lab where he worked at St. Mary's, it simply wasn't equipped to so the kind of work that he was doing. There were very few chemists working there and overall it was much, much less funded than the chemical dye companies who microbiologists were partnering with in Germany. So there was only so much Fleming could do. But where Fleming left off Florey picked up. In 1935 Australian Howard Florey was professor of pathology and fellow of Lincoln College at Oxford and was leading a research group to investigate why bacteria could not penetrate the wall of the GI tract or did not seem to. He suspected lysozymes which you remember from Fleming's discovery and to carry out his research he realized he needed to form a strong collaboration with chemists.
	And honestly Erin I think I had no idea that this episode was going to be propaganda for chemistry but I'm won over, I'm there. I'm here for it. Okay. And anyway he ended up partnering with a couple of chemists, one named E. A. H. Richards who would purify lysozyme and another named Ernst Chain who would identify its substrate. These efforts were part of a larger goal of the lab that had begun in 1937 which was to survey, this is like a massive undertaking, to survey all of the antibacterial substances produced by microorganisms. Just all of them.

Erin Allmann Updyke	Just all of them by all of the microorganisms.
Erin Welsh	Yep.
Erin Allmann Updyke	Cool, cool, cool. Yeah. How did that work out for them?
Erin Welsh	Actually pretty great because Penicillium molds were on that long list. But in the 8 years since Fleming had published his paper in 1929, not much substantial work had been done on penicillin and Fleming had all but abandoned it. At one lab group afternoon tea Florey was talking about what a difficult time Fleming and a biochemist named Raistrick had in trying to stabilize penicillin. And Chain was like, 'Well they must not have been very good chemists' and took it as a personal challenge to try to do it himself.
Erin Allmann Updyke	I relate to that. (laughs)
Erin Welsh	But Chain at the time had his hands full with a bunch of other chemical experiments on penicillin so he called on biochemist Norman Heatley who by all accounts was the nicest, most humble, kind person in this group. So he wanted Heatley to work on growing enough of this Penicillium mold to make research feasible.
Erin Allmann Updyke	Okay.
Erin Welsh	And where Fleming had lacked the equipment and chemical minds to make forward progress with penicillin, Florey's lab just lacked money, period. And eventually the Rockefeller Foundation awarded a nice grant to help them along and it was just in the nick of time because the penicillin project had been yielding some very promising results.
Erin Allmann Updyke	Ooh.
Erin Welsh	So Heatley had been able to extract larger quantities of penicillin as well as stabilize it which made experiments much easier to perform. And finally on May 25th, 1940 penicillin was injected into 4 of 8 mice that had been infected with Strep. pyogenes and the 4 that received treatment survived and seemed absolutely 100$ healthy. And the 4 that did not, died.
Erin Allmann Updyke	Whoa.
Erin Welsh	Yeah. And this was great news. I mean not for the mice that died but for the world. But there was still this huge problem of production.
Erin Allmann Updyke	Right.
Erin Welsh	Because although Heatley had made amazing progress in the stabilization of penicillin he simply could not make enough of it to keep up with demand and it's not like the lab had the funds to supply the materials or equipment to ramp up production. So he resorted to stealing. He took bedpans from the hospital supply cabinet and baking trays from the kitchen to supply this huge amount of space that he needed to grow the mold and he basically MacGyvered his way through the purification process through a collection of just random junk that he found in the lab.
Erin Allmann Updyke	I love that.

Erin Welsh	It is amazing.
Erin Allmann Updyke	He just literally needed containers to grow mold in.
Erin Welsh	Yep.
Erin Allmann Updyke	He's like, 'I'll use this, I'll use that. Ooh great, you're not using this? You haven't baked in a while!'
Erin Welsh	Yeah. (laughs) Yeah, it's really impressive. And in the book that I read there are some figures that show his setup and it's just amazing, it's amazing.
Erin Allmann Updyke	That's awesome.
Erin Welsh	There's so much more to all of these stories and this is already a very long story. But it's just fun and it's fun to know about the individuals themselves and the personalities and how much personality plays a role.
Erin Allmann Updyke	And the ingenuity. Yeah.
Erin Welsh	Yeah. It's very cool.
Erin Allmann Updyke	Yeah.
Erin Welsh	So in August of 1940, Florey felt that he had enough to go public with this penicillin news and he published this research in an article in The Lancet titled 'Penicillin as a chemotherapeutic agent.' But at this point penicillin hadn't yet been tested in humans, just mice. First up was someone with terminal cancer but no bacterial infection. After receiving a shot of penicillin she developed a high fever and seizures and so that led them to realize that the process of concentrating penicillin also had this unintended side effect of concentrating impurities. So they fixed it. But instead of just testing to see whether it was safe for injection, they also needed to test whether it was effective. They needed someone with an infection. And they found one in an Oxford policeman named Albert Alexander who had scratched his face with a rosebush while gardening. Over the course of a few months that small cut, that tiny cut led to massive infections all over his body. He was quote "oozing pus everywhere".
Erin Allmann Updyke	Gross.
Erin Welsh	His eyes, everywhere. Just everywhere. One day after just one injection of 200 mg of less than 5% pure penicillin he had made a miraculous improvement. The pus had stopped flowing and the fever had gone. But the problem was still in keeping up the supply. So the researchers had known that penicillin is excreted by the kidneys so they started to collect the policeman's urine and rush it back to the lab to repurify and then back to the hospital to reinject. But they just couldn't keep up with the demand, they couldn't do it. And so the policeman died 5 days after once they ran out of penicillin which is sad. But another person, a young kid that they treated survived later on. And so this was proof enough that it worked as long as you had enough of penicillin. But there was never enough. (singing) Never, never.
Erin Allmann Updyke	(laughs)

Erin Welsh	Florey and Heatley needed more funds if they were ever going to make penicillin a feasible treatment for infections and so they turned to a country that had the funds and the agricultural research infrastructure that they needed: the United States. After meeting with some friends/fellow researchers about the work that they wanted to do, they came to the conclusion that they needed to head to the foremost site of agricultural research in the country, the Northern Regional Research Lab in Peoria, Illinois.
Erin Allmann Updyke	Peoria!
Erin Welsh	Yeah! Peoria is the site for penicillin from when it went from a novel, potential thing to-
Erin Allmann Updyke	To actual penicillin.
Erin Welsh	Actual penicillin.
Erin Allmann Updyke	Yeah. Wow. Peoria, yeah.
Erin Welsh	Peoria.
Erin Allmann Updyke	I wonder if they have that just blazoned everywhere, like all their bridges. Penicillin!
Erin Welsh	Penicillin was found here! It wasn't found there but...
Erin Allmann Updyke	I've only been to the outskirts of Peoria, I've never traversed the city streets.
Erin Welsh	So how exactly did penicillin go from medical curiosity to world-changing substance?
Erin Allmann Updyke	Yeah.
Erin Welsh	There are three big developments that would make this transformation possible. Number one, finding the strains of Penicillium that produced the most penicillin. Number two, developing the best protocol to rapidly grow the mold. And number three, improving the fermentation process that actually led to penicillin. To find these turbo strains of Penicillium, a bacteriologist at the lab named Mary Hunt went to Peoria's markets like every day, every weekend to find moldy fruits and vegetables.
Erin Allmann Updyke	Like the veg market!
Erin Welsh	Yep.
Erin Allmann Updyke	She's just like walking around the farmer's market like, 'Don't worry, I'm here for work.'
Erin Welsh	Yep. And she hit paydirt, she hit absolute paydirt in 1943 with the moldy cantaloupe.
Erin Allmann Updyke	Cantaloupe!
Erin Welsh	The mold on that cantaloupe was so powerful that it became the source for basically all of the world's penicillin. And as for a better growth medium the Midwest US is known for what type of food, Erin?

Erin Allmann Updyke	Corn?
Erin Welsh	Corn, yeah.
Erin Allmann Updyke	Corn.
Erin Welsh	Corn.
Erin Allmann Updyke	Corn and corn.
Erin Welsh	Corn, corn, and corn. And it turned out that growing Penicillium in something called corn steep liquor plus sugar produced 1000 times more penicillin than the previous method.
Erin Allmann Updyke	Wow.
Erin Welsh	That's a lot.
Erin Allmann Updyke	Way to go corn!
Erin Welsh	Way to go corn.
Erin Allmann Updyke	Corn for the win.
Erin Welsh	But once again it came time to ground truth penicillin. On Valentine's Day 1942 a woman named Anne Miller was in the hospital in New Haven, Connecticut after experiencing a miscarriage. She had developed blood poisoning, aka hemolytic streptococcal septicemia. She had fevers of 107, she was not coherent, it was really bad. And her doctor ended up through a series of pleading phone calls, he ended up securing a small glass vial containing 5.5 grams of penicillin which had come from that research lab. 5.5 grams at the time, 1942, was half of the entire amount of penicillin in the US.
Erin Allmann Updyke	Whoa!
Erin Welsh	He guessed at a dosage cause there were no guidelines, right.
Erin Allmann Updyke	No.
Erin Welsh	And he injected the drug into Anne. She survived the night and the next day and the next day and the next 57 years after that.
Erin Allmann Updyke	Wow.
Erin Welsh	Yep. Her medical chart from this time is actually in the Smithsonian's National Museum of American History. So you can go and see it.
Erin Allmann Updyke	I love that museum.
Erin Welsh	Yeah, oh it's amazing.

Erin Allmann Updyke	Yeah.
Erin Welsh	And this marked a real turning point in penicillin's history. By 1942 and 1943, many pharmaceutical companies in the US including Merck and Pfizer got into the penicillin biz and worked on their own production processes meaning that mass manufacturing was just around the corner and so were massive profits, just like heaps and tons and loads of money.
Erin Allmann Updyke	Money, money, money.
Erin Welsh	Money, money, money. And whereas the successful treatment of Anne Miller with penicillin had led to the biomedical industry taking note of the drug, a horrific fire would wake up the public to it. On November 28th, 1942 an artificial palm tree at a Boston nightclub called The Coconut Grove caught fire and within minutes the entire club was consumed.
Erin Allmann Updyke	Whoa.
Erin Welsh	492 of the over 1000 partiers died. It's one of the deadliest fires in American history.
Erin Allmann Updyke	That's terrifying.
Erin Welsh	It's horrible. And hundreds more were horrifically burned. At Mass General Hospital the doctors decided that rather than debridement of the burns, they would try to administer penicillin and sulfa drugs. And it's hard to say whether the penicillin did perform the miracles on these burn victims as the newspapers later claimed but regardless it had now become firmly established in the public's eye as a wonder drug. Demand for penicillin reached new heights but the mounds of this dried brown powder weren't just there for anyone to use cause there was a war going on, right. And so basically until the war was over, penicillin was strictly reserved for troops, Allied troops, with most of the drug being used for wounds received in battle and also gonorrhea. Most civilians, and there were a few exceptions, wouldn't enjoy the benefits of penicillin until after the war was over and Australia would actually be the first company to open up its use to the public.
	Even with all of these amazing advancements in penicillin production there was still one big piece of the puzzle that had yet to be solved, that of its structure. So Chain had made some progress towards this but only in that he could produce crystallized degradation products which he offered up to biochemist Dorothy Crowfoot Hodgkin who had already made amazing, amazing discoveries about the structure of many large complex organic molecules such as cholesterol, testosterone, pepsin, insulin, and many others using X-ray crystallography. So with the crystals that Chain had given her, Hodgkin used X-ray crystallography to get a clearer idea of the different components of penicillin. But a clearer idea is not the same thing as a clear idea and without that clear idea, penicillin would never be synthesized in a lab where it could be produced in larger quantities that were more stable, more pure, and more efficient.
Erin Allmann Updyke	Right.
Erin Welsh	The breakthrough came when Hodgkin proposed a beta lactam ring.
Erin Allmann Updyke	Yeah.
Erin Welsh	It's amazing. And by all accounts, just like Heatley, she was not only North America amazingly brilliant researcher, she was also really kind and well-loved by everyone who knew her.

Erin Allmann Updyke	Aw. I have a baby book that she is the letter 'D' is for Dorothy Hodgkin.
Erin Welsh	Oh nice! Oh that makes me happy, yay. So anyway penicillin wouldn't be synthesized until 1957 but knowing its structure was integral to not just making it in a lab but also in trying to look for other compounds that have similar structures and could be used to treat bacterial infections. So the reason that I spent so much time talking about penicillin in this history of antibiotics as a whole is because it provided this new framework for thinking about what these different antibiotics might look like and where you could look for them.
Erin Allmann Updyke	Right.
Erin Welsh	So namely in the existence of other living things. But as you talked about, Erin, penicillin acts on only a subset of bacterial species, gram-positives. It's not effective against gram-negatives like Yersinia pestis or Chlamydia trachomatis or Vibrio cholerae or acid-fast bacteria like microbacterium tuberculosis. And up until the early 1940s the second leading cause of death in the US was bacterial pneumonia which could be caused by a variety of gram-positive and gram-negative bacteria. And tuberculosis wasn't much farther down the list, it took 6th place.
Erin Allmann Updyke	Yeah.
Erin Welsh	So the war on bacteria was far from over and it's - spoiler - still not over today. And besides penicillin the war years yielded more than just penicillin in terms of antibiotics. Two other drugs named tyrothricin and gramicidin were developed from compounds produced by a soil bacterium. But there's a reason that those two might not sound that familiar even though they're still occasionally used today. So one of these works by stopping proteins by being made as you had described and the other makes cell membranes impermeable. Both of these things will definitely kill bacterial cells but in these cases they also killed animal cells. So their use was pretty limited. But one important thing they did was give people a reason to look in soil for other possible antibiotics and whereas Fleming's discovery of penicillin had been a possibly fortuitous accident, the hunt for antibacterial compounds in soil was a grueling, systematic, trial and error search full of long days of hard work which is, let's face it, that's how most scientific developments actually happen.
Erin Allmann Updyke	Right.
Erin Welsh	One of the people doing these long days of work and often even sleeping in the lab was a PhD candidate named Albert Schatz. His particular obsession was with actinomycetes which is a group of soil bacteria and trying to find a compound to kill microbacterium tuberculosis. And if you listened to our tuberculosis episode from way back in Season 1, this story may sound a bit familiar to you. As we know, tuberculosis is very deadly and at the time there was no cure. So Schatz was basically exiled to do his work in the basement by himself.
Erin Allmann Updyke	Oh gosh.
Erin Welsh	Cause it was dangerous. He was like culturing tuberculosis.
Erin Allmann Updyke	Oh culturing tuberculosis, okay.
Erin Welsh	Yeah. But this isolation I guess paid off. On October 19, 1943 he discovered that a bacterium named Streptomyces griseus produced a substance that killed microbacterium tuberculosis.
Erin Allmann Updyke	Oh yeah.

Erin Welsh	And that substance is what we know as streptomycin.
Erin Allmann Updyke	Streptomycin!
Erin Welsh	But just like with the early days of penicillin, there was a production issue. How do you make enough of the stuff to actually perform meaningful experiments? And that's where Schatz' advisor Selman Waksman leaned on the lab's connection with Merck to enlist their help in ramping up production. So eventually they were able to make enough streptomycin to test ti on guinea pigs infected with tuberculosis. And guess what? It worked.
Erin Allmann Updyke	It worked!
Erin Welsh	It also worked on humans which was again viewed as a miracle, the common thread in this. But who would get credit for this miracle?
Erin Allmann Updyke	Not the PhD student, never.
Erin Welsh	No, no. And interestingly Merck gave up the patent rights to Schatz and Waksman and they filed the patent on behalf of Rutgers University on February 9th in 1945, both swearing under oath that they were co-discoverers of the drug. But if you were living then and following the news about streptomycin during this time you only heard one name: Waksman. Waksman had dozens of newspaper articles calling him a hero and man of the soil while Schatz' name was nowhere to be found. Schatz was not okay with this and told Waksman this but what he got in reply was, quote: "You must therefore be fully aware of that fact that your own share in the solution of the streptomycin problem was only a small one, you were one of many cogs in a great wheel in the study of antibiotics in this laboratory. There were a large number of graduate students and assistants who helped me in this work, they were my tools, my hands if you please."
Erin Allmann Updyke	Oh gosh. That's why doing a PhD's so depressing with advisors like that.
Erin Welsh	Yep. It's not a very great way to look at your students.
Erin Allmann Updyke	Nope.
Erin Welsh	And this conflict spilled over into the financial side of things, so they both had supposedly signed away their patent rights for $1 each but Waksman had made a side deal that had earned him 20% of the profits if he got Schatz to sign away his rights.
Erin Allmann Updyke	What?
Erin Welsh	Yeah!
Erin Allmann Updyke	That's so gross.
Erin Welsh	I know, I know, I know. And of course there was the fact that Waksman was awarded the Nobel Prize and Schatz was not mentioned either by the committee or in Waksman's acceptance speech as more than just 20 graduate students or lab techs.
Erin Allmann Updyke	This is the problem with Nobel Prizes, guys.

Erin Welsh	It is true that Waksman was a brilliant scientist who made many other incredible discoveries and he is actually the one who came up with the term 'antibiotic' but this is not great visuals.
Erin Allmann Updyke	Not great visuals.
Erin Welsh	In any case by the late 1940s there were now two super powerful antibiotics at work, completely reshaping the health of the world. And the dive into soil bacteria kind of opened the floodgates even more than penicillin. The first broad spectrum antibiotics that worked on both gram-positive and gram-negative bacteria were discovered, so called tetracyclines, and then in 1949 came erythromycin also from a soil bacterium, Streptomyces erythreus. Chloramphenicol was another that came along that received immediate popularity but its popularity wouldn't last too long because when people realized that the super powerful antibiotic resulted in some people developing aplastic anemia, it was kind of not used as much.
Erin Allmann Updyke	Yeah. Aplastic anemia means that your body stops making blood cells, like all of them.
Erin Welsh	Yeah.
Erin Allmann Updyke	So it's really bad news.
Erin Welsh	It's really bad.
Erin Allmann Updyke	It's also very deadly for babies.
Erin Welsh	Yeah, yeah, especially in kids they were seeing this happen. And then in 1948 came the discovery that there was a massive, so far untapped market for antibiotics: agriculture.
Erin Allmann Updyke	Oh gosh, yeah.
Erin Welsh	Oh gosh. An early experiment showed that chickens receiving broad spectrum antibiotics grew much bigger, much more quickly than those that didn't get the drug and almost immediately pharmaceutical firms jumped on this, producing tetracycline-derived nutritional supplements.
Erin Allmann Updyke	Yeah.
Erin Welsh	Up to 25% of all of the antibiotics ever manufactured have been for use in animals.
Erin Allmann Updyke	Wow. That is a lot.
Erin Welsh	It's a lot. And the doses that they were given were not therapeutic doses like the amount needed to cure an infection.
Erin Allmann Updyke	Right.
Erin Welsh	These were super tiny doses given to promote growth. And those tiny amounts of antibiotics led to massive amounts of resistance which I'm gonna talk more about in our next episode but this is a great trend towards antibiotic use throughout the 20th century. Do you have any sort of complaint or infection whether it be viral or bacterial?

Erin Allmann Updyke	Sprinkle some antibiotics on it!
Erin Welsh	Sprinkle, sprinkle. And the philosophy was that even if it might not be... And the philosophy still kind of is, I think some people thought that even if it's not a bacterial infection that antibiotics couldn't hurt, right? But that's where we might be wrong.
Erin Allmann Updyke	We are definitely wrong.
Erin Welsh	Because not only does this overuse of antibiotics and misuse lead to antibiotic resistance but we're also finding fewer and fewer effective antibiotics and so we're literally running out of the ones that we have. And we're also, as I mentioned earlier, learning a lot more about our own microbiome and the huge role that it plays in our health.
Erin Allmann Updyke	Right.
Erin Welsh	So I mean my story kind of stops there because it's just like antibiotics continue to be developed and then now it's kind of fallen off. And I'll pick up in our next episode on the history of resistance which is a fascinating one that definitely bleeds into current times.
Erin Allmann Updyke	Oh definitely, big time.
Erin Welsh	But for now, Erin, tell me what's going on in the world of antibiotics.
Erin Allmann Updyke	I can't wait to. We'll take a quick break first.
TPWKY	(transition theme)
Erin Allmann Updyke	A lot of the modern story of antibiotics is antibiotic resistance and we're not gonna talk about that today. So we'll just sort of focus on what is the status of sort of antibiotic development today. So how do we use antibiotics today? You mentioned in agriculture. The use of antibiotics in agriculture is so intense and so massive. In 2010 there were something like 63,000 tons of antibiotics used in livestock. And it's on the rise, it's projected that by 2030 it'll be 105,000 tons.
Erin Welsh	Yeah. It is eye-opening to be sure.
Erin Allmann Updyke	Yes, most definitely. So what about in humans? How do we use antibiotics? Between 2000 and 2015, overall antibiotic consumption has increased 65%.
Erin Welsh	Really?
Erin Allmann Updyke	Yeah, we're just increasing our use of antibiotics even though we know we shouldn't be.
Erin Welsh	So penicillin when it was first introduced, you could just go to the store and buy it.
Erin Allmann Updyke	And in a lot of places that's still the case, right.
Erin Welsh	That's true.

Erin Allmann Updyke	A lot of parts of the world you can still buy antibiotics just over the counter. So yeah, overall global consumption is really increasing and this is concerning for a lot of reasons, resistance chiefly among them. But the other thing is, and you kind of mentioned this a little bit Erin, we haven't been good at coming up with new antibiotics for a really long time. So after the discovery of penicillin and then quickly after that the discovery of so many other groups of antibiotics right, streptomycin that acts in a totally different way we know, protein synthesis rather than the cell wall. Once we came up with those four classes of antibiotic mechanism there haven't really been new classes of antibiotics approved. Between 1960 and 2000, no new classes of antibiotics were approved.
Erin Welsh	Oof.
Erin Allmann Updyke	Right? And that means that while we came up with plenty of new antibiotics, they were just variations on a theme.
Erin Welsh	Right. Which means that resistance might be an easier leap than it would be for a whole new class of antibiotics?
Erin Allmann Updyke	Exactly, right. So it's basically just trying to one up the resistance that we're seeing, right. Let's change the structure of that beta lactam just a little, etc.
Erin Welsh	Yeah.
Erin Allmann Updyke	The good news is that between 2000 and 2014 there have been a couple of new antibiotics approved including a few new classes. The oxazolidinones that I mentioned earlier, linezolid, so that's a synthetic drug, it still inhibits protein synthesis but its mechanism of action is different than other classes like the aminoglycosides and macrolides. And another one called diarylquinoline, hadn't hear of that before, it's a drug that we use for TB I guess, it's relatively new, and this disrupts energy metabolism in some new way that I don't know a lot about.
Erin Welsh	Cool.
Erin Allmann Updyke	So we're making progress. But there's still a lot of challenges, right. You mentioned how we used to screen and discover new antibiotics, right, by digging through soil samples and trying to isolate compounds, etc.
Erin Welsh	Yeah. It's a grueling process.
Erin Allmann Updyke	It's a very long and drawn out-
Erin Welsh	Labor intensive.
Erin Allmann Updyke	Labor intensive, expensive, difficult process.
Erin Welsh	Yeah.
Erin Allmann Updyke	So I guess one of the big questions is have we come up with any better ways to identify antibiotics?
Erin Welsh	I think that we have.

Erin Allmann Updyke	We have which is absolutely thrilling. And we don't wanna tell you about it, we wanna have someone tell you about it who knows more about it. So for that we're very, very fortunate to interview doctor Jonathan Stokes who has worked on antibiotics extensively and who recently published a paper about his work on discovering new antibiotics using entirely different methods than what we have used in the past.
Erin Welsh	It is so mind blowing, I love it.
Erin Allmann Updyke	It's very, very cool.
Erin Welsh	It's like this is the future.
Erin Allmann Updyke	It is the future.
Erin Welsh	We are living in the future.
Erin Allmann Updyke	We are. (robot voice) The year 2000, the distant future. Okay?
Erin Welsh	(laughs) Yes!
Erin Allmann Updyke	So we'll let him introduce himself.
Jonathan Stokes	So I'm Jon Stokes, I'm a postdoctoral fellow in Jim Collin's lab at MIT in the Broad Institute. I did my PhD at McMaster University in Hamilton, Ontario under the supervision of Eric Brown. So by training I consider myself more of a biochemist, like an antimicrobial biochemist and I find myself now in Jim's lab doing a lot more systems biology type work which is cool because it kind of compliments what I did during my PhD nicely I feel.
Erin Welsh	That's awesome.
Erin Allmann Updyke	That sounds awesome. So we talk in our episode about how antibiotic discovery has kind of decreased over the last few decades but of course the need for antibiotics hasn't decreased, if anything it's getting worse. So could you talk a little bit about some of the challenges in the traditional ways that we search for new antibiotics?
Jonathan Stokes	Yeah. So historically we had our heyday of antibiotic discovery between the 1940s and the 1960s, the golden era of antibiotics where we were able to find a wide structural and functional collection of antibiotics through screening secondary metabolites from soil-dwelling microbes, right. However come the mid 1960s we ran into a problem in that we kept rediscovering the same antibiotics over and over again. And so in more recent decades we've shifted to high-throughput screening of synthetic chemical libraries in an attempt to find new antibiotics. However for I would guess perhaps 30 years maybe of high-throughput screening we haven't found a single new clinically used antibiotic through that approach.

	The other one beyond the scientific drawbacks are the economic drawbacks, right. So if you're a drug company, it costs just as much money to make an antibiotic as it does to make a blood pressure medicine or an anticoagulant or something, like something that patients are gonna be on for decades as opposed to you're gonna take a 10 day course on an antibiotic. And furthermore if let's say we start a company, we find the best antibiotic the world has ever seen, right. Physicians aren't gonna wanna use it, they're gonna wanna save it, right. So you've invested a billion dollars or 2 billion dollars or something to develop this drug that is never going to be able to generate the revenue to recoup your costs. So the economic model for antibiotic discovery and development is fundamentally broken as well.
Erin Welsh	So you mentioned high-throughput screening for potential antimicrobial or antibiotic compounds. What are some of the other innovative new ways that people have looked for new antibiotics?
Jonathan Stokes	Yeah so for my old lab one approach that we took in our lab was looking for molecules that have unconventional activities. Another one is adjuvant screens right, so instead of looking for one molecule at a time that inhibits the growth of a bacterium of interest, what happens if we start combining two at a time? There's been some work looking into phage therapy for the treatment of bacterial infections but not just using what we would call a natural phage or wild-type phage but there have been instances where investigators have engineered phage to deliver toxic payloads. What else? Antivirulence molecules, in this case you're not eradicating your population, you're just inhibiting bacterial cell process such that it won't make you sick. So there's a whole lot of ways that people are trying to kind of address this problem outside of conventional antibiotic discovery as we would define it.
Erin Allmann Updyke	Awesome. So we found you through your recent paper that was published in Cell that was on deep learning approaches to antibiotic discovery. I had literally never heard of the term 'deep learning' before reading this paper so could you tell us a little bit about this project specifically and kind of explain maybe for us who have no idea what machine learning is?
Jonathan Stokes	Yeah. So at a high level machine learning is the discipline in which computers are programmed to learn patterns in data sets. In our specific case we can use that as a concrete example. We want it to predict antibiotics. What we wanted was a case where if we showed a computer the structure of a molecule it would be able to predict whether it was antibacterial or not, right, like at the simplest form. In order to develop a machine learning model that can do that, what you first have to do is train that model.
	So the model that we trained is called Chemprop, it was developed a couple years ago maybe by Regina Barzilay in her lab. So in our case we had 2500 molecules that we trained on and each one of those molecules had a bioactivity score. Did it inhibit the growth of E. coli? Yes or no? So during training the model was walking around all 2500 molecules and then learning the relationships between the structures of every molecule and whether it inhibited the growth of bacteria or not. And then we ran predictions in a library that's housed at the Broad Institute which is called the Broad Repurposing Hub. It's about 6000 molecules either in preclinical development, in phase 1, 2, 3 trials or on the market.

	And all the model did was look at every molecule in that library cause we have the structures for all 6000 molecules and then for every one it gave us a number between 0 and 1. If it was close to 0 it was unlikely to be antibacterial. If it was close to 1 it was likely to be antibacterial based on the model that we trained using the 2500 compounds. So the way that we did it through the message-passing approach, it was not user-defined, like the model could find any molecular features within any given molecule that would be strongly predictive or not predictive of antibacterial activity, right. It was not human constrained, it was free to explore the molecule and interpret the molecule in any way that it found fit to make right predictions. But yeah, so they can make all of these interesting quote unquote "discoveries" even though we don't necessarily know what they're doing, they know what they're doing. They predict molecules to be antibiotic that you would not necessarily think to be antibiotic just looking at the structure.
Erin Allmann Updyke	Wow.
Erin Welsh	That is amazing. So you mentioned that this model did find a couple of potential antibiotic compounds. Can you talk a little more about those and how they work to either inhibit the growth or kill bacteria and what kind of bacteria they seem to be effective against?
Jonathan Stokes	Yeah, yeah. So the first one that we found was halicin, right. So that was actually from the drug repurposing hub, that library of 6000 I just mentioned. So we have set out to find molecules, we wanted to predict molecules that were antibacterial but also we wanted to find molecules that didn't look like conventional antibiotics, right. Cause we wanted to find structurally and functionally new stuff that would be able to overcome a lot of the currently existing resistance mechanisms. When we ran out predictions on the drug repurposing hub we actually put two constraints on it. The first thing was obviously it had to have a high prediction score, the molecules had to be strongly predicted to be antibacterial but the second constraint was they couldn't look like conventional antibiotics. And then we trained another model to predict toxicity, right, so we wanted the molecules to be nontoxic, predicted to be nontoxic.
	The molecule that fit the bill for those three criteria was halicin. And halicin was a really interesting molecule to study. So we trained our model in E. coli, we also found that it was bactericidal in E. coli rather than bacteriostatic, so it killed E. coli cells rather than simply inhibiting their growth. One of the coolest features to me about the molecule is that it had this really interesting ability to eradicate antibiotic tolerant cells. So most antibiotics, they were discovered using growth inhibition. However there are states in which bacteria can reside in which they aren't undergoing a lot of biological activity so what happens is if you take those cells in stationary phase or nutrient-deprived or whatever in any other way and you expose them to a bactericidal antibiotic, most of the time with a couple of exceptions, those bactericidal antibiotics don't work because the biological processes that they inhibit aren't doing anything.
Erin Allmann Updyke	Oh man.
Erin Welsh	That is amazing.
Jonathan Stokes	It's like trying to crash a parked car, it doesn't really work.
Erin Welsh	Oh my god, that's a great analogy.
Erin Allmann Updyke	Yeah.

Jonathan Stokes	Thank you. But we found we could take E. coli, put it in conditions that didn't have food in it, they weren't dividing, they weren't doing anything, and we were observing that halicin was still eradicating these cultures. So to me that was like okay, there's not a whole lot of molecules that do this and that's what got me pretty excited about this compound.
Erin Allmann Updyke	Wow.
Erin Welsh	That is so cool.
Erin Allmann Updyke	That is really, really cool.
Erin Welsh	We're gonna set a record for the number of times we say 'that's so cool' I think, Erin.
Erin Allmann Updyke	(laughs) Oh gosh.
Jonathan Stokes	So that was all in E. coli. So that's fine and good but it's lab strain E. coli so the next question becomes does it work in clinically problematic species, right. So can we take isolates that are multidrug resistant, Klebsiella, Acinetobacter, staph, microbacterium tuberculosis, like these types of bugs. So we ask does halicin retain activity in multidrug resistant versions of all of these really nasty pathogens? And it did. We observed that it retained activity in multidrug resistant carbapenem-resistant Enterobacteriaceae, right.
Erin Allmann Updyke	Whoa, that's major.
Jonathan Stokes	Multidrug resistant Acinetobacter baumannii, MRSA, microbacterium tuberculosis, C. difficile.
Erin Welsh	That is amazing.
Erin Allmann Updyke	I don't wanna just say 'that's so cool' again but like oh! Oh man.
Jonathan Stokes	So when we saw this it became very obvious that the next thing that we hope to do is figure out how it was working. And I kinda have a playbook for how I like to figure out how molecules work and the first thing that I like to do is evolve resistance to it, right, because if you can evolve resistance to something sometimes, not all the time, but sometimes it'll point you in a direction to tell you what it's doing, right, what is the target. However something that was super cool and at the time slightly annoying occurred and it was in the laboratory in liquid culture over the course of 30 days I ran just like a typical evolution experiment in liquid medium and I couldn't evolve resistance to halicin over the course of 30 days. And the control experiment for that was ciprofloxacin, right. So I ran the same experiment in parallel with ciprofloxacin and over the course of the same 30 day experiment I think we evolved resistance to cipro like 200 fold or something like that, like it was outrageous.
Erin Allmann Updyke	Oh my gosh.

Jonathan Stokes

So then we also did an experiment where we tried to evolve resistance on solid media and again we were unable to see colonies after 7 days on halicin supplemented media whereas we were able to get dozens or something on cipro. All right so we wouldn't be able to play the evolution trick to figure out how halicin was working. So what we did next was an RNA sequencing, right. W just asked what is the cellular response when you take E. coli and expose it to halicin? And what we observed, it was a very obvious transcriptional response. What was happening is cells were immediately downregulating genes involved in motility and flagellar biosynthesis and it turns out that that's a very common transcriptional response when you dissipate the proton gradient across the cytoplasmic membrane.

So it appeared based on a whole bunch of experiments that we did that halicin was dissipating that proton gradient that is essential for viability across the cytoplasmic membrane. So cells did not have that proton gradient that is essential to do things like turn flagellar motors, couldn't turn ATP synthase so they couldn't make ATP, and that proton gradient is important for moving solutes across the membrane. So it was all these different functions that are dependent on this proton gradient weren't working that resulted in a loss of viability.

Erin Allmann Updyke

Oh my god.

Erin Welsh

That's amazing. So in terms of having a very clear effect, like a clear bactericidal effect on those cells, would it have a similar effect or did you test for a similar effect on eukaryotic cells?

Jonathan Stokes

So we didn't test specifically but we did put it in a mouse. So we tested halicin in two mouse models, right. So we tested it in an Acinetobacter baumannii skin infection model and we also tested it in a C. difficile gut infection model. So the A. baumannii model we set up an abrasion on the dorsal surface of mice, so just on their back, we infected it with A. baumannii and then we treated topically with halicin. We had 6 mice in the control group and 6 mice in the treatment group and I think in 24 hours or 25 hours, 5 of the 6 mice that received halicin, the number of Acinetobacter baumannii cells was below the limit of detection. And the control group, they had like 10 to the 8 cells per gram of tissue or something like that, like it was a lot.

Erin Allmann Updyke

Wow.

Jonathan Stokes

And then in the C. diff model we tested it against both a vehicle control group and metronidazole and we observed that halicin eradicated the C. difficile infection in mice within like I think it was 4 days, something like that.

Erin Welsh

That's huge.

Jonathan Stokes

Yeah, yeah. So that was cool. But then after that right, we have this model that appeared to work fairly well, better than fairly well I should say. Quite well. So then we wanted to test it on a very large chemical library, right, so we tested it against the ZINC15 database. So the ZINC15 database is a virtual repository of 1.5 billion molecules.

Erin Allmann Updyke

Whoa.

Jonathan Stokes

Right? But instead of running predictions on the entire 1.5 billion, we curated 107 million we predicted that they had antibiotic-like physical chemical properties. So then we ran predictions on that 107 million. It took us 4 days to run predictions on that. And I actually did a calculation not too long ago. So the model jogged through those molecules in 4 days and I calculated that if I had access to 107 million molecules which nobody does and I screened all day, every day, it would take something like I think I calculated it was like 14.5 years to screen that many molecules empirically.

Erin Allmann Updyke	Oh my gracious.
Jonathan Stokes	Yeah, right?
Erin Allmann Updyke	Wow.
Jonathan Stokes	Yeah.
Erin Welsh	We are living in the future. This is incredible.
Erin Allmann Updyke	The year 2000. Wow.
Jonathan Stokes	Right? So when we ran predictions on the ZINC it was like a similar game plan as we did for the drug repurposing hub, it was run predictions, take the molecules that are the most likely to be antibacterial, and find molecules that are structurally dissimilar from conventional antibiotics. Based on those criteria we were able to curate 23 of such molecules for testing in the lab. 8 of those 23 ended up working against one panel of different species of bacteria. And 2 of them are actually really cool that we're continuing to study, they're both bactericidal and they're both broad spectrum. And right now those are at the stage of mechanism of action, elucidation. So that's what we're focused on right now with those two.
Erin Allmann Updyke	Wow. Oh my goodness. So then what are the next sort of steps? And does the fact, was that a choice that you used this repository where you're maybe further along in that drug development pathway, was that intentional? And so what are the kind of next steps for halicin?
Jonathan Stokes	Yeah so we're trying to design analogs of halicin that work better against TB because we saw this really rapidly bactericidal bioactivity against TB. We're seeing if we can both increase its potency, so decrease the concentration of drug required to do that as well as perhaps explore chemical spaces around the core structure of halicin such that it can eradicate those cells even faster. But also we have some late stage preclinical work that we're aiming to finish up with halicin, so these would be experiments that would allow for an IND filing prior to hopefully moving into phase 1 if everything looks good. And we've also recently started a large initiative leveraging this platform to find antibiotics against a wide phylogenetic spectrum of species. So basically we took the top 7 bacteria pretty much on the WHO hit list and we're trying to find new antibiotics against each of those using this machine learning approach and other similar ones as well.
Erin Welsh	That's awesome.
Erin Allmann Updyke	Oh man.
Erin Welsh	So machine learning, I think Erin and I are both completely won over.
Erin Allmann Updyke	Oh I'm sold.
Erin Welsh	We're all in for machine learning. And it seems like there are so many different amazing applications. Are there any drawbacks to machine learning that you could shed some light on?

Jonathan Stokes	Yeah. So pros: for drug discovery anyway it's cheaper, you don't have to run giant screens, it's faster, 4 days vs 14 years or whatever, and you can "assay" quote unquote, virtually assay orders of magnitude more molecules than you could feasibly do in the lab, right. Cons: at least for drug discovery it's way better to run predictions for molecules that have some biological activity in chemical spaces that the model recognizes, right. So imagine like a giant circle, right. So that giant circle is the chemical space of all the molecules that you wanna run predictions on, let's say. And let's say when you train your model it's like a tiny, tiny little circle within that larger circle at the bottom edge of it, right. If the model has only seen a very narrow substructure of the possible chemistry within the prediction space, it's not going to be able to do something called generalize very well, right. So another con and I wouldn't even say it's a con, it's more just something to be aware of is ML is like the epitome of garbage in, garbage out. Like if the data that you're using for training isn't pristine and you have complete control over it, you don't know what your model's gonna be predicting. And that's something that I developed a very strong appreciation for throughout the course of this project.
Erin Allmann Updyke	Wow. So I know your background is in kind of antibiotics more specifically but I wonder if you could just briefly talk about some of the other ways it seems like this type of machine learning could be used for obviously a lot more than just antibiotic discovery.
Jonathan Stokes	Oh yeah, for sure. So one thing that a team at MIT is working on now is using this approach to find molecules for COVID, right, so antivirals is another obvious one. And something else that we're also working on is building algorithms not only to predict whether something is antibacterial or not but being able to predict the mechanism of action of that molecule. But I mean ML in general, I mean it's permeated every aspect of our life, right. So like we have self-driving cars and what we wanna watch next on Netflix, that's all thanks to ML algorithms, right. So it's just natural that ML is gonna permeate obviously biomedical research and healthcare too. If you pick your favorite biological problem, I'm sure you can envision a way that ML might be able to assist if not now, then in some number of years when we get better at this.
TPWKY	(transition theme)
Erin Welsh	Thank you so much Dr. Stokes, that was great. I sit okay to say we were stoked to talk with you?
Erin Allmann Updyke	Oh! I hope it's okay to say that cause that's really good.
Erin Welsh	How many times have people made that joke? (laughs)
Erin Allmann Updyke	Oh really though, that was phenomenal. It's one of those ingenious things, you know, you're like how come people didn't think of it? Well maybe they did but they didn't have the technology for it.
Erin Welsh	Right, right.
Erin Allmann Updyke	It's amazing.
Erin Welsh	It's so cool, it's so cool. Awesome. Well, sources.
Erin Allmann Updyke	Sources.

Erin Welsh	So I mostly leaned on a couple of books that were great. One was called 'Miracle Cure' by William Rosen and this is such an excellent book that talks about the history of antibiotics, I loved it, it was really well written. And then another one was called 'Big Chicken' as I mentioned earlier by Maryn McKenna. And then I also read a book called 'Missing Microbes' by Martin Blaser which talks mostly about sort of the overuse of antibiotics and their microbiome disruption. And there are a few papers that I'll also post.
Erin Allmann Updyke	I also used books this time which is rare for me. But there are a couple of books I found actually very useful and they have a lot more detail if you're interested on the different mechanisms of antibiotics. One was by Rosaleen Anderson et al, that was the editors, it's called 'Antibacterial Agents: Chemistry, Mode of Action, Mechanisms of Resistance, and Clinical Applications'. And the other one, he actually has two books and I kind of used them both but one is more recent, it's by Christopher Walsh and Timothy Wencewicz called 'Antibiotics: Challenges, Mechanisms, Opportunities'. He also has one from 2003 that's 'Antibiotics: Actions, Origins, Resistance.' They're both great. And then a number of papers. If you'd like to read Jonathan Stokes' paper it's called 'A deep learning approach to antibiotics discovery' and we'll post that on our website as well.
Erin Welsh	Mm-hmm, we will. Awesome. Well thank you to Bloodmobile for providing the music for this episode and all of our episodes.
Erin Allmann Updyke	Thank you again to Dr. Stokes for coming on the podcast to tell us everything about their work.
Erin Welsh	And thank you listeners for listening to this very long episode about antibiotics.
Erin Allmann Updyke	If you made it this far, we love you.
Erin Welsh	We love you. Even if you didn't make it this far, we still love you.
Erin Allmann Updyke	But they won't ever hear that.
Erin Welsh	I know but it still exists, Erin, the feeling is still there.
Erin Allmann Updyke	True, it's true.
Erin Welsh	(laughs) Okay well until next time, wash your hands.
Erin Allmann Updyke	You filthy animals.