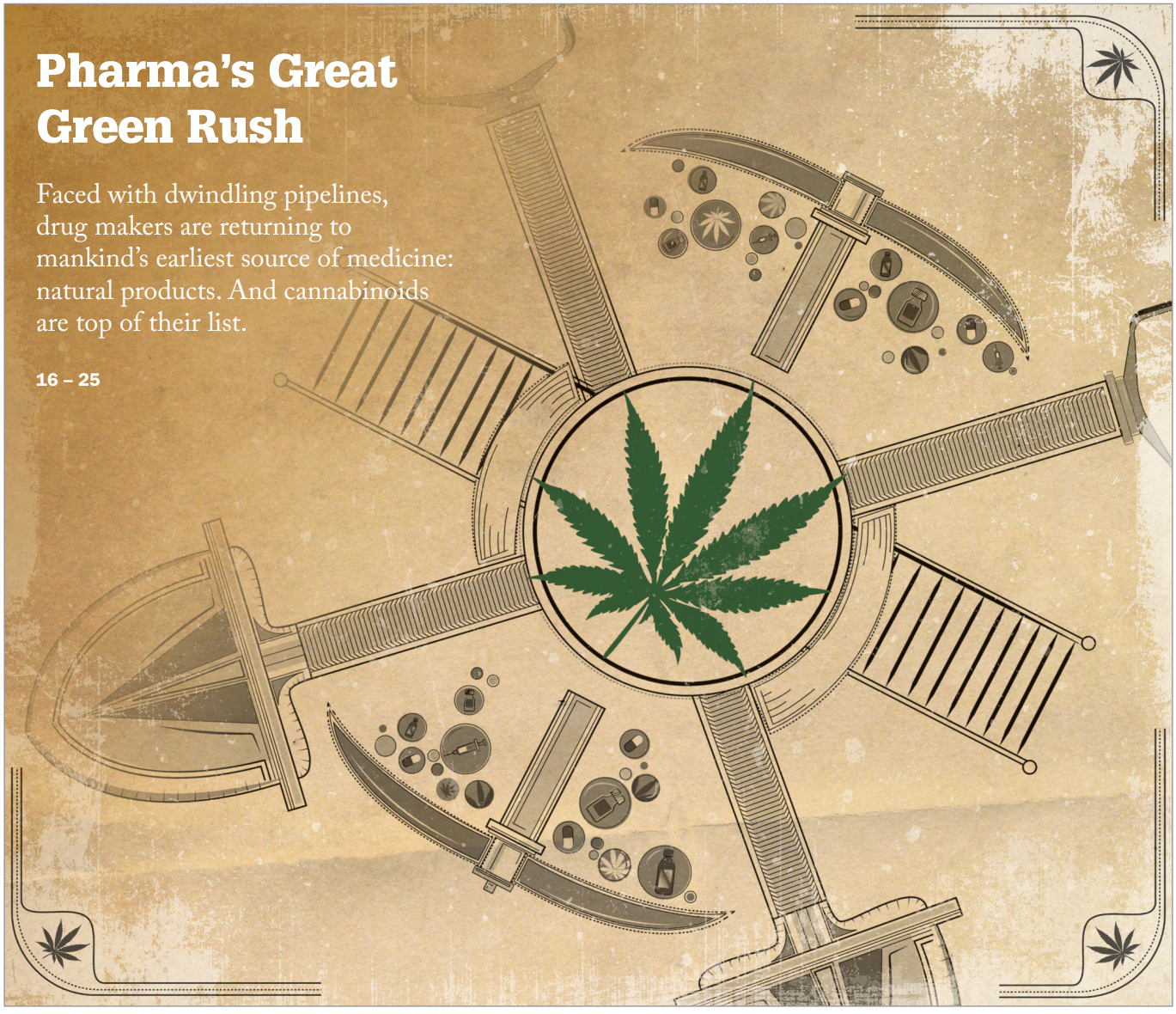
AUGUST 2017 # 03

the Cannabis Scientist



Pharma's Great Green Rush

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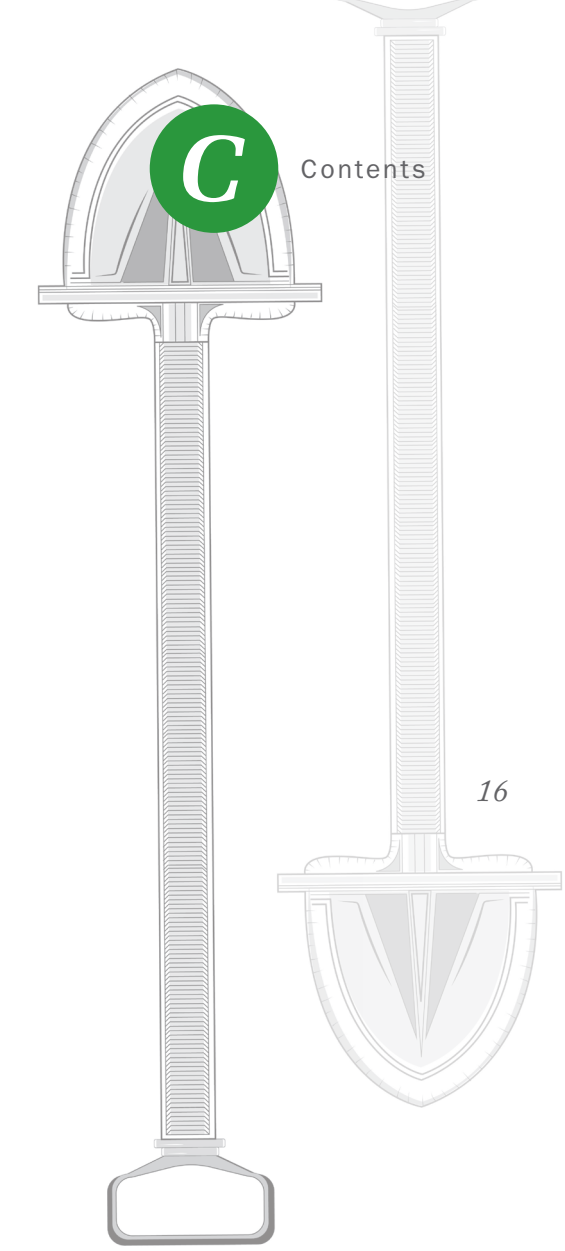

16

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26

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Heroes and Villains

What's the role of big pharma in medical cannabis research?

As the endocannabinoid system starts to give up its secrets, drug companies are increasingly focusing on cannabinoids, with the hope of patenting novel pain killers, anti-inflammatories and even cancer drugs. Many whole-leaf advocates believe it's impossible (and unnecessary) to recreate the beneficial effects of the hundreds of cannabinoids found in a cannabis plant, and so pharma is often painted as the big, bad, greedy gate-crasher.

Clearly, pharmaceutical companies are not charities (and nor, for that matter, are cannabis growers or dispensaries...); they won't invest in a drug unless there is a realistic chance of making a profit. However, the simple truth is that while cannabis may have been legalized for medical use in many regions, it is highly unlikely that it will ever be approved as a pharmaceutical. The multitude of cannabinoids, which make the plant so intriguing as a potential medicine, also make it a regulator's nightmare. The drug development scientists in our cover feature believe the only path to make true medicine from cannabis lies in isolating individual cannabinoids, just as we once isolated aspirin from the willow tree.

Those who favor a pharmacological approach point out that natural doesn't always mean better; what modern doctor would suggest boiling willow bark to treat pain? To make it onto the market, a drug must be not just safe, but a proven improvement on existing therapies.

If pharma companies are successful in developing more cannabinoid drugs, does that mean that there is no longer a place for medical cannabis? Certainly not. After all, there are plenty of widely used natural remedies that offer relief to patients, even if they don't come in pill form. And if some patients prefer medical cannabis over yet another pill, why shouldn't they have that choice? Plus, doesn't the very fact that the pharmaceutical industry is proactively pursuing cannabinoid-based drugs speak to the medical power of the cannabis plant?

Whether developing standards for safety testing, producing better strains, or working in the lab to produce cannabinoid-inspired drugs, cannabis scientists are united by a common goal: to help patients by unlocking the secrets of this extraordinary plant.

What does big pharma's move into in the cannabis space mean to you? Let me know: charlotte.barker@texerepublishing.com

Charlotte Barker
Editor

Upfront

Reporting on research, personalities, policies and partnerships that are shaping cannabis science.

We welcome information on interesting collaborations or research that has really caught your eye, in a good or bad way. Email: charlotte.barker @texerepublishing.com

Inflammatory News

New research sheds light on how cannabinoids fight inflammation.

Population studies suggest that diets rich in omega-3 fatty acids have a positive impact on the heart and brain, and damp down inflammation. The anti-inflammatory action is mediated by conversion of omega-3 fatty acids into endocannabinoids, but the precise mechanisms have been unclear.

The endocannabinoid system is still relatively unstudied. "The first endocannabinoid receptors were only isolated in 1991 and 1993 and the endogenous ligands were discovered in 1992 and 1994, so there is still a lot of work needed," says Aditi Das, Assistant Professor at the University of Illinois Urbana-Champaign, who led a new study into omega-3 fatty acid-derived endocannabinoids.

"We were investigating the metabolism of omega-3 fatty acids to form anti-inflammatory metabolites, and separately studying endocannabinoid metabolism," says Das. It was a natural progression for the group to begin studying omega-3 endocannabinoid metabolism.

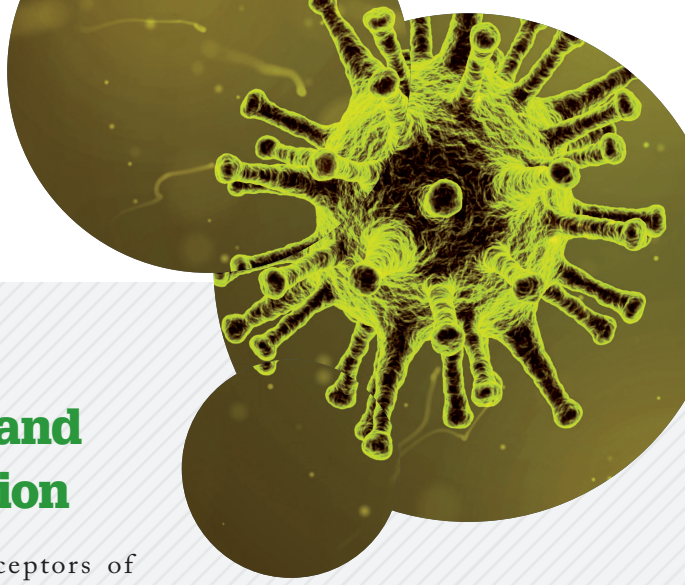
In their recently published study (1), the team identified potent anti-inflammatory lipids (fats) produced by a cascade of enzymes from omega-3 derived endocannabinoids. These omega-3 endocannabinoid epoxides were found to

interact with cannabinoid receptor type 2 (CB2; see sidebar), and have positive effects on inflammatory molecules in animal tissues. "The key finding was that omega-3 fatty acids, when converted to omega-3 endocannabinoids, can produce similar anti-inflammatory effects to cannabis," says Das.

The work suggests the possibility of developing drugs to target omega-3 pathways, which could gain targeted medicinal benefits of cannabis with no psychotropic effects. "Next, we need to make stable derivatives of these compounds and test their efficacy as anti-inflammatory and anti-pain drugs," says Das. *CB*

Reference

1. *DR McDougle et al., "Anti-inflammatory ω-3 endocannabinoid epoxides", PNAS, 114, E6034–E6043 (2017)*



CB2, Immunity and Inflammation

The two main receptors of cannabinoids – whether endogenous, plant-derived or synthetic – are (rather unimaginatively) known as cannabinoid receptor types 1 and 2. While CB1 is responsible for the "high" of THC and has a range of effects in the gastrointestinal, nervous and cardiovascular systems, CB2 is thought to be involved in cannabinoid action in the immune system, including potential functions in controlling inflammation and pain.

CB2 is a G protein coupled receptor, expressed predominantly in immune cells (for example, T cells, macrophages monocytes, and B cells). It's also expressed in keratinocytes in the skin, the peripheral nervous system, and microglia in the brain.

Chilled Out?

Regular cannabis users appear to take stress test in their stride

Cannabis users often report using the drug to combat stress. Now, new evidence suggests that regular cannabis users have a different response to stress compared with non-users – even when they aren't under the influence (1).

Carrie Cuttler, Clinical Assistant Professor at Washington State University Department of Psychology and co-author of the study, says, "One of the most common reasons cannabis users report using cannabis is to cope with stress. In support of this, previous research has shown that acute administration of THC or cannabis dampens affective responses and subjective stress ratings. We wanted to determine whether the stress relieving properties of cannabis would extend beyond the period of intoxication."

To find out, the group recruited approximately 40 self-identified regular cannabis users and 40 non-users (all of whom were asked to abstain on the day of the study) for a psychological study. Participants were randomly assigned to take part in a high-stress or no-stress version of the same task – the Maastricht Acute Stress Test. Those in the high-stress group had to plunge their hand into ice cold water for around a minute, before being asked to do some tricky mental math. To pile on the pressure, they were verbally corrected if they got an answer wrong, and were monitored by a camera which displayed their own image to them. Participants selected for the no-stress version had a much easier time, simply placing their hand in lukewarm water before counting from 1 to 25.

All trial subjects gave a saliva sample before and after the test, so that researchers could measure their levels of stress hormone cortisol. "The most important finding was the discovery of a blunted stress response in chronic cannabis users. More specifically, we found that levels of cortisol were much higher in non-users subjected to an acute stressor relative to non-users who were not subjected to a stressor. In contrast, the cortisol levels of cannabis users who were subjected to an acute stressor were comparable to the levels found in cannabis users who were not subjected to the stressor," says Cuttler.

The fact that cannabis users showed little change in cortisol after a stressful task suggests that the drug has an impact on physiological responses to stress beyond the immediate period of intoxication.

So what's next? "We plan to corroborate these findings in animals," says Cuttler. "One of the limitations of our research is that because we did not manipulate cannabis use we cannot conclude that cannabis caused the muted stress response. It is possible that there is something else that differs between cannabis users and non-users that is driving this effect (e.g., personality differences). However, we can manipulate cannabis use in animals and then examine their stress response."

Assuming the results are borne out in animal studies, it's an intriguing finding, but further work will be needed to determine whether this blunted response to stress has a positive or negative effect on cannabis users' health.

Reference

1. *C Cuttler et al., "Blunted stress reactivity in chronic cannabis users", Psychopharmacology, 234, 15 (2017).*

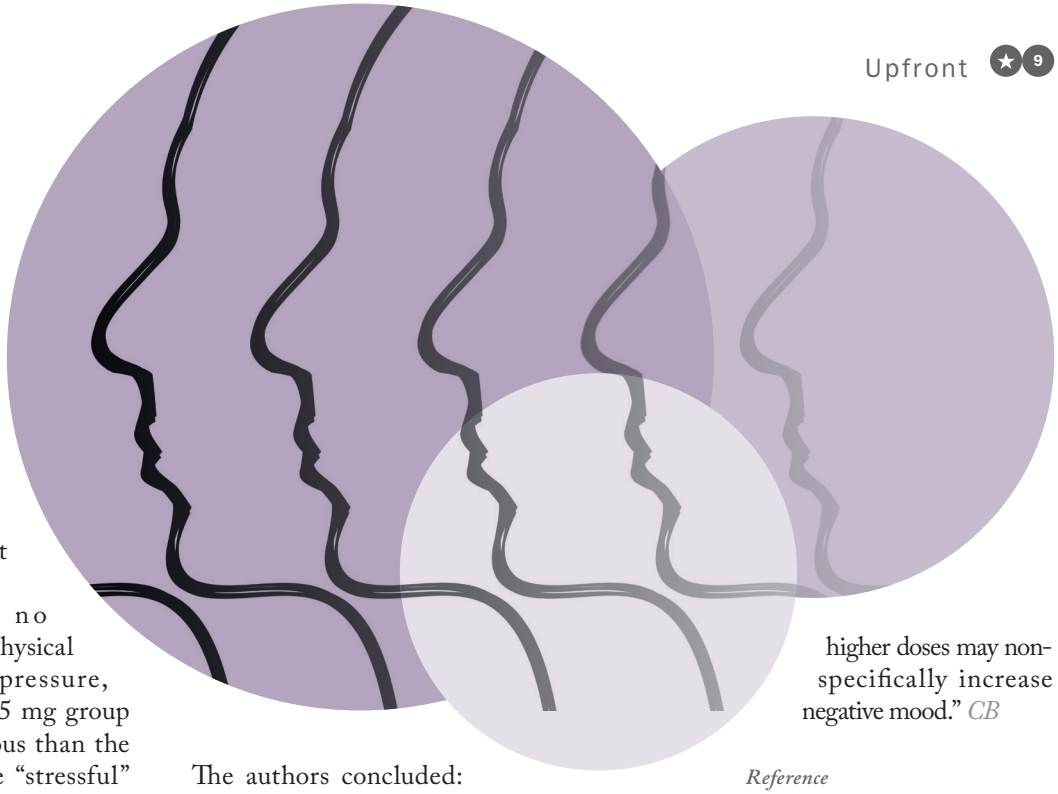
Too Much of a Good Thing?

A little THC reduces stress, but higher doses have the opposite effect.

Cannabis may live up to its reputation for reducing stress – but only at the very lowest doses, according to researchers in Chicago (1). Once levels of THC climbed enough to produce a mild "high", anxiety increased during a psychological stress test.

The scientists divided 42 healthy volunteers, all of whom had tried cannabis but were not regular users, into three groups. The low-dose group received 7.5 mg THC orally, the moderate-dose group received 12.5 mg, while the third group received a placebo pill.

In two separate sessions, the volunteers came to the lab, took their assigned pill and relaxed for two hours before completing various tasks. The first set of tasks, known as the Trier Social Stress Test, was designed to raise stress levels, with a mock interview and a stress test favorite – a math test (counting backwards from a five digit number by subtracting 13). Five days later, the volunteers returned for an



easier assignment – talking to lab assistants about a favorite book or movie, then playing solitaire.

Before, during and after each test, participants rated their stress levels and feelings about the tasks, and researchers recorded their blood pressure, heart rate and cortisol levels.

While there were no significant differences in physical signs of stress (blood pressure, etc), participants in the 7.5 mg group reported feeling less anxious than the placebo group during the "stressful" task. However, the 12.5 mg group reported more negative feelings about the test than the other two groups, and showed impaired performance.

The authors concluded: "Our findings suggest that a low dose of THC produces subjective stress-relieving effects in line with those commonly reported among cannabis users, but that higher doses may non-specifically increase negative mood." *CB*

Reference

1. E Childs, JA Lutz, H de Wit, "Dose-related effects of delta-9-THC on emotional responses to acute psychosocial stress", Drug Alcohol Depend, 177, 136–144 (2017).

The Importance of Being Earnest

Suspiciously low failure rates have earned a Washington-based cannabis testing lab a suspension

Following an audit, the Washington Liquor and Cannabis Board has suspended certification of Peak Analytics, a cannabis testing laboratory in Bellingham, Washington, USA.

The suspension came after a complaint about an unusually low rate of failures for microbial contamination in samples sent to the lab, according to the Seattle Times (1). An analysis of results over a three month period revealed that while the average microbial failure rate for certified labs in the state was 11 percent, Peak Analytics recorded a rate of 1.69 percent.

Data analyst Jim MacCrae has drawn attention to discrepancies in potency and failure rates of different labs in the state (2), and suggests that the labs with the highest potency and lowest failure rates get more business. Another Washington lab, Testing Technologies, was suspended last year for failure to follow good laboratory practices, but has since been recertified.

Peak Analytics released a statement explaining that it is addressing the issues and has submitted a microbial validation study for technical review, going on to say, "The current circumstance are being viewed as an opportunity to improve and build on our operating procedures".

Auditors RJ Lee Group recommend that the lab's certification be renewed once the concerns have been addressed.

Many states are tightening legislation for cannabis testing labs – read more in our interview with lab assessor Susan Audino on page 26.

References

1. *R Young, "Bellingham pot lab Peak Analytics suspended after auditors question testing practices". Available at: http://bit.ly/2fpeyL9. Accessed on 8 August 2017.*
2. *J MacCrae, "Labbiness in Washington Labs: Where is truthiness when you need it?". Available at: http://bit.ly/2ukvc0S. Accessed on 8 August 2017.*

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In My View

In this opinion section, experts from across the world share a single strongly-held view or key idea.

Submissions are welcome. Articles should be short, focused, personal and passionate, and may deal with any aspect of cannabis science. They can be up to 700 words in length and written in the first person.

Contact the editors at edit@texerepublishing.com

The Thin Green Line

To ensure the safety of cannabis consumers, regulators must walk the tightrope between rigor and pragmatism.

By Heather Krug, State Marijuana Laboratory Sciences Program Manager, Colorado Laboratory Services Division, Department of Public Health and Environment, Denver, CO, USA.

On January 1, 2014, Colorado became the first state to allow legal sales of retail (non-medical) cannabis for adults over age 21. Colorado regulators working across multiple state agencies are tasked with oversight of the emerging cannabis industry. The numerous challenges regulators face must be addressed in the absence of guidance from the federal government – and there is little precedent. Testing of cannabis presents a unique set of challenges, and execution of some key regulatory requirements for laboratories, such as proficiency testing, has been complicated by the infancy of regulated cannabis testing, the limitations imposed by marijuana's schedule I classification, and its tightly controlled legal structure.

How are Colorado regulators addressing some of these challenges? When it comes to cannabis testing, product safety and accurate labeling information are the foremost concerns. However, when trying to define testing requirements there are substantial gaps in knowledge, largely due to a lack of research data; historically, most of the limited research conducted was focused on adverse effects.

"The numerous challenges regulators face must be addressed in the absence of guidance from the federal government – and there is little precedent."

A prime example of a challenge arising from a lack of data is implementation of regulatory requirements for pesticide residue testing. No pesticides are registered for use on cannabis and, therefore, the potential health hazards posed by applying pesticides to marijuana have not been assessed, nor have tolerance limits been defined. Evidence from multiple states shows that some marijuana cultivators have resorted to illegal application of pesticides to save their valuable crops when threatened by insect infestation or fungal overgrowth. Because the potential health effects of these pesticides are unknown, Colorado has taken the position that until scientific studies are conducted to officially

establish that a particular pesticide may be applied to cannabis, and tolerance limits established as necessary, the presence of any banned pesticide will be considered a threat to public health and safety.

In an effort to address the fact that no pesticides are currently registered for use specifically on cannabis, the Colorado Department of Agriculture (CDA) has adopted rules establishing criteria to determine which pesticides may be allowed in cannabis cultivation. These criteria specify that the pesticide must have broad label language allowing it to be used on unspecified crops, must be suitable for use at the intended site of application (for example, a greenhouse), and must be approved for plants intended for human consumption. CDA maintains a list of approved pesticides based upon these criteria (1); any pesticide not on the list is banned for use on cannabis.

The Colorado Department of Public Health and Environment (CDPHE), in collaboration with the other state agencies and stakeholders, has been working to implement requirements for pesticide testing. Again, because health-based tolerance limits have not been established – and it is not analytically possible for a laboratory to test to a concentration of zero – it is necessary for states to define actionable or reporting limits for pesticides to ensure consistency of result reporting across laboratories, as each will have a unique limit of detection. Colorado, like some other states, has decided to base these limits upon laboratory detection capability, and we have conducted three multi-lab detection limit studies using cannabis matrices with several private marijuana laboratories and the CDA laboratory. The final study was conducted in June 2017 and those results will be used to inform Colorado's reporting limits for banned pesticides in cannabis.

"Standard analytical methodologies for testing marijuana and the vast array of marijuana products do not currently exist, so labs are developing their own methods."

Pesticide testing is only one example of the many challenges facing cannabis regulators. Gaps in knowledge and data also exist when it comes to defining testing requirements for other potential contaminants, such as microbials and residual solvents. Standard analytical methodologies for testing marijuana (a very complex matrix) and the vast array of marijuana products (edibles, tinctures, concentrates, and so on) do not currently exist, so labs are developing their own methods – each of which is considered intellectual property. Even the application of standard methods from other industries requires careful method optimization and validation due to the differences in the sample matrices and huge variety of matrix types – marijuana can be added to virtually anything! This is one reason that laboratory accreditation/certification is an essential requirement – accurate laboratory testing not only ensures accurate labeling, but protects consumers and patients, and also can protect a cultivator or manufacturer from possible liability.

"With time, I'm confident that standardization of cannabis related processes and regulations will be achieved."

Regulators must tread carefully when defining cannabis testing requirements, to balance protection of public health and safety with the risk of making testing too expensive, potentially driving black market activities. Regulatory testing requirements are best constructed upon evidence-based science, but that can be difficult given the lack of currently available data. This has resulted in many regulatory changes during these first few years of legalized marijuana, and the system will certainly continue to change as knowledge advances. Nonetheless, with time, I'm confident that standardization of cannabis related processes and regulations will be achieved. Colorado's state regulatory agencies continue to work diligently through these challenges, using the expertise of industry members, scientists and technical experts, and other interested parties, to ensure competent cannabis testing and protect public health and safety.

Reference

1. *Colorado Department of Agriculture, "Pesticide Use in Cannabis Production Information". Available at http://bit.ly/1W4LXVr. Accessed 25 July 2017.*

Opportunity Knocks…

But will science answer? There is growing evidence that cannabis has medically beneficial properties; however, to understand its true potential we need rigorous, unbiased research.

By Neil Mahapatra, Managing Partner, Kingsley Capital Partners, London, UK.

One of Richard Nixon's lesser-known misdemeanors came in 1970, when he made cannabis a Schedule 1 substance – a decision that has frustrated research into the plant's medical applications. It wasn't until November 2016 that the US lifted a federal cultivation restriction that hindered cannabis research by US academic institutions.

The easing of cultivation restrictions for US academic institutions is indicative of the larger shift by the pharmaceutical industry back towards re-examining natural products (produced by animals, plants, bacteria and fungi) as a source of medicine. The 1990s saw a "green rush" of bioprospecting by the pharmaceutical industry as it sought to mine Earth's biodiversity. Despite significant success (the vast majority of today's antibiotics are derived from natural products), by the 2000s most pharmaceutical companies had terminated their natural product discovery programs, citing difficulties in obtaining samples at sufficient yields, ecological and legal restrictions, and insufficient capital investment.

However, recent years have seen the industry move back towards natural products as a source of new drugs. Because they occur naturally, candidate drugs derived from natural products have quicker development times, and are more likely to pass toxicity studies. Natural product pathways are highly complex, which makes them challenging to synthesize in the lab, and has led to several drugs consisting of standardized plant-based extracts (containing a number of quantified compounds) gaining regulatory approval.

In this context, the three primary species of cannabis (Cannabis sativa, Cannabis indica, and Cannabis ruderalis) are of great interest as a potential source of medicines. Firstly, historic restrictions stymied much of the research that might otherwise have occurred in the 1990s, when many other natural products were investigated for medical use – there is significant catching up to do. Secondly, most research on cannabis has focused on the known major cannabinoids (in particular THC and CBD) and on two key receptors (CB1 and CB2 receptors – found in every mammal). Pharmaceutical companies have tended to focus on screening their current libraries of molecules against these two receptors, but have not studied the effects of other molecules (e.g., naturally occurring minor cannabinoids). In my view, it is here where the most exciting medical potential lies.

Beyond some excellent studies in Israel, little work has been conducted into the effects of minor cannabinoids and their combinations on CB1 and CB2 receptors. Huge improvements in cannabinoid extraction, purification and isolation techniques mean that additional minor cannabinoids are still being identified. At a time where natural product research is again in vogue and where whole plant extracts form the basis of regulator-approved medication, the effects of minor cannabinoids deserve to be explored in detail.

Over the last ten years, evidence has been presented that cannabinoids could have therapeutic effects in a number of major diseases, such as multiple sclerosis, epilepsy and cancer. However, as both last year's independent UK review and this year's US National Academy of Sciences study point out, the restrictions imposed on cannabis research mean that many of these studies were carried out without adequate control trials, or with inadequate sample sizes, rendering them largely anecdotal.

"The 1990s saw a 'green rush' of bioprospecting by the pharmaceutical industry."

Whatever the medical applications of cannabinoids turn out to be, it would be a missed opportunity not to explore them. It is for this reason that Oxford University and Kingsley Capital Partners have launched a pioneering research program to investigate the role of cannabinoids in biology and medicine (read more on page 23).

We hope this research program will prove a step in the right direction, building on initiatives led by companies and academic institutions across the world. Cannabis has already been used as a therapeutic aid for centuries – now is the time to harness 21st century science to understand its mode of action and explore its potential in the most rigorous way possible.

Optimization of cannabis extraction yield by controlled milling

The Fritsch PULVERISETTE 19 is utilized to finely mill cannabis plant material in preparation for SFE processes. The efficient and precise reduction in particle size optimizes oil output and formulation.

Dr. Markus Roggen, OutCo, El Cajon, California, USA.
Blake Grauerholz, OutCo, El Cajon, California, USA.

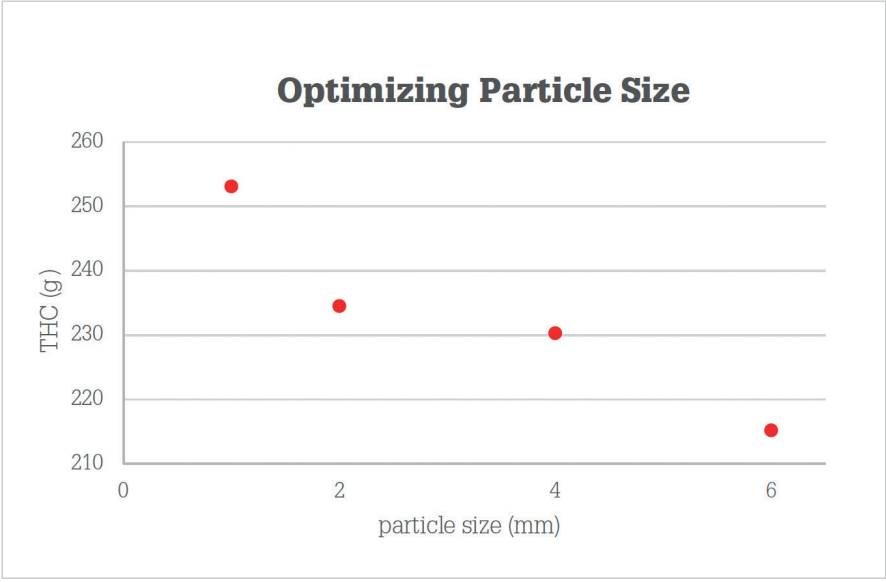


Figure 1: Extraction efficiency for different particle sizes. All other inputs are identical, e.g. type of material, weight of material (2.0 kg), and extraction parameters, like temperature (34°C), pressure (124 bar), and run time (6 h).

The fast-growing field of cannabis extraction still holds many process inefficiencies, that can be easily overcome. A bottleneck often encountered is the packing density, or lack thereof, of cannabis plant material in the extraction vessel. Low packing density leads to a decrease in extraction efficiency and increase in output variability. Non-milled cannabis plant material generally experiences packing densities of 100-125 g/L, whereas milled material packs at 225-250 g/L.

The Fritsch PULVERISETTE 19 is an efficient tool to quickly comminute large volumes of cannabis plant material to a precise particle size. Plant material is fed into the PULVERISETTE 19 through large funnel for fast throughput. The negative pressure in the milling system ensures a continuous flow through the cutting rotor and the selected sieve cassette for precise particle sizing, and prevents any system clogging. The high throughput of up to 60 L/h is supported by large collection vessels of up to 10 liters. Fast processing is further supported by unrestricted accessibility of the cutting chamber, quickly removable cutting rotor and sieve cassette, and generally easy-to-clean grinding chamber.

In this application note we describe the general process employed at OutCo for sample preparation in their SFE production operation. This will include particle size distribution data and experimental data on extraction yield increases due to particle size reduction.

After testing (Figure 1), we chose the 2mm screen size, as it allows for a high packing density, increased extraction speed, optimized oil constitution, while allowing the operator to constantly feed material into the mill itself, thus increasing work efficiency. The blade speed of 300 rpm was found to be optimal for narrow particle size distribution. Furthermore, this low blade speed avoids thermal damage and loss of volatiles for the sample. It is important that the moisture content of the material being milled is dry, below 15%, as the milling sieve will clog in the presence of moisture. One full extraction load of 4.5 kg can be milled before stopping the machine to clean the sieve and behind the milling wheel to prevent buildup of chlorophyll and cannabis residue. If there is not enough of a single cultivar to facilitate a full extraction run, a blend of strains can be homogenized using the Fritsch mill. Strains selected for a blend should have complimentary flavor profiles and can also be chosen to enhance therapeutic effects.

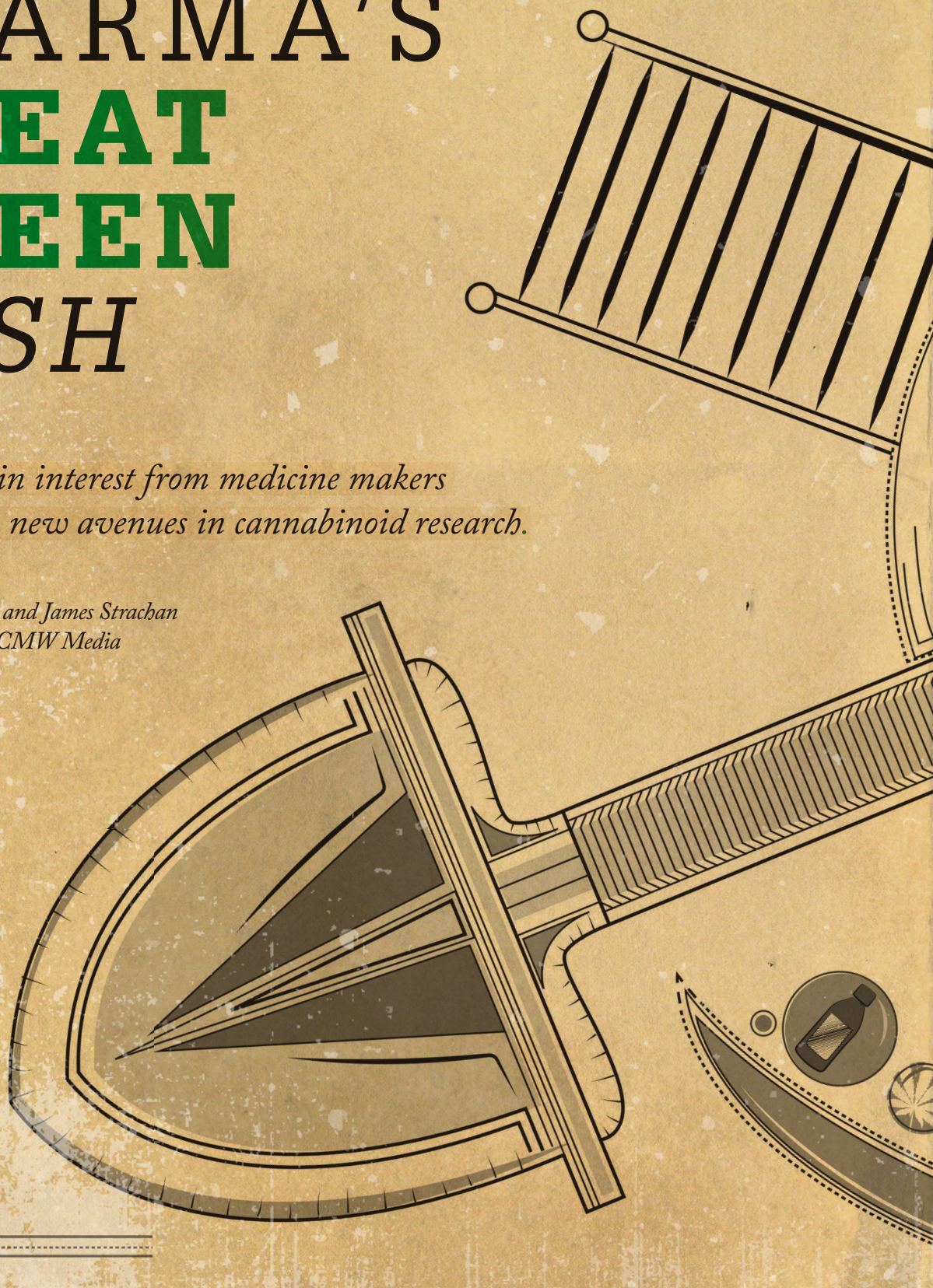
Other applications OutCo uses the mill for is sample preparation for rosin pressing and milling of flower for pre-roll production. It was found that different particle sizes optimize draw behavior or item stability.

The Fritsch PULVERISETTE 19 critically supports OutCo's extraction operations by providing fast milling of powders with precise particle size distribution and minimal degradation of raw material.

PHARMA'S **GREAT GREEN** *RUSH*

A resurgence in interest from medicine makers is opening up new avenues in cannabinoid research.

By Stephanie Sutton and James Strachan
Images Courtesy Of CMW Media



Cannabis has been used for centuries to treat a variety of ailments, but there are currently only a handful of approved cannabis-derived pharmaceuticals on the market. However, faced with dwindling pipelines, drug makers are returning to mankind's earliest source of medicine: natural products. And as scientists learn more about the role of the human endocannabinoid system in regulating sleep, appetite and pain, cannabis is an increasingly attractive target.

We find out more about pharma's "green rush", and discover how scientists are seeking to translate knowledge about the body's own cannabinoids into new treatments.

UNCOVERING CANNABINOIDS

Roger Pertwee reflects on 50 years studying the pharmacology of cannabinoids.

Roger Pertwee, Emeritus Professor at the University of Aberdeen, UK, and Director of Pharmacology at GW Pharmaceuticals, has made major contributions to the field of cannabinoid research, including the co-discovery of the first endocannabinoid – anandamide – and thus the endocannabinoid system. He is a co-founder of the International Cannabinoid Research Society and has received numerous awards for his work, including the 2011 Wellcome Gold Medal by the British Pharmacological Society. Here, Pertwee tells us about his career in cannabinoids and considers what's next for the field.

How did you first become interested in pharmacology?

I was reading for a degree in biochemistry at the University of Oxford where the Head of Department was the famous scientist, Hans Krebs. During that time, I joined the OU Officer's Training Corps (Royal Engineers), which gave me the opportunity to spend a couple of weeks at Marchwood, near Southampton, to undergo training as an army shallow water diver. As a result, I became aware of the phenomenon of inert gas narcosis ("raptures of the deep") – early signs of general anesthesia that can be induced by compressed air when

inhaled by a diver at certain depths. I was intrigued by this then little-investigated phenomenon to the extent that once I had obtained my degree (in the summer of 1965), I approached the Royal Naval Physiological Laboratory at Portsmouth for advice on how I might begin research into inert gas narcosis. I was directed back to Oxford – to Professor Bill Paton, Head of the Department of Pharmacology and a world-renowned expert on the pharmacology of anesthetics. He took me on as a student in October 1965.

And how did you come to focus on cannabinoids?

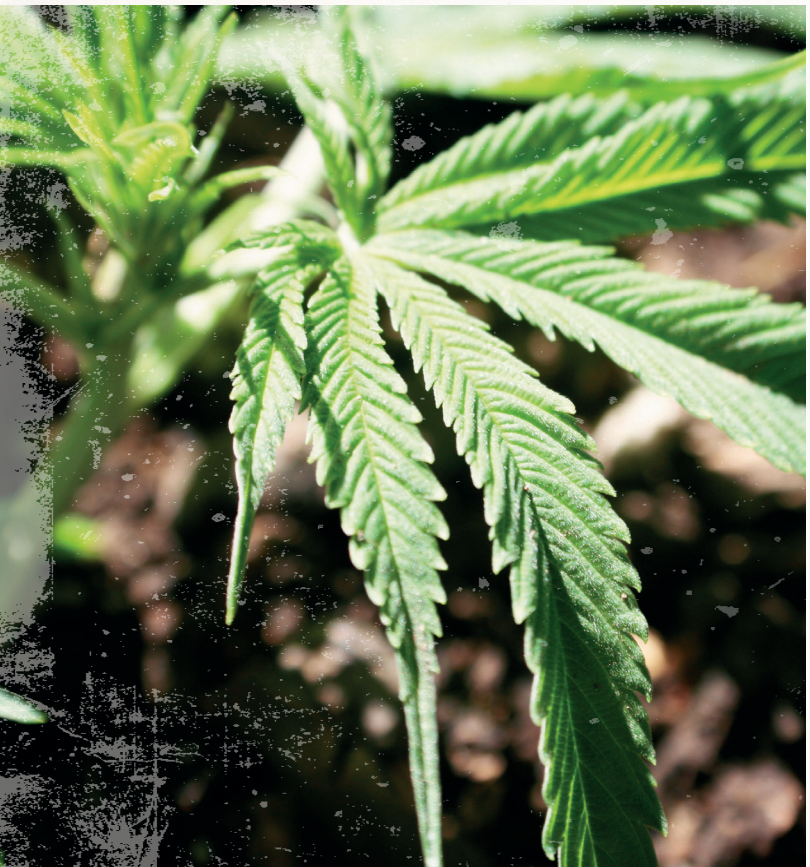
Around the time I was carrying out my DPhil research, cannabis had just emerged in the UK as a significant recreational drug, prompting the need for research to investigate its then largely unexplored pharmacology. Since the constituents of cannabis were known to be very lipid-soluble, Paton was interested in investigating the possibility that one or more of these constituents might affect brain function (for example, to produce a "high") by acting like a general anesthetic (at a sub-anesthetic dose) potentially by affecting the "fluidity" of cell membranes. Because I had been working on the pharmacology of general anesthesia for my DPhil, Paton took me on as a post-doctoral research assistant to work on the pharmacology of cannabis and some of its constituents. I was very lucky to enter what turned out to be a fascinating field of research, at a time when so little was known about cannabinoid pharmacology.

What challenges have you faced in the field?

There is a good system in place in the UK for obtaining a license that allows a scientist to perform valid research with cannabinoids, so there haven't been any regulatory hurdles. One challenge I have faced, however, was the fading interest in cannabinoid pharmacological research in the mid/late 1980s – many felt, at the time, that there was nothing new that needed to be, or could be, learned about cannabis. However, all that changed with the discovery of cannabinoid receptors – and the cloning of the CB1 receptor in 1990 – along with the subsequent discovery, in 1992, that we humans produce cannabinoids (endocannabinoids) in our own bodies that can activate cannabinoid receptors. The first of these endocannabinoids, anandamide, was discovered partly in my lab, in a project led by Raphael Mechoulam, and generated important new reasons for carrying out cannabinoid research.

A key question is whether whole-plant extracts or individual cannabinoids make better therapeutics. What are your thoughts?

The goal – and challenge – is to develop new cannabinoid medicines with optimal benefit-to-risk ratios. In my view,



A Career in Cannabinoids

A sample of the many cannabinoid-related discoveries made by Roger Pertwee and colleagues.

- The development of new bioassays for exploring the pharmacology of cannabinoids.
- The co-discovery of anandamide and the endocannabinoid system – an important potential therapeutic target.
- The discovery of an allosteric site on the cannabinoid CB1 receptor.
- The discovery of some of the pharmacological actions of certain chemicals (phytocannabinoids) present in cannabis, and hence of new potential therapeutic uses for some of these compounds – for example, the elucidation of the mechanisms of action and unique therapeutic potential of delta-9-tetrahydrocannabivarin (THCV) and of cannabidiolic acid (CBDA).
- Contributing to the eventual development of a cannabis-based medicine (Sativex) for multiple sclerosis (MS) – first, by interacting in the 1990s with MS patients who were self-medicating with cannabis, and with MS scientists and clinicians, and publishing findings generated by these interactions; and, second, by presenting information about cannabinoids by invitation, for example, to the British Medical Association, and to the Science and Technology Committee of the House of Lords, again in the 1990s.
- In collaboration with others, the pharmacological characterization of synthetic cannabinoids now widely used as experimental tools – e.g. AM281, AM630, methanandamide, ACEA and ACPA
- The co-development of a water-soluble synthetic cannabinoid that can activate cannabinoid receptors.

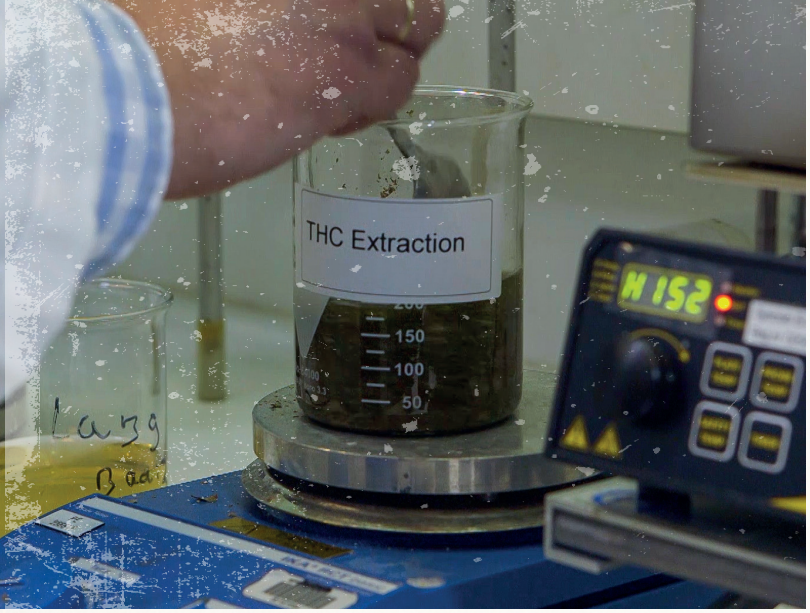
this will most likely be achieved using individual synthetic or plant-derived cannabinoids, either by themselves or in combinations of two or more cannabinoids, in optimized ratios. The development of such medicines will take time.

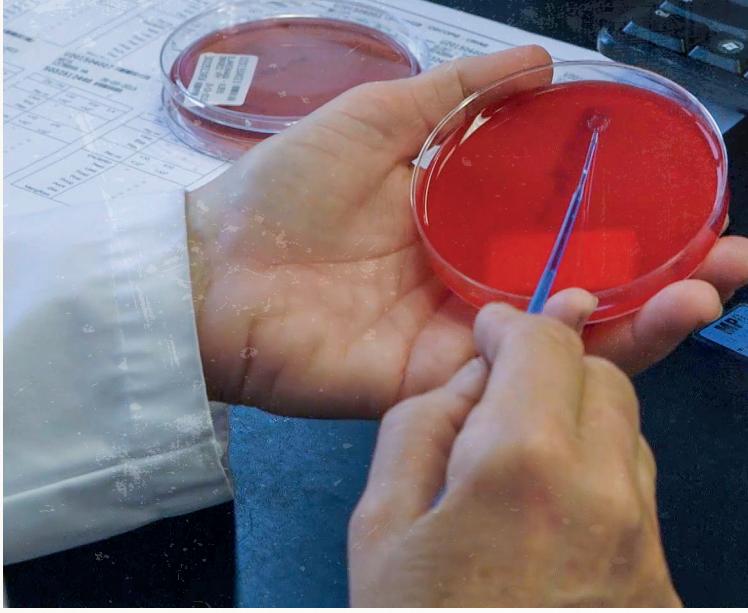
There is also the question of whether to develop synthetic cannabinoids or botanical cannabinoids as medicines. I believe there is a place for both. Examples of a synthetic cannabinoid medicine could include an inhibitor of endocannabinoid metabolism or a positive allosteric modulator of cannabinoid receptors that augments "autoprotection" resulting, for example, from the activation of cannabinoid receptors by endogenously released endocannabinoids; a structural analogue of a plant cannabinoid that displays greater stability; a peripherally-restricted synthetic cannabinoid that cannot enter the brain to target central cannabinoid receptors, but can still activate or block cannabinoid receptors located outside the brain to produce various effects, including therapeutically beneficial ones; and/or a medicine with a set of pharmacological properties that give it a particularly high benefit-to-risk ratio.

What is needed to help boost cannabinoid research?

More clinical research is needed to establish the accuracy of the vast amount of preclinical evidence predicting new therapeutic areas for cannabinoids. That said, there remains a need for yet more preclinical pharmacological research directed at exploring the pharmacological actions of known cannabinoids more completely, as well as developing new cannabinoids, and exploring their pharmacology and therapeutic potential. There is also a need for more extensive research into the central and peripheral roles of the endocannabinoid system.

Roger Pertwee is Emeritus Professor at the University of Aberdeen, UK, Director of Pharmacology at GW Pharmaceuticals and one of the founders of the International Cannabinoid Research Society.





CANNABIS COMPLEX

Discovering a cannabinoid with therapeutic effects is just the start of the journey for drug companies – we also need to find ways to deliver the drug into the body safely and effectively. The cannabis plant contains hundreds of different compounds – and most are difficult to formulate – but if overcoming the complexities means new medicines for unmet needs, it's worth it.

By George E. Anastassov

Some researchers take the viewpoint that cannabis should be used in its whole form because the mixture of different compounds are what give the plant its intriguing medical properties. However, the scientific community still does not understand what every substance in the plant does, which will make it very difficult to turn the plant into a regulated medical product. The active pharmaceutical ingredient in most medicines is a single molecule that can easily be characterized. If a medicine contains two or more active molecules then development is more difficult because you must investigate the interactions between the molecules – and if you have 700 different compounds then thorough investigation becomes virtually impossible. The consequences of getting it wrong are severe. In 2016, there was a disastrous clinical trial in France involving the testing of a fatty acid amide hydrolase (FAAH) inhibitor, which resulted in a patient death. FAAH inhibitors aren't based on cannabinoids, but play a role in the breakdown of endo- and exocannabinoids. Such disasters highlight the challenge of synthesizing safe compounds when you do not fully understand the biochemistry and pharmacology involved.

Chewing over challenges

I became interested in cannabis around 15 years ago, when a colleague and I were looking for novel classes of painkillers. We were very dissatisfied with what was on the market at the time (and little has changed since then) – yes, we have opioids and non-steroidal anti-inflammatories, and combinations of the two, but these drugs can have severe side effects. Eventually, we became interested in cannabinoids, partly because cannabis has been used for pain relief for thousands of years in many different cultures.

Today, I am the Chairman, Chief Executive Officer and President of AXIM Biotechnologies, which is developing a variety of pharmaceuticals, nutraceuticals and cosmetic products. One of our main focuses is on cannabinoids, and we are working on nine different formulations for fourteen different indications, including pain, eczema, psoriasis, vitiligo, dry eye, and irritable bowel syndrome – results from our Phase II clinical trial for irritable bowel syndrome (being conducted in the Netherlands) are expected very soon.

When it comes to formulation and drug delivery, cannabinoids tend to be very hydrophobic and challenging to work with, but some can be more difficult than others; THC, for example, is extremely volatile and oxidizes at room temperature. Much industry attention has focused on inhalation as a delivery method, but we wanted to investigate alternative approaches and have seen success with a more unconventional drug delivery format: chewing gum. Chewing gum presents challenges in terms of formulation and manufacture, but it also has a number of inherent advantages. For instance, the act of chewing itself is thought to offer neuroprotective properties. The scientific literature suggests that chewing can improve cerebral circulation, boost memory, and reduce stress. Importantly, the use of chewing gum as a drug delivery vehicle bypasses the gastrointestinal system. Some cannabinoids can be transformed into toxic metabolites when they reach the gut or liver. Inhalation, of course, can bypass this issue, but so too can chewing gum, where the active chemical enters circulation via the trans-oromucosal system.

As well as developing our own innovative medicines, we are also investigating how we can enhance older medicines. The first cannabinoid-based medicine approved by the FDA was Marinol (manufactured by AbbVie) in 1985. Marinol contains a synthetic form of THC (dronabinol) and is approved for loss of appetite and

An Alternative Approach

Could amplifying the body's own endocannabinoid system give us the medicinal benefits of cannabis, without the pharmacological and regulatory headaches?

By Alan Ezekowitz

The endocannabinoid system is a signaling network in the brain and periphery that plays a role in maintaining the balance of many of our physiological functions, including sleep, appetite, pain, memory, and mood. The system is also the target of the psychoactive substance in the cannabis plant, tetrahydrocannabinol (THC), which produces its effects through activation of two cannabinoid receptors, CB1 and CB2. In the brain, CB1 activation reduces excessive neurotransmission, while CB2 activation reduces inflammatory signaling. The naturally occurring activators of the cannabinoid receptors are the endogenous cannabinoids, or endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG).

Since the discovery of the endocannabinoid system, researchers have made significant advances in understanding the role it plays in health and disease. Hard-to-treat neurological disorders, such as multiple sclerosis, Tourette Syndrome, Alzheimer's disease, epilepsy, ADHD and pain, are natural areas of exploration for molecules that target the endocannabinoid system. However, the scientific community is only just beginning to identify how to safely manipulate the endocannabinoid system to modify disease and provide therapeutic benefit.

Some researchers are seeking to achieve this with cannabis-based products – after all, the therapeutic benefit of cannabis has been well-recognized for centuries in patients suffering from a wide variety of ailments. Appropriate and precise delivery of therapeutically active doses of cannabis, however, is a challenge. In addition, cannabinoids act indiscriminately, activating every accessible cannabinoid receptor pathway in the body, which can cause severe side effects. As a result, it would be wise for the industry to consider alternative molecules, such as those that amplify the action of the body's own endocannabinoids by preventing their enzymatic degradation.

This is something Abide Therapeutics has been focusing on. We have combined our activity-based protein profiling platform with a library of structurally unique small molecules to help identify previously inaccessible drug targets within the under-explored serine hydrolase superfamily, which act through the endocannabinoid pathway. Using this approach, we have developed ABX-1431 (in collaboration with Celgene, which holds ex-US rights to ABX-1431). ABX-1431 is an oral, first-in-class inhibitor of monoacylglycerol lipase (MGLL) with the potential to treat neurological disorders, pain and neuroinflammation.

MGLL is responsible for terminating endocannabinoid signaling by breaking down the primary endocannabinoid, 2-AG. The inhibition of MGLL results in increased 2-AG levels, enhanced signaling through the cannabinoid receptors, and regulation of neurotransmission and inflammation. Preclinical studies conducted so far demonstrate that ABX-1431 mimics the beneficial therapeutic effects of cannabis without negatively affecting cognition or behavior, due to its specificity towards MGLL and effects on CB1 receptors in active signaling pathways. The molecule is now being studied in a variety of patient populations.

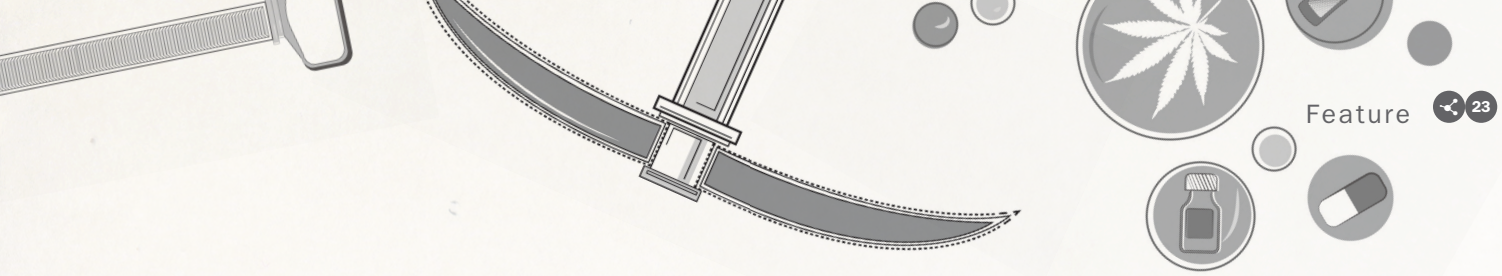
Alan Ezekowitz is President, Co-Founder and CEO of Abide Therapeutics, San Diego, CA, USA.

nausea. The drug is administered via a gel capsule, but can cause a number of side effects due to first-pass metabolism in the liver, where 90 percent of the active is metabolized. We are currently developing a bioequivalent of Manitol in chewing gum form – and so far we've shown a significant increase in bioavailability (over 70 percent).

A new leaf

It's clear that cannabinoids have captured the interest of the industry and although there are still significant challenges hindering research, attitudes are slowly changing. In January 2017, the United States National Academy of Sciences released a substantial report – over 400 pages long – that reviews the scientific research conducted around cannabis and cannabis-derived products since 1999. The report includes information on indications where there is clinical evidence of a therapeutic effect with cannabis. And there are certainly many – perhaps the most exciting prospect is that cannabinoid research may lead to new medicines for diseases that currently have no effective treatment – brain cancer, stroke, myocardial infarction, and epilepsy to name just a few.

George E. Anastassov is Chairman, Chief Executive Officer and President of AXIM Biotechnologies, New York, USA.



SMASHING THE STIGMA WITH SCIENCE

Cannabis can be a turn off for investors but, via a collaboration with Oxford University, Neil Mahapatra and his investment firm are showing the world that there's nothing to fear – and much to gain.

Neil Mahapatra was interviewed by Stephanie Sutton

Why cannabis?

It started with biology and business. Both of my parents were doctors and I read biology at the University of Oxford. After graduation, I started work at Morgan Stanley, then went to the US to study for an MBA at Harvard Business School. After that, I worked directly for Lord Rothschild – managing the Rothschild family's and RIT Capital Partners' investments. Most recently, I set up Kingsley Capital Partners with some friends, which is a private equity and venture capital firm headquartered in London.

Shortly after we set up Kingsley, my mother – who had never touched a cigarette in her life – was diagnosed with stage-four lung cancer, and 18 months later she sadly passed away. At the time, I was seeking anything – a novel piece of research or any left-of-field treatment – that could potentially help. It was in this context that I came across cannabinoid medicines. I read a number of personal stories of people who had seen their cancer growth slow or disappear after taking cannabis, but they were just anecdotes – right now, nobody truly understands the mechanism by which cannabis may act – in any indication. In the US, President Nixon placed cannabis in the "Schedule I" category in 1972 – limiting the amount of research that could be done. At the time, scientists were mining many natural products for potential pharmaceuticals, but cannabis was left out.

I wanted to make a difference. I went back to my own plant biology professor at Oxford, and told him my investment firm were considering entering the legal cannabis space in the US, and conducting research in the UK. He was interested and introduced me to the wider business development team within Oxford's medical sciences division. This kicked off one and a half years of discussions with the university, culminating in the announcement of a research program in March 2017.

What details can you share about the program?

We have established a portfolio company called Oxford Cannabinoid Technologies (OCT). We will be initially investing £10 million and the ultimate goal is to identify and deliver new therapies for sufferers of acute and chronic conditions around the world by finding out how cannabinoids work. OCT will be working in close collaboration with Oxford University, and will be involved in the implementation and monitoring of the research projects.

The real benefit will only emerge in three to four years. In the meantime, we'll be screening a variety of cannabinoids, in different combinations, against a variety of indications.

We will be looking at both synthetic and natural cannabinoids. Personally, I am very interested in naturally occurring cannabinoids. Extraction is challenging, but mainly because resources have not yet been placed into optimizing isolation and extraction technologies.

What were the challenges in getting the project up and running?

Regulation has been something of a challenge. In the UK you have to get a series of licenses to do research with cannabis or cannabinoids – which takes time. We also had to get a license to export cannabis to our extraction partner on the continent. That aside, it hasn't been too difficult – especially when you compare the situation with what US-based firms have to deal with. You can't even transport cannabis or cannabinoids across state lines in the US.

I expect to face challenges relating to the stigma of cannabis; however, the vast majority of people we have spoken with up until now have been very supportive of the medical potential. You might think that an esteemed university like Oxford, with their centuries of history, would be concerned about damaging their reputation by working with cannabis: not so! Oxford are brilliant, supportive partners and there are some deeply clever people working on this program. What excites me is that we have all these experts in different fields now turning their attention to cannabinoid medicines.

What is needed to advance the cannabinoid space?

Changes in the scheduling of cannabis at a federal level would make the US a far more attractive destination for international research; there are many companies I would love to work with in the US, but can't because of the regulatory barriers. More generally, I think we need to see even more experienced and professional people moving into the space: the clinical potential is clearly there, but the stigma surrounding cannabis still puts people off. OCT is not in the business of "peddling weed" – we are trying to create drugs that will help millions of people worldwide.

A VIEW FROM THE BIOSYNTHETIC BRIDGE

Could biosynthetic approaches to cannabinoid production prompt regulators to rethink restrictions on medical research

By Jeff Korentur

Teewinot Life Sciences focuses on the biosynthetic production of pharma-grade cannabinoids.

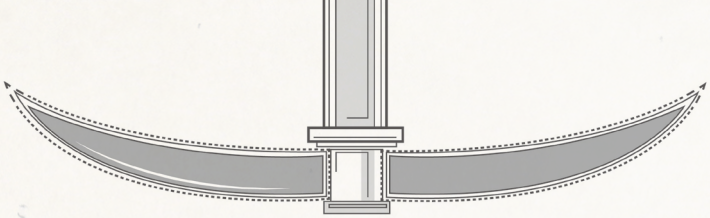
Fantastic research around cannabinoids is being conducted worldwide on a variety of indications, including pain, cancer, metabolic disease, psychiatric disease and much more. It seems clear that there is huge potential for cannabinoids for many unmet medical needs. We still need more research and more methods to investigate the remaining cannabinoids – at the moment, THC and CBD are receiving the most attention, but given that that are over a hundred other cannabinoids, we are only scratching the surface of what could potentially be achieved. Many cannabinoids have no known chemical synthesis route and occur only fractionally in plants, making them commercially unviable to extract. A researcher may hypothesize that a certain cannabinoid will benefit a certain patient population, but unless they can get their hands on the cannabinoid, they will never find out.

We are headquartered in Tampa, Florida (which implemented the Florida Medical Marijuana Legalization Initiative in 2016); we conduct our internal research and development in Canada under license from Health Canada, and all our existing and planned intellectual property are housed by our subsidiary in Ireland.

Genesis through biosynthesis

To create biosynthetic cannabinoids, we use a production environment that replicates the internal cellular function of the cannabis plant. We can currently produce 18 different cannabinoids, which are identical to those produced by the plant. This is really important because many cannabinoids have multiple chiral centers and when chemically synthesizing cannabinoids it is difficult to avoid stereoisomers, which can have very different effects to their chemical cousins. Our processes use the same mechanism as the plant, so they end up producing the same compounds as the plant. Our approach can include a mixture of organic chemistry, synthetic biology and biocatalysis. The synthetic part refers to changing the structure of an organism, such as modifying a single-celled microbe, to encode performance of a new function required for the overall process. Biocatalysis involves using enzymes to react with a starting material to produce the desired end product. Essentially, we have engineered microorganisms to produce specific synthase enzymes found in the cannabis plant. We react these synthases with the same starting material the plant uses, which – depending on the conditions set up within the bioreactor – yields different cannabinoids.

Synthetic biology and biocatalysis are not new to the pharma



industry – and are already used extensively for producing medicines. One of the benefits of our process is that it can be used to enhance a naturally occurring molecule. For example, we have developed a pro-drug of CBD that has a half-life of more than 12 hours. Standard CBD has a half-life of around 70 minutes, which means that patients may have to dose many times a day to maintain a particular blood plasma level, whereas boosting the half-life would allow for just a twice daily dose.

Compared with the botanical approach to cannabinoids, the advantages of synthesis are speed and diversity. Botanical extractions usually take between three to four months, whereas chemical synthesis takes around two months to create a limited number of specific end products. Our process takes between two and seven days, which demonstrates the speed that a biosynthetic approach can bring to cannabinoid production. In addition, a biosynthetic approach removes the need to test for the various contaminants and impurities that may exist within a botanically grown plant. Plants, after all, are living things, which makes them prone to variability, although some companies are seeking to mitigate this reality by using plant clones.

High times

For the future, my hope is that our population will recognize there is more to cannabis than getting high. I can, of course, appreciate regulators wanting to limit exposure of psychotropic drugs to the general population, but only a few cannabinoids are psychotropic, which means that a blanket ban does a tremendous disservice to the wider patient population that could be well served by the remaining non-psychotropic compounds. We are currently making the case to a variety of regulatory bodies about rethinking the categorization and scheduling of these non-psychotropic molecules to accelerate the development of new medicines.

Jeff Korentur is CEO of Teewinot Life Sciences, Tampa, FL, USA.

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Making Cannabis Labs Accountable

Sitting Down With... Susan Audino, Assessor and Instructor at A2LA, and Chemistry Laboratory Consultant at S.A. Audino & Associates, LLC, Wilmington, Delaware , USA.

You had an unusual route into analytical chemistry…

I was originally a psychologist, but after several years working as a counselor in emergency rooms, I was burnt out and ready for a change. I was accepted onto a course to re-train as a chiropractor, provided I first took classes in organic chemistry, biochemistry and physics. I enjoyed my chemistry classes so much that I decided to continue them. I can remember a specific day when I was studying the visible light spectrum in the laboratory and I was filled with fascination about the underlying physics. It sparked a passion for spectroscopy and physical chemistry that eventually led to a new career. While studying undergraduate chemistry, I won an award to do independent research and got the chance to work with the US Secret Service in their forensic chemistry division. After that, I decided to apply for graduate studies in chemistry instead of chiropractic school.

How did you get involved in the cannabis industry?

Around eight years ago, I was working as a chemist in the Department of Agriculture when I got a call from a family friend, who asked me to join the quality assurance team of their up-and-coming dispensary in Rhode Island. My first reaction was that I wanted nothing to do with cannabis, which I believed should be illegal. But the conversation prompted me to start reading about the chemistry of cannabis and I became increasingly intrigued. The turning point came when I met Raphael Mechoulam – the eminent Israeli chemist who discovered the endocannabinoid system – at a conference. He helped to change my mind about medical cannabis, and as I learnt more about the cannabis industry, I became aware of its shortcomings and wanted to make positive changes. Cannabis is an amazing topic – it's economics, law, politics, botany, biology, chemistry and math all rolled into one. There are ups and downs – misconceptions and shortsightedness – but I believe we're moving in the right direction.

What is your role?

I work as a laboratory auditor for A2LA and teach courses on ISO standards. I'm also a consultant to chemical and biological labs. Some of my work is with cannabis labs, some with labs testing other substances, like jet fuel or compressed gas. In my remaining time, I serve as the Chair of the AOAC Cannabis Advisory Panel and Cannabis Working Group, which is establishing requirements for standard test methods for analytical laboratories. I'm also on the executive committee of the new Division 37 of ASTM, whose goal is to set standards within various subgroups (for example, laboratory, cultivation, packaging). The cannabis industry is changing; it's beginning to recognize the need for metrics to ensure quality, consistency and verification.

Why are standard methods for cannabis testing so important?

As part of my work, I go to a lot of dispensaries. When I flip over a packet and ask the staff what the numbers on the label mean, very few are able to tell me and, even if they can, the numbers don't always add up. Consumers generally trust the information on product labels, but the reality is that we often have no idea. Why? There are no standard test methods for cannabis, so it's impossible to verify test results. We could send one sample to three different labs and get three different results – from three different methods. Though it is worrying that five states require no testing whatsoever, I believe inadequate testing is worse than no testing at all because it gives a false sense of security. Imagine your doctor prescribing 10mg of heart medicine, but the pill turned out to contain 30 mg; you might well end up sicker than if you'd been prescribed nothing. If results aren't reliable and reproducible, then it's irresponsible.

Regulators are in a unique position with cannabis. One day they are prosecuting people for growing cannabis, the next day they have to regulate legal use. Given that most state regulators are not scientists, it's no easy task. Regulators are now beginning to require labs to follow ISO 17025 – an international standard that sets out the principles for running a quality laboratory and also mandates auditing by an appropriate accreditation body. It relieves a bit of the pressure on regulators and adds another layer of accountability for laboratories.

What needs to change?

I believe the answer lies in more standardized test methods and better regulation. I would love to see more regulatory harmonization between states and districts. In other industries, we have federal bodies overseeing individual state programs, but we don't have that luxury with cannabis, which means we have 29 separate systems; not ideal. Laboratories will always say that they are using the best science available to them – and while the majority of labs have good intentions, less scrupulous operators engage in 'dry-labbing' (supplying fictional but plausible results) or keep their prices low by employing inadequate equipment and untrained staff.

Much more research is required to turn cannabis into a true medicine. We have a remarkable opportunity to understand the science of a very interesting plant and harness its potential for medical use. But with opportunity comes responsibility, and we need to move forward with integrity and accountability.

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