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Surname	Other names

Pearson Edexcel Level 3 GCE

Centre Number	Candidate Number

Biology A (Salters-Nuffield)

Advanced

Paper 3: General and Practical Applications in Biology

Monday 18 June 2018 – Morning
Time: 2 hours

Paper Reference
9BN0/03

You must have:
Calculator, HB pencil, ruler and a copy of the scientific article adapted from several sources (enclosed)

Total Marks

Instructions

- Use **black** ink or ball-point pen.
- **Fill in the boxes** at the top of this page with your name, centre number and candidate number.
- Answer **all** questions.
- Show your working in any calculation questions and include units in your answer where appropriate.
- Answer the questions in the spaces provided
  – *there may be more space than you need.*
- You may use a scientific calculator.
- In questions marked with an **asterisk (*)**, marks will be awarded for your ability to structure your answer logically, showing how the points that you make are related or follow on from each other where appropriate.

Information

- The total mark for this paper is 100.
- The marks for **each** question are shown in brackets
  – *use this as a guide as to how much time to spend on each question.*

Advice

- Read each question carefully before you start to answer it.
- Try to answer every question.
- Check your answers if you have time at the end.

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Answer ALL questions.

Write your answers in the spaces provided.

1 A deer was found dead on National Trust land. Some people thought that the wounds that led to the deer's death could have been caused by a big cat such as a black panther.



(a) Samples of DNA from the wounds of this deer were collected.

Investigators used the polymerase chain reaction (PCR) to increase the quantity of DNA in the samples.

(i) Describe how one PCR cycle would increase the quantity of DNA present.

(3)

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(ii) One PCR cycle takes two minutes.

Show that the quantity of DNA would have been amplified over one million times after 40 minutes.

(2)

(b) The DNA produced by PCR was analysed to find out if a black panther was involved.

Explain how gel electrophoresis could be used to find out if this DNA came from a black panther.

(5)

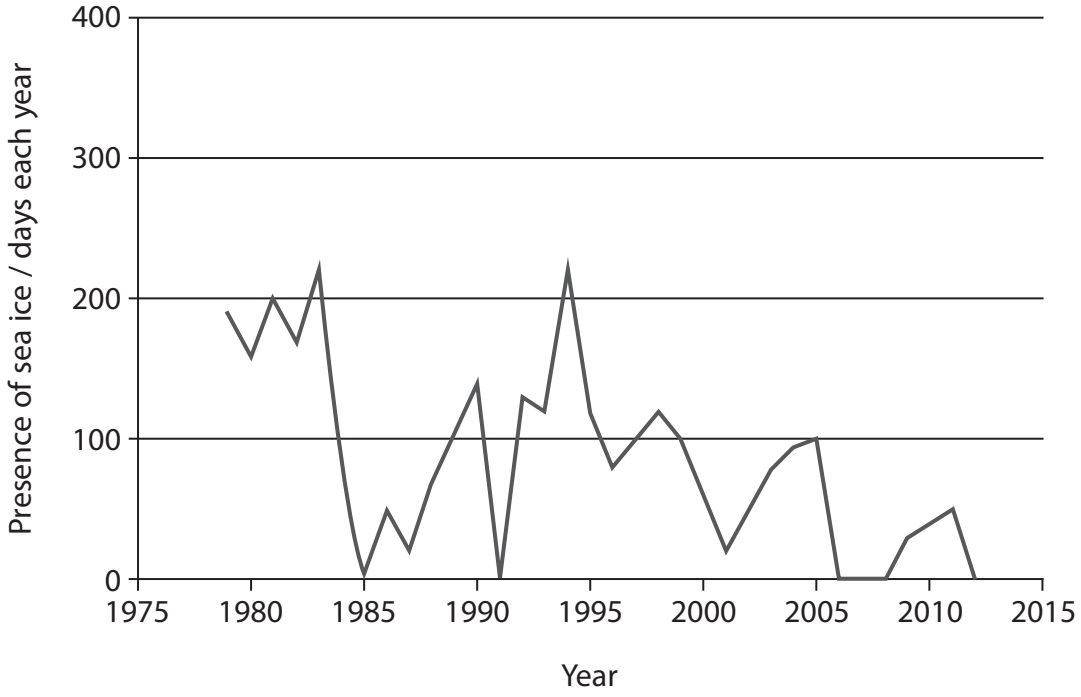
(Total for Question 1 = 10 marks)

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2 Scientists are concerned that climate change may be affecting the Arctic environment and the polar bears that live there.

(a) In a study, scientists recorded the length of time ice was present each year in the sea around the west coast of Spitsbergen, an island in the Arctic.

The graph shows the number of days each year when sea ice was present.



(i) Explain how the data could support a conclusion that climate change is occurring in the Arctic.

(2)

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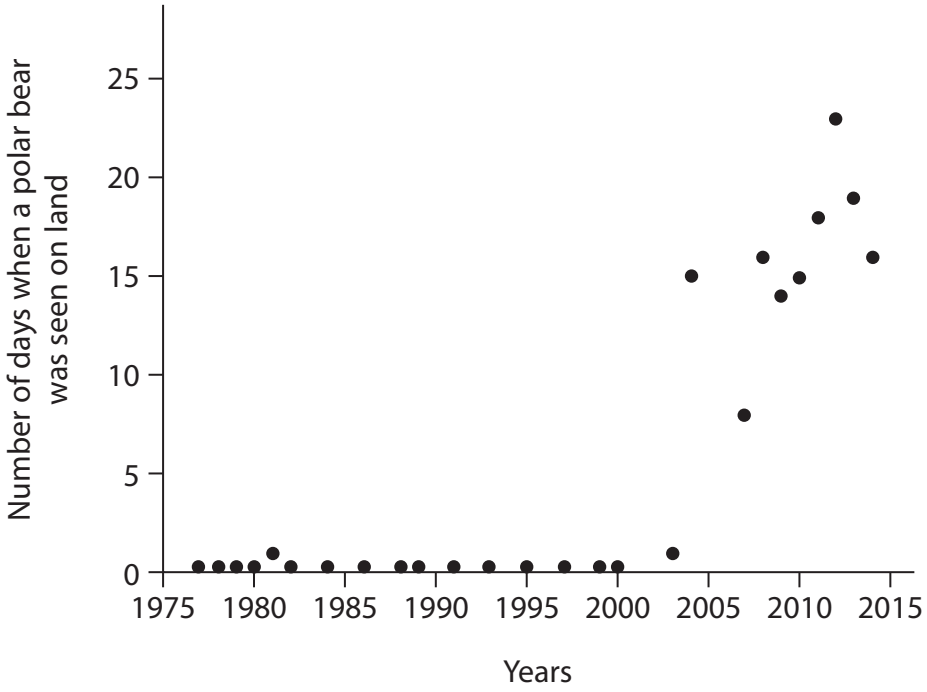
(ii) Explain why the data may not be useful for predicting future climate change.

(4)

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(b) The scientists also recorded data from west Spitsbergen on polar bear behaviour.

The graph shows the number of days polar bears were seen on land.



Sketch a trend line onto this graph.

(1)

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(c) Birds, such as barnacle geese, use the land on Spitsbergen as nesting sites.

Polar bears usually hunt for seals from sea ice. They have also been seen feeding on eggs from barnacle geese nests.

Assess the relationship between the presence of sea ice and the number of eggs taken by polar bears since 2000.

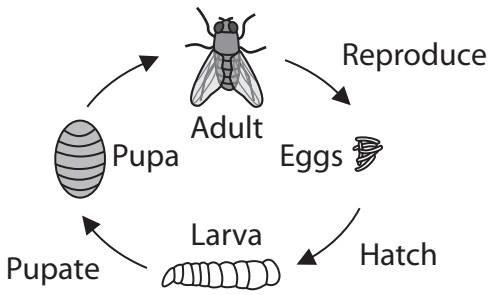
(4)

(Total for Question 2 = 11 marks)

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3 Blowflies are found in many parts of the world, including Africa.

The diagram shows the life cycle of one species of blowfly (species A) found in Africa.



An investigation was carried out to find the temperature at which 50% of the larvae of this species survive. This investigation was repeated for a further six species of African blowfly larvae, B to G. All other variables were kept constant.

In another investigation, the temperature of sand that the larvae selected when ready to pupate was recorded.

(a) A student used the data from these investigations to find out if there is a statistically significant correlation between the two sets of temperatures.

To do so, a Spearman's rank correlation coefficient can be calculated.

(i) Complete the table to rank all the data and to calculate d and d^2 for species E to G.

(3)

Blowfly species	Mean temperature at which 50% of larvae survive / °C	Rank for mean temperature for 50% larvae survival	Mean temperature of sand selected / °C	Rank for mean temperature of sand selected	Difference in rank (d)	Difference in rank squared (d^2)
A	49.0	5	26.1	7	–2	4
B	47.5	2	23.2	3	–1	1
C	48.5	3	24.7	6	–3	9
D	42.9	1	16.6	1	0	0
E	48.8		23.6			
F	50.1		24.2			
G	49.2		23.1			

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(ii) Calculate the Spearman's rank correlation coefficient (r_s) using the equation:

(3)

$$r_s = 1 - \frac{6\sum d^2}{n(n^2 - 1)}$$

where $\Sigma d^2 = 34$ and n is the number of blowfly species.

Answer .....

(iii) The table shows critical values for r_s.

	Probability		
n	**0.10**	**0.05**	**0.01**
5	0.900	1.000	1.000
6	0.829	0.886	1.000
7	0.714	0.786	0.929
8	0.643	0.738	0.881
9	0.600	0.683	0.833

Deduce whether the data showed a statistically significant correlation.

(2)

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(b) A number of observations were made about a dead rhinoceros:

- adults of all seven species of blowfly (A to G) were observed near the rhinoceros
- large numbers of living larvae of species F were present inside the rhinoceros
- mean temperature in this group of larvae was 49 ± 1.1 °C
- mean temperature of the air surrounding the rhinoceros was 33 ± 3.0 °C.

(i) Determine how the mean temperature of 49 °C was found.

(2)

(ii) It was observed that all the living larvae in the rhinoceros belonged to species F.

The metabolic activity of the larvae of species F increases the temperature within the dead rhinoceros.

Explain the advantages for this species of blowfly of increasing the temperature within the dead rhinoceros.

(3)

(Total for Question 3 = 13 marks)

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4 Seed banks store seeds to conserve different varieties of plants.

Seeds are stored for long periods of time in conditions that allow them to be germinated when required.

(a) An investigation was carried out to study the effect of storage time on wheat seeds of two different varieties.

Wheat seeds of one variety, P, were collected and divided into six groups, each with the same number of seeds. Each group of seeds was stored in an atmosphere of 80% humidity at a temperature of 42 °C.

Each group was stored in these conditions for different lengths of time. One group of seeds was germinated immediately after collection. This was the control group.

After storage, the seeds were planted in suitable conditions to allow germination. The number of seeds that germinated was found and the mean percentage germination calculated.

This was repeated using wheat seeds of a different variety, Q.

All other variables were kept constant.

The results are shown in the table.

Storage time / hours	Mean percentage germination (%)	
	Wheat variety P	Wheat variety Q
0 (control)	97	99
24	96	99
48	95	98
72	94	81
96	60	66
120	57	63

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(i) Describe the effect of storage time on the germination of seeds for these two varieties of wheat.

(3)

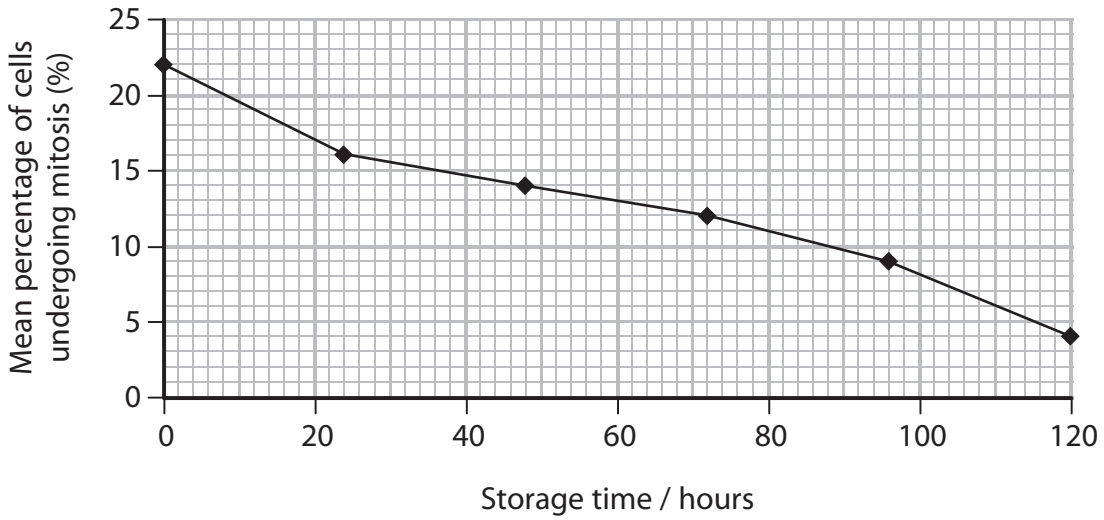
(ii) Explain how the results for the control were useful in this investigation.

(2)

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(b) Root tips were removed from the seedlings of variety Q. The percentage of cells from these root tips undergoing mitosis was recorded.

The results are shown in the graph.



(i) Describe the appearance of the chromosomes in the cells undergoing the metaphase stage of mitosis.

(3)

(ii) Analyse the data to explain how storage time affects the growth of the seedlings of variety Q.

(2)

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(c) It is claimed that the percentage of cells in a root tip undergoing mitosis during the day is different from the percentage during the night.

Devise an investigation to test this hypothesis.

(5)

(Total for Question 4 = 15 marks)

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5 Calcium ions are required for muscle contraction.

(a) Describe the role of calcium ions in the contraction of muscle fibres.

(3)

(b) An investigation was carried out to study the effect of calcium ion concentration on muscle fibres.

Individual fibres, from one sample of muscle, were provided with different calcium ion concentrations. The force produced by each fibre was recorded and a mean force was calculated for each calcium ion concentration.

All other variables were kept constant.

(i) Give one advantage and one disadvantage of using muscle fibres from the same sample of muscle.

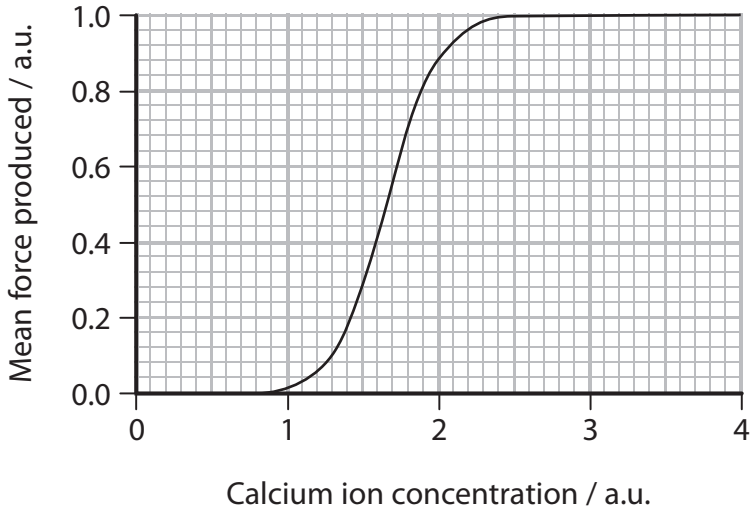
(2)

Advantage:

Disadvantage:

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(ii) The results are shown in the graph.



Explain the effect of increasing calcium ion concentration on the mean force produced by muscle fibres.

(4)

(Total for Question 5 = 9 marks)

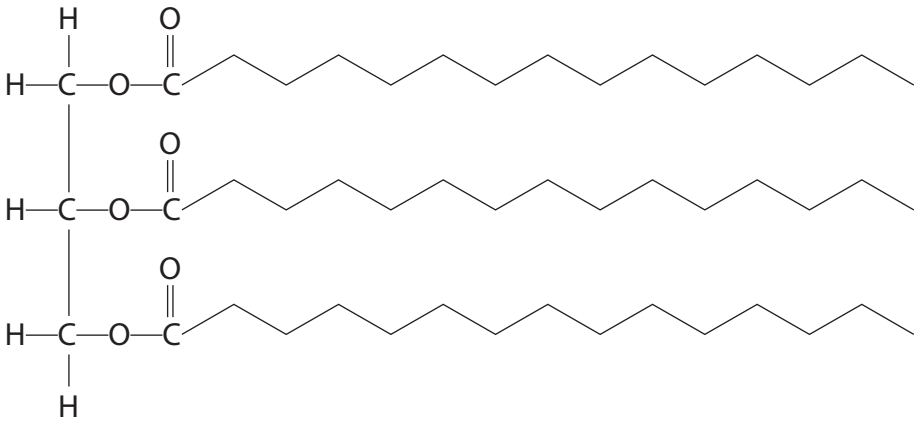
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6 Lipids and carbohydrates are found in both plants and animals.

(a) The diagram shows a triglyceride.



(i) On the diagram, draw a circle around an ester bond.

(1)

(ii) The fatty acids shown on the diagram are saturated.

Describe how the diagram would be different if one of the fatty acids was unsaturated.

(2)

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*(b) The carbohydrate content of vegetables ranges from 3 to 35%. However, meat contains little to no carbohydrate. Milk is the only food source from animals that contains a significant amount of carbohydrate.

Although plant material contains a higher proportion of carbohydrate than animal tissues, it has been claimed that carbohydrates are more important to animals than they are to plants.

Assess the relative importance of carbohydrates to plants and animals.

(9)

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(Total for Question 6 = 12 marks)

7 The scientific article you have studied is adapted from several sources.

Use the information from the scientific article and your own knowledge to answer the following questions.

(a) 'The hunger system is mediated by hormones from the gut and from fat cells' (paragraph 6).

Describe how these fat cells could release hormones.

(2)

(b) The reward system in humans involves the neurotransmitter dopamine pathway (paragraph 7).

Describe how dopamine acts as a neurotransmitter.

(4)

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(c) The hormone ghrelin may cause epigenetic changes to the *FTO* gene leading to weight gain (paragraph 9).

Describe how ghrelin could cause epigenetic changes.

(2)

(d) Probiotics include live bacteria that are ingested for health benefits.

Explain how probiotics may regulate food cravings (paragraphs 18, 19 and 20).

(3)

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(e) Tabor used enzymes to genetically modify a 'harmless species of *Escherichia coli*' (paragraph 26).

Describe the functions of the enzymes used to genetically modify bacteria.

(4)

(f) Give two reasons why doctors want to prevent people gaining excess weight (paragraph 28).

(1)

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(g) 'Bacterial molecules pass into the bloodstream where they do not belong, triggering an immune response' (paragraph 32).

Explain how the bacterial molecules could trigger a specific immune response.

(4)

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(h) Explain why genetically modified bacteria delivering drugs 'to the exact tissue in the body where they're needed and nowhere else' would decrease side effects (paragraph 34).

(2)

(i) There are 'trillions of microbes' found in the gut, 'as well as others living elsewhere in and on the body'.

Describe the role of the microbes that are normally found on the skin surface of humans (paragraph 40).

(2)

(j) The bacteria present in and on the body of a vertebrate 'can weigh as much as six pounds, and they make up a sort of organ' (paragraph 40).

State two properties of an organ.

(2)

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(k) Compare and contrast the structure of a bacterium, such as the one that causes neurological syphilis, with the Human Immunodeficiency Virus (HIV) (paragraph 49).

(3)

(l) Name one process that would have validated Pettersson's paper before it was published in the journal known as the Proceedings of the National Academy of Science (paragraph 58).

(1)

(Total for Question 7 = 30 marks)

TOTAL FOR PAPER = 100 MARKS

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Biology A (Salters-Nuffield)

Advanced

Paper 3: General and Practical Applications in Biology

June 2018

Scientific Article for use with Question 7

Paper Reference

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Scientific article for use with Question 7

Craving control: how food messes with your mind

1. You've just had a hearty lunch, but the doughnuts next to your desk are winking at you. You can't shake the thought of what the glazed, soft dough would taste like – and know that you won't be able to get on with your day until you have it.

2. On a basic level our relationship with food is simple – signals between the gut and the brain tell us when we're hungry, and when we are full. But experience shows us that the drive to eat is much more tangled and irrational. Some of that is down to the reward hit – the feeling of pleasure, mediated by the brain's reward centre – that we get from eating calorie-dense food like that glistening doughnut. Indeed, the effect of such foods has led some to liken our desire for them to drug addiction.

3. But we now know the gut itself, and also the microbes inside it, manipulate what we crave, painting a much more complex picture of the forces that determine the way we see food. Cravings could even be contagious – literally. When it comes to food, we're not as in control as we might think.

4. "People think we have much more conscious control over our eating behaviour than we do. There's a lot going on behind the scenes and it makes it very difficult to exert control on it," says Tony Goldstone, an endocrinologist at Imperial College London.

5. Even so, knowing about the forces that manipulate the way we think about food opens up new ways to regain control – for instance by retraining the brain or altering our gut flora. Fresh approaches would be more sensible than just expecting people to eat better, says Goldstone: "We don't just tell asthmatic people to breathe more."

6. What, when and how much we eat has typically been explained by two systems, one based on hunger and one on reward. The hunger system is mediated by hormones from the gut and from fat cells, which send information to the brain via the gut's own nervous system about when we last ate and how hungry we should feel. "We can eat very little one day, and a great deal the next, but this system works to ensure that body weight is relatively stable across the years," says John Menzies, a neurobiologist at the University of Edinburgh, UK.

7. The reward system is more concerned with what type of food we eat. At its heart is the dopamine pathway, which seems to respond most strongly to foods that are high in fat and sugar. This is natural and necessary – it evolved to prompt us to seek out such food, helping us survive. "If we see a high-energy food, it pays to get it while it's available – a famine may be round the corner," says Menzies. "However, in our modern environment where food is abundant and cheap, the reward system may work against us, pushing us towards eating sweet and fatty foods even though we already have plentiful energy stores."

8. The brain even has its own calorie counter that drives our choices without us knowing, according to a recent study. Participants were shown pictures of 50 foods and asked how many calories they thought each contained, and then invited to bid in an auction for a chance to eat the foods. Regardless of their calorie estimations, which were often inaccurate, the individuals were more likely to bid for the foods that were truly the most calorific. MRI scans showed that activity in reward regions of the brain correlated with the true calorific content of foods – the more calories, the greater the reward.

9. Although these hunger and reward systems sound very different, there's a growing awareness of how interconnected they are. Some clues come from genetics. A gene called *FTO* is strongly linked to weight gain, and one variant of it raises a person's risk of becoming obese by 70 per cent. A recent study showed that such people have higher than normal levels of the hormone ghrelin, which is released by the gut, telling them they are still hungry after eating, but their reward system works differently too. MRI studies showed that this group's brains responded differently when they were shown pictures of food: the most pronounced differences being in the reward regions. The reward pathways in the brains of obese people have also been shown to respond less strongly to food – which could be driving them to seek out even more each time.

10. More evidence of the link comes from people who have had gastric bypass surgery – which reduces the capacity of the stomach and makes food pass more quickly into the small intestine. After surgery, not only do people want to eat less, they experience a profound change in what they want to eat, finding they are drawn to much less calorie-dense foods. And brain scans of people before and after gastric bypass surgery showed altered activity in their reward centres. That contrasts with people who have a gastric band inserted. One explanation for these effects is that after a gastric bypass, food reaches the bowel much more quickly, so there's a faster hormone response, whereas a gastric band has no effect on hormone levels.

11. "These hormones are normally released after a meal to make us feel full, but as we're discovering, they also have effects on the way the brain works, to regulate the hedonic responses, the pleasure from food," says Goldstone. "The bypass patient will say, 'I'm not hungry, and I also don't want or like the food'. The band patient will say: 'I'm not hungry, but I could murder the chocolate cake'."

12. What if you could recreate these effects without the surgery? Susan Roberts, at Tufts University in Medford, Massachusetts, has designed a diet in which foods look like the kinds of calorie-dense treats people have learned to crave, but with a twist. "We basically confused people's reward system by giving them foods that had the flavour and appearance of high-calorie foods that are easily digested, but in fact they were lower calorie, slowly digested versions," she says. For instance, her diet includes a lower-calorie, slowly digested pizza, made with added fibre.

13. In a small trial, she scanned the brains of a group of overweight people before and after putting them on a six-month diet based on these foods. At the end of the study, the scans showed an increase in activity of reward pathways when the participants looked at pictures of healthy, low-calorie foods, compared with a group not eating the diet.

Risky rewards

14. "We were effectively retraining their brains," says Roberts. "You can think of pizza and you start craving pizza because you anticipate that rush of calories. If you eat the food and you fail to get the rush of calories, over time the reward circuitry adapts so it's no longer expecting a great zoom of carbohydrate coming in," she says.

15. The added fibre helped recondition cravings by making people feel full, but Roberts says it's also important that the participants only ate when they were truly hungry, to strengthen the reward they got from the food. And if dieters cheat and tuck into old favourites, it would strengthen the old reward pathways. Roberts is now beginning two larger clinical trials, and has commercialised the diet plan.

16. So we can retrain our brains to desire different foods.



17. While the brain clearly has a huge influence over what we eat, the influence of gut bacteria might be surprisingly large, too, and they can even affect our minds.

18. Joe Alcock at the University of New Mexico in Albuquerque and his colleagues published a review of research on the microbiome and came to an intriguing conclusion – gut microbes don't just flourish on certain diets, they may also control our food cravings and preferences to serve their own purposes.

19. There are several ways they could do this. Animals' gut flora has been shown to affect their taste receptors, which changes their food preferences. And many gut microbes can produce proteins that mimic gut hormones. Alcock's team even thinks that changes in food preferences that people experience after bariatric surgery might be down to changes in gut microbes, not hormones.

20. That means interventions like probiotics, which help to change the composition of the microbiome, might be useful tools in regulating food cravings. And it suggests a varied diet would make it harder for any one type to flourish and exert control.

21. Because the faecal and oral microbiomes of families under the same roof are more similar than people who don't live together, the idea that food cravings are influenced by gut bacteria also raises the intriguing possibility that through the spread of these microbes, cravings could even be contagious. Of course, this similarity could be because the members of a household have the same diet. But it might also be that gut bacteria are spread person to person. We already know people are much more likely to become obese if they have a friend who is obese, leading some to speculate that the effect is not down to social contagion, but the spread of microbes.

22. More needs to be done to work out how strong all these effects are, but this new appreciation for the hidden forces influencing our perception of food has wide-reaching implications. Goldstone even wonders whether tapping into the connection between the hunger and reward pathways could alter appetites of a different kind. Animal studies have already shown that ghrelin increases intake of alcohol, nicotine and other drugs, while "fullness" hormones reduce intake.

23. He suspects the same is true for humans. "We've shown that your nutritional state modifies the way the brain responds not just to food but also to winning money, and to stress," he says. "That's because the same reward circuitry is involved. There's evidence that gut hormones modify not only reward and consumption of food but also any drug of abuse – such as nicotine, cocaine, alcohol," he says. They are now beginning a large study.

24. At the very least, all this suggests that expecting people to rely purely on willpower to control what they eat, especially if they are obese, is misguided. "There's a cabal of obesity researchers that have turned up their hands and said the only thing you can do is rely on willpower," says Roberts, "I don't think it's worked for the last 30 years and it's not going to work next year either. Which is why we're trying to do it in a different way."

U.S. Navy Recruits Gut Microbes to Fight Obesity and Disease



25. In his lab at Rice University synthetic biologist Jeff Tabor is creating a kind of Lilliputian naval academy. The midshipmen are so small they can't be seen with the naked eye. But they're part of a vital mission to protect U.S. naval forces from internal enemies, ranging from metabolic disorders to anxiety and depression.

26. In 2014 Tabor received a three-year grant from the U.S. Office of Naval Research (ONR) to genetically modify a harmless species of *Escherichia coli* bacteria normally found in the human gut. The goal is to create an edible probiotic organism that can hone in on developing disease and stave it off, even before symptoms take hold. He has recently succeeded in engineering *E. coli* with sensors that can detect the presence of chemicals signaling disease – at least in the mouse gut.

27. His ultimate aim is to design "a precision gut bacterium that manipulates the intestinal environment in humans to keep it healthy," he says. This involves rewiring the genes of *E. coli* to transform the cells into predictable and reliable microbial medics loaded with engineered genetic circuits that can sense specific chemical disturbances and fire off a battery of molecules to neutralize them. The cells would live only a short time in the gut, perhaps six hours or so, "just long enough to do their job," Tabor says. Then they would die naturally or self-destruct.

28. Tabor's initial target: obesity and related metabolic issues. "We want to use a genetically engineered *E. coli* cell to sense the chemicals that signal gut disturbances linked with obesity," Tabor says, "and then deliver beneficial molecules to prevent weight gain."

29. Tabor's work represents the fruitful collision of two hot fields: synthetic biology, the engineering of microorganisms to make useful products; and microbiomics, the study of the microbes living on and inside humans and other animals, collectively known as the microbiome. "There's great potential in this area because there are so many widespread chronic diseases associated with the gut," says Pamela Silver of the Wyss Institute for Biologically Inspired Engineering at Harvard University, which published a report of the first synthetic engineered gut microbe in 2014.

30. The 100 trillion bacterial cells that reside in our guts play a major role in nearly every aspect of human biology – digesting food, guiding the immune system, even dictating mental health by sending signals to the brain that affect mood, cognition and behavior. It's not surprising, then, that disruption of these gut microbial communities can lead to disease, including obesity and related problems.

31. Tabor's project is part of a larger program on the microbiome funded by the ONR to help U.S. naval forces be more robust in the face of stressors – changes in diet or environment, fearful situations, sleep loss or disrupted circadian rhythms from shifting time zones or living in a submarine. "We're interested in how gut microbiota respond to these stresses," says Linda Chrisey, program officer in the ONR's Warfighter Protection and Application Division. "Are they contributing to the host's response? If so, can we tweak the microbiota to insulate the host from the stress?"

32. Tabor chose to focus on obesity "because we already know a lot about it at the molecular level," he says, "so it's a good model to test the concept." Our microbiota act like a kind of metabolic 'organ,' that affects calorie and nutrient absorption, manages energy balance and controls body weight. (Scientists aren't sure what shapes microbiomic composition. Increasing evidence suggests that it's determined before birth and has to do with genetics, maternal diet and mode of delivery.) It's clear that some bacteria make molecules that disrupt the balance within, causing obesity and other disorders. Studies have shown that the gut bacteria of healthy people churn out compounds that strengthen the intestinal wall but those of obese people make compounds that weaken the wall. This allows bacterial molecules to pass into the bloodstream where they do not belong, triggering an immune response. The resulting chronic inflammation is correlated with a laundry list of ailments, from inflammatory bowel disease to mental health disorders, such as anxiety and depression.

33. It's still early in the game, but Tabor has already isolated several sensors, reengineered them and put them into a single *E. coli* bacterium. He has fed the modified cells to mice and shown that the sensors have been activated inside the mouse gut, suggesting they have detected the target chemicals.

34. Tabor plans to have a single *E. coli* bacterium carry up to a dozen sensors so it can detect multiple signals at one time for a more accurate diagnosis. Ultimately, he plans to engineer these cells to produce drugs when and where they're called for – highly targeted antibiotics designed to bind with and deactivate those bacterial chemicals that might otherwise leak into the blood from the intestine – thereby preventing the changes that lead to obesity, inflammation and associated ills. Delivering these drugs to the exact tissue in the body where they're needed and nowhere else would both decrease side effects and increase efficacy.

35. However, "these are genetically engineered organisms, so there will be a long debate about them," Silver says. "We'll have to weigh the risks versus the potential benefits. But we're working to develop ways to make these organisms inherently safe. And I think the concern over risks will be neutralized by the benefits, especially for people who suffer from chronic disease."

36. So far, Tabor has altered only mouse microbiota. But, he says, "it's hard to imagine a future where we aren't diagnosing and treating, possibly curing, many diseases in humans by manipulating gut bacteria in this way – diabetes, autoimmune disorders, cancer, neurological disorders," and, yes, weight issues.

37. In fact, the Navy may find creative ways to deploy these synthetic probiotics not just to avoid obesity and its attendant problems but to quickly shift body weight and metabolism as necessary, Tabor suggests. "Imagine you have a team of marines going from a temperate environment, say, at sea level, to a really cold environment, like up on top of a mountain, in a short period of time. You want them to be able to put on some fat quickly to be more robust in the cold environment."

38. The solution? A dose of yogurt laced with synthetic probiotics that change warfighters' metabolism to increase fat for a couple of weeks – and after that another dose to take it off when they return to sea level.

Can the Bacteria in Your Gut Explain Your Mood?

39. Eighteen vials were rocking back and forth on a squeaky mechanical device the shape of a butcher scale, and Mark Lyte was beside himself with excitement. "We actually got some fresh yesterday – freshly frozen," Lyte said to a lab technician. Each vial contained a tiny nugget of monkey feces that were collected at the Harlow primate lab near Madison, Wis., the day before and shipped to Lyte's lab on the Texas Tech University Health Sciences Center campus in Abilene, Tex.

40. Lyte's interest was not in the feces per se but in the hidden form of life they harbor. The digestive tube of a monkey, like that of all vertebrates, contains vast quantities of what biologists call gut microbiota. The genetic material of these trillions of microbes, as well as others living elsewhere in and on the body, is collectively known as the microbiome. Taken together, these bacteria can weigh as much as six pounds, and they make up a sort of organ whose functions have only begun to reveal themselves to science. Lyte has spent his career trying to prove that gut microbes communicate with the nervous system using some of the same neurochemicals that relay messages in the brain.

41. Inside a closet-size room at his lab that afternoon, Lyte hunched over to inspect the vials, whose samples had been spun down in a centrifuge to a radiant, golden broth. Lyte, 60, spoke fast and emphatically. "You wouldn't believe what we're extracting out of poop," he told me. "We found that the guys here in the gut make neurochemicals. We didn't know that. Now, if they make this stuff here, does it have an influence there? Guess what? We make the same stuff. Maybe all this communication has an influence on our behavior."

42. Since 2007, when scientists announced plans for a Human Microbiome Project to catalog the micro-organisms living in our body, the profound appreciation for the influence of such organisms has grown rapidly with each passing year. Bacteria in the gut produce vitamins and break down our food; their presence or absence has been linked to obesity, inflammatory bowel disease and the toxic side effects of prescription drugs. Biologists now believe that much of what makes us human depends on microbial activity. The two million unique bacterial genes found in each human microbiome can make the 23,000 genes in our cells seem paltry, almost negligible, by comparison. "It has enormous implications for the sense of self," Tom Insel, the director of the National Institute of Mental Health, told me. "We are, at least from the standpoint of DNA, more microbial than human. That's a phenomenal insight and one that we have to take seriously when we think about human development."

43. Given the extent to which bacteria are now understood to influence human physiology, it is hardly surprising that scientists have turned their attention to how bacteria might affect the brain. Micro-organisms in our gut secrete a profound number of chemicals, and researchers like Lyte have found that among those chemicals are the same substances used by our neurons to communicate and regulate mood, like dopamine, serotonin and gamma-aminobutyric acid (GABA). These, in turn, appear to play a function in intestinal disorders, which coincide with high levels of major depression and anxiety. Last year, for example, a group in Norway examined feces from 55 people and found certain bacteria were more likely to be associated with depressive patients.

44. At the time of my visit to Lyte's lab, he was nearly six months into an experiment that he hoped would better establish how certain gut microbes influenced the brain, functioning, in effect, as psychiatric drugs. He was currently compiling a list of the psychoactive compounds found in the feces of infant monkeys. Once that was established, he planned to transfer the microbes found in one newborn monkey's feces into another's intestine, so that the recipient would end up with a completely new set of microbes – and, if all went as predicted, change their neurodevelopment. The experiment reflected an intriguing hypothesis. Anxiety, depression and several pediatric disorders, including autism and hyperactivity, have been linked with gastrointestinal abnormalities. Microbial transplants were not invasive brain surgery, and that was the point: changing a patient's bacteria might be difficult but it still seemed more straightforward than altering his genes.

45. When Lyte began his work on the link between microbes and the brain three decades ago, it was dismissed as a curiosity. By contrast, last September, the National Institute of Mental Health awarded four grants worth up to $1 million each to spur new research on the gut microbiome's role in mental disorders, affirming the legitimacy of a field that had long struggled to attract serious scientific credibility. Lyte and one of his longtime colleagues, Christopher Coe, at the Harlow primate lab, received one of the four. "What Mark proposed going back almost 25 years now has come to fruition," Coe told me. "Now what we're struggling to do is to figure out the logic of it." It seems plausible, if not yet proved, that we might one day use microbes to diagnose neurodevelopmental disorders, treat mental illnesses and perhaps even fix them in the brain.

46. In 2011, a team of researchers at University College Cork, in Ireland, and McMaster University, in Ontario, published a study in Proceedings of the National Academy of Science that has become one of the best-known experiments linking bacteria in the gut to the brain. Laboratory mice were dropped into tall, cylindrical columns of water in what is known as a forced-swim test, which measures over six minutes how long the mice swim before they realize that they can neither touch the bottom nor climb out, and instead collapse into a forlorn float. Researchers use the amount of time a mouse floats as a way to measure what they call "behavioral despair." (Antidepressant drugs, like Zoloft and Prozac, were initially tested using this forced-swim test.)

47. For several weeks, the team, led by John Cryan, the neuroscientist who designed the study, fed a small group of healthy rodents a broth infused with *Lactobacillus rhamnosus*, a common bacterium that is found in humans and also used to ferment milk into probiotic yogurt. *Lactobacilli* are one of the dominant organisms babies ingest as they pass through the birth canal. Recent studies have shown that mice stressed during pregnancy pass on lowered levels of the bacterium to their pups. This type of bacteria is known to release immense quantities of GABA; as an inhibitory neurotransmitter, GABA calms nervous activity, which explains why the most common anti-anxiety drugs, like Valium and Xanax, work by targeting GABA receptors.

48. Cryan found that the mice that had been fed the bacteria-laden broth kept swimming longer and spent less time in a state of immobilized woe. "They behaved as if they were on Prozac," he said. "They were more chilled out and more relaxed." The results suggested that the bacteria were somehow altering the neural chemistry of mice.

49. Until he joined his colleagues at Cork 10 years ago, Cryan thought about microbiology in terms of pathology: the neurological damage created by diseases like syphilis or H.I.V. "There are certain fields that just don't seem to interact well," he said. "Microbiology and neuroscience, as whole disciplines, don't tend to have had much interaction, largely because the brain is somewhat protected." He was referring to the fact that the brain is anatomically isolated, guarded by a blood-brain barrier that allows nutrients in but keeps out pathogens and inflammation, the immune system's typical response to germs. Cryan's study added to the growing evidence that signals from beneficial bacteria nonetheless find a way through the barrier. Somehow – though his 2011 paper could not pinpoint exactly how – micro-organisms in the gut tickle a sensory nerve ending in the fingerlike protrusion lining the intestine and carry that electrical impulse up the vagus nerve and into the deep-brain structures thought to be responsible for elemental emotions like anxiety. Soon after that, Cryan and a co-author, Ted Dinan, published a theory paper in Biological Psychiatry calling these potentially mind-altering microbes "psychobiotics."

50. It has long been known that much of our supply of neurochemicals – an estimated 50 percent of the dopamine, for example, and a vast majority of the serotonin – originate in the intestine, where these chemical signals regulate appetite, feelings of fullness and digestion. But only in recent years has mainstream psychiatric research given serious consideration to the role microbes might play in creating those chemicals. Lyte's own interest in the question dates back to his time as a postdoctoral fellow at the University of Pittsburgh in 1985, when he found himself immersed in an emerging field with an unwieldy name: psychoneuroimmunology, or PNI, for short. The central theory, quite controversial at the time, suggested that stress worsened disease by suppressing our immune system.

51. By 1990, at a lab in Mankato, Minn., Lyte distilled the theory into three words, which he wrote on a chalkboard in his office: Stress → Immune → Disease. In the course of several experiments, he homed in on a paradox. When he dropped an intruder mouse in the cage of an animal that lived alone, the intruder ramped up its immune system – a boost, he suspected, intended to fight off germ-ridden bites or scratches. Surprisingly, though, this did not stop infections. It instead had the opposite effect: stressed animals got sick. Lyte walked up to the board and scratched a line through the word "Immune." Stress, he suspected, directly affected the bacterial bugs that caused infections.

52. To test how micro-organisms reacted to stress, he filled petri plates with a bovine-serum-based medium and laced the dishes with a strain of bacterium. In some, he dropped norepinephrine, a neurochemical that mammals produce when stressed. The next day, he snapped a Polaroid. The results were visible and obvious: the control plates were nearly barren, but those with the norepinephrine bloomed with bacteria that filigreed in frostlike patterns. Bacteria clearly responded to stress.

53. Then, to see if bacteria could induce stress, Lyte fed white mice a liquid solution of *Campylobacter jejuni*, a bacterium that can cause food poisoning in humans but generally doesn't prompt an immune response in mice. To the trained eye, his treated mice were as healthy as the controls. But when he ran them through a plexiglass maze raised several feet above the lab floor, the bacteria-fed mice were less likely to venture out on the high, unprotected ledges of the maze. In human terms, they seemed anxious. Without the bacteria, they walked the narrow, elevated planks.

54. Each of these results was fascinating, but Lyte had a difficult time finding microbiology journals that would publish either. "It was so anathema to them," he told me. When the mouse study finally appeared in the journal Physiology & Behavior in 1998, it garnered little attention. And yet as Stephen Collins, a gastroenterologist at McMaster University, told me, those first papers contained the seeds of an entire new field of research. "Mark showed, quite clearly, in elegant studies that are not often cited, that introducing a pathological bacterium into the gut will cause a change in behavior."

55. Lyte went on to show how stressful conditions for newborn cattle worsened deadly *E. coli* infections. In another experiment, he fed mice lean ground hamburger that appeared to improve memory and learning – a conceptual proof that by changing diet, he could change gut microbes and change behavior. After accumulating nearly a decade's worth of evidence, in July 2008, he flew to Washington to present his research. He was a finalist for the National Institutes of Health's Pioneer Award, a $2.5 million grant for so-called blue-sky biomedical research. Finally, it seemed, his time had come. When he got up to speak, Lyte described a dialogue between the bacterial organ and our central nervous system. At the two-minute mark, a prominent scientist in the audience did a spit take.

56. "Dr. Lyte," he later asked at a question-and-answer session, "if what you're saying is right, then why is it when we give antibiotics to patients to kill bacteria, they are not running around crazy on the wards?"

57. Lyte knew it was a dismissive question. And when he lost out on the grant, it confirmed to him that the scientific community was still unwilling to imagine that any part of our neural circuitry could be influenced by single-celled organisms. Lyte published his theory in Medical Hypotheses, a low-ranking journal that served as a forum for unconventional ideas. The response, predictably, was underwhelming. "I had people call me crazy," he said.

58. But by 2011 – when he published a second theory paper in Bioessays, proposing that probiotic bacteria could be tailored to treat specific psychological diseases – the scientific community had become much more receptive to the idea. A Canadian team, led by Stephen Collins, had demonstrated that antibiotics could be linked to less cautious behavior in mice, and only a few months before Lyte, Sven Pettersson, a microbiologist at the Karolinska Institute in Stockholm, published a landmark paper in Proceedings of the National Academy of Science that showed that mice raised without microbes spent far more time running around outside than healthy mice in a control group; without the microbes, the mice showed less apparent anxiety and were more daring. In Ireland, Cryan published his forced-swim-test study on psychobiotics. There was now a groundswell of new research. In short order, an implausible idea had become a hypothesis in need of serious validation.

59. Late last year, Sarkis Mazmanian, a microbiologist at the California Institute of Technology, gave a presentation at the Society for Neuroscience, "Gut Microbes and the Brain: Paradigm Shift in Neuroscience." Someone had inadvertently dropped a question mark from the end, so the speculation appeared to be a definitive statement of fact. But if anyone has a chance of delivering on that promise, it's Mazmanian, whose research has moved beyond the basic neurochemicals to focus on a broader class of molecules called metabolites: small, equally druglike chemicals that are produced by micro-organisms. Using high-powered computational tools, he also hopes to move beyond the suggestive correlations that have typified psychobiotic research to date, and instead make decisive discoveries about the mechanisms by which microbes affect brain function.

60. Two years ago, Mazmanian published a study in the journal Cell with Elaine Hsiao, then a graduate student [in the lab of Paul Patterson] and now a neuroscientist at Caltech, and others, that made a provocative link between a single molecule and behavior. Their research found that mice exhibiting abnormal communication and repetitive behaviors, like obsessively burying marbles, were mollified when they were given one of two strains of the bacterium *Bacteroides fragilis*.

61. The study added to a working hypothesis in the field that microbes don't just affect the permeability of the barrier around the brain but also influence the intestinal lining, which normally prevents certain bacteria from leaking out and others from getting in. When the intestinal barrier was compromised in his model, normally "beneficial" bacteria and the toxins they produce seeped into the bloodstream and raised the possibility they could slip past the blood-brain barrier. As one of his colleagues, Michael Fischbach, a microbiologist at the University of California, San Francisco, said: "The scientific community has a way of remaining skeptical until every last arrow has been drawn, until the entire picture is colored in. Other scientists drew the pencil outlines, and Sarkis is filling in a lot of the color."

62. Mazmanian knew the results offered only a provisional explanation for why restrictive diets and antibacterial treatments seemed to help some children with autism: altering the microbial composition might be changing the permeability of the intestine. "The larger concept is, and this is pure speculation: is a disease like autism really a disease of the brain or maybe a disease of the gut or some other aspect of physiology?" Mazmanian said. For any disease in which such a link could be proved, he saw a future in drugs derived from these small molecules found inside microbes. (A company he co-founded, Symbiotix Biotherapies, is developing a complex sugar called PSA, which is associated with *Bacteroides fragilis*, into treatments for intestinal disease and multiple sclerosis.) In his view, the prescriptive solutions probably involve more than increasing our exposure to environmental microbes in soil, dogs or even fermented foods; he believed there were

wholesale failures in the way we shared our microbes and inoculated children with these bacteria. So far, though, the only conclusion he could draw was that disorders once thought to be conditions of the brain might be symptoms of microbial disruptions, and it was the careful defining of these disruptions that promised to be helpful in the coming decades.

63. The list of potential treatments incubating in labs around the world is startling. Several international groups have found that psychobiotics had subtle yet perceptible effects in healthy volunteers in a battery of brain-scanning and psychological tests. Another team in Arizona recently finished an open trial on fecal transplants in children with autism. (Simultaneously, at least two offshore clinics, in Australia and England, began offering fecal microbiota treatments to treat neurological disorders, like multiple sclerosis.) Mazmanian, however, cautions that this research is still in its infancy. "We've reached the stage where there's a lot of, you know, 'The microbiome is the cure for everything,'" he said. "I have a vested interest if it does. But I'd be shocked if it did."

64. Lyte issues the same caveat. "People are obviously desperate for solutions," Lyte said when I visited him in Abilene. (He has since moved to Iowa State's College of Veterinary Medicine.) "My main fear is the hype is running ahead of the science." He knew that parents emailing him for answers meant they had exhausted every option offered by modern medicine. "It's the Wild West out there," he said. "You can go online and buy any amount of probiotics for any number of conditions now, and my paper is one of those cited. I never said go out and take probiotics." He added, "We really need a lot more research done before we actually have people trying therapies out."

65. If the idea of psychobiotics had now, in some ways, eclipsed him, it was nevertheless a curious kind of affirmation, even redemption: an old-school microbiologist thrust into the midst of one of the most promising aspects of neuroscience. At the moment, he had a rough map in his head and a freezer full of monkey fecals that might translate, somehow, into telling differences between gregarious or shy monkeys later in life. I asked him if what amounted to a personality transplant still sounded a bit far-fetched. He seemed no closer to unlocking exactly what brain functions could be traced to the same organ that produced feces. "If you transfer the microbiota from one animal to another, you can transfer the behavior," Lyte said. "What we're trying to understand are the mechanisms by which the microbiota can influence the brain and development. If you believe that, are you now out on the precipice? The answer is yes. Do I think it's the future? I think it's a long way away."

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