STRAIGHT FROM THE GUT

Stephanie is the first to admit that she never had the guts for life. She was born with familial adenomatous polyposis, a genetic disorder in which thousands of polyps form in the colon. By the age of 22, much of the organ had to be removed. Four years later, a massive benign tumour choked off the blood supply to her small intestine, so doctors cut out all but a metre of it. For the next six years, she was fed by a tube every night until the feeding left her liver badly scarred and fighting recurring infections. "I was given a month to live," she says.

That's when doctors referred Stephanie to Georgetown University Hospital in Washington DC. There, on 17 April 2006 , surgeons cut out her stomach and what was left of her small and large intestine and replaced it with new organs from a donor who had died days earlier in Tennessee. "Oesophagus to anus, her entire gastrointestinal tract was in the garbage can," says Tom Fishbein, who directed the surgery. "She got a brand new one."

All organ transplants are complicated, but there are only a handful of centres in the United States that have the expertise to transplant a small intestine, the seven metres of coiled tissue connected up to the stomach at one end and the large intestine at the other. The technique is complicated because the gut is teeming with trillions of bacteria and other microbes, plus the bulk of the body's lymphocytes. Before such transplants, the donor's intestine has normally been flushed with antibiotics. But rates of infection and rejection from such transplants are very high because, it is thought, some foreign bacteria and immune cells survive the cleaning process and are thrust into an immunosuppressed recipient.

"Oesophagus to anus, her entire gastrointestinal tract was in the garbage can."
— Tom Fishbein

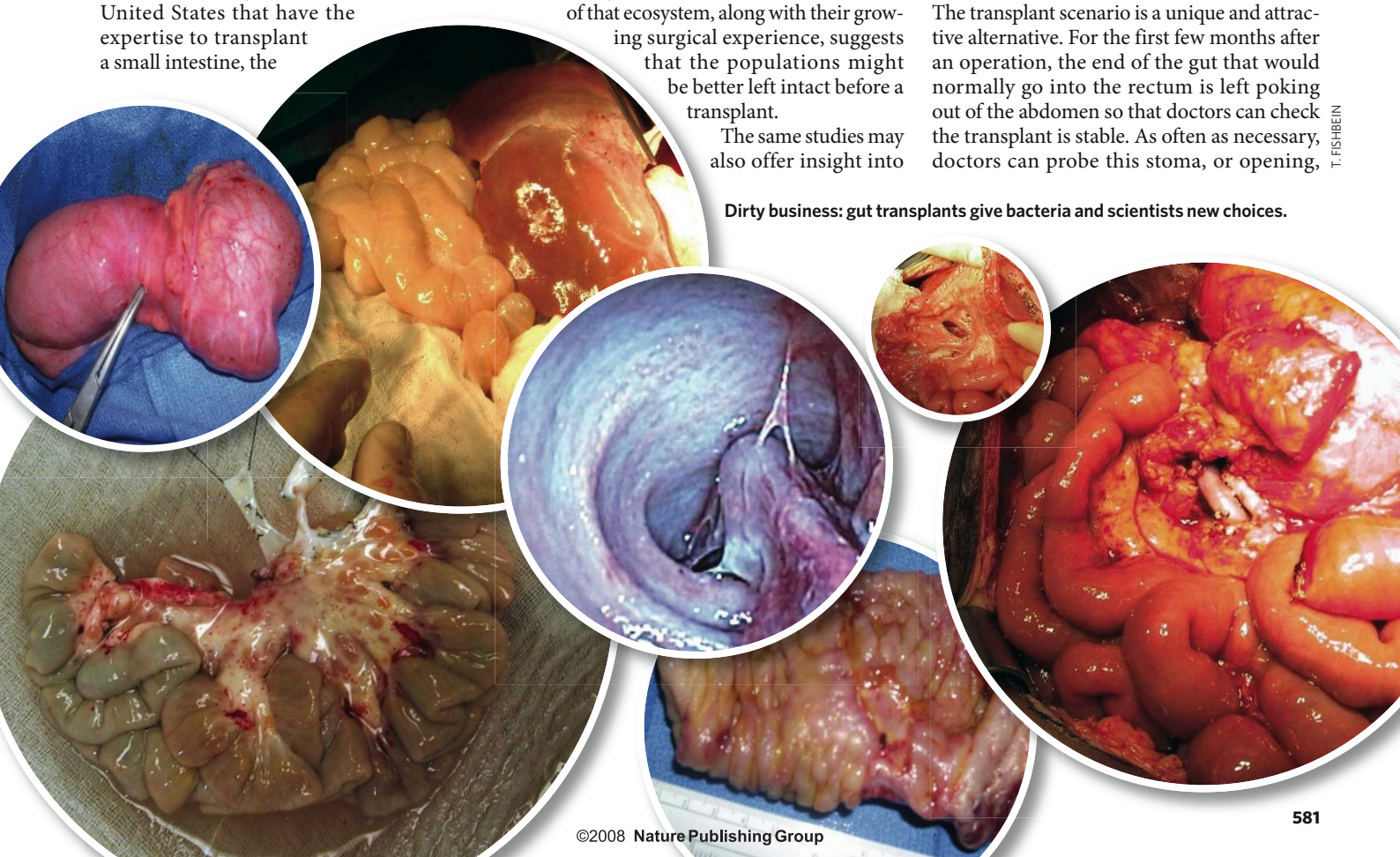
The idea that these intestinal bacteria are a menace is now under review. By teaming up with microbiologists, the surgeons are taking advantage of a rare chance to study microbes as they colonize the walls of the gut after transplanting an intestine: which ones arrive first, and how they restore the ravaged microbial communities. "An all new ecosystem of organisms had to populate that bowel from scratch," says Fishbein. Their new appreciation of that ecosystem, along with their growing surgical experience, suggests that the populations might be better left intact before a transplant.

The same studies may also offer insight into how the gut is first populated by microbes after birth, how it recovers from the damage done by a heavy course of antibiotics and, perhaps, how to minimize that damage. "Most people study this in animal models, but this is a real-person model," says Brett Finlay, a microbiologist at the University of British Columbia in Vancouver, Canada. "It's an artificial system in some sense, but it's a neat model."

How the moist, pink intestinal tubing lives in such harmonious contact with bacteria has puzzled scientists for decades. But "it's hard to get in there, especially in a healthy person", says David Relman, who studies microbiology and immunology at Stanford University, California. "And to do it in a way that doesn't perturb the system, and to do it every week or every day, well, forget it." For this reason, most researchers interested in the contents of human innards have had to collect and filter faeces.

Beautiful opportunity

The transplant scenario is a unique and attractive alternative. For the first few months after an operation, the end of the gut that would normally go into the rectum is left poking out of the abdomen so that doctors can check the transplant is stable. As often as necessary, doctors can probe this stoma, or opening,



T. FISHBEIN

Dirty business: gut transplants give bacteria and scientists new choices.

CAMERON/CORBIS

An opportunity to track gut colonization.

with an endoscope to pinch off biopsies of the intestinal wall with its microbial community intact. Yellow faeces and glistening, pink skin are signs that the new intestine has successfully taken. "That is beautiful stuff," says Stuart Kaufman, medical director of the intestinal-transplantation programme at Georgetown. "We live for poop like this."

The adult samples are also beautiful stuff for researchers who want to chart the arrivals of the different bacterial species. The material is put on ice and shipped to the lab of Jonathan Eisen, a microbial geneticist at the University of California, Davis. There, graduate student Amber Hartman identifies the inhabitants by analysing distinguishing gene sequences that vary slightly between different species of bacteria.

It's far too early to draw firm conclusions from their data; Fishbein and his collaborators have only studied 15 patients over the past 2 years. But what they have found suggests that the gut is populated first by enterobacteriaceae, a large family of facultative anaerobes, which can grow with or without oxygen. This suggests that the transplanted tissue has higher oxygen levels than the normal gut, where anaerobic bacteria dominate. Inflammation may boost oxygen levels, giving enterobacteria the advantage.

Hartman and Eisen have also found that each person studied so far has had different proportions of the various microbial species, and that these oscillate rapidly in the first few months after a transplant. Their preliminary observations suggest that the more chaotic the variations over time, the worse the outcome of the transplant. "One thing that becomes very obvious is that the amount or degree of fluctuation is much greater in the sicker patient," Hartman says. They now want to know whether the microbiota becomes more stable and reaches equilibrium as a patient recovers, but that requires following more patients over longer times, and intestinal transplants are rare.

A different bloody, messy procedure arrives all too often though: a birth. Here, too, researchers see a fascinating opportunity to explore how microbes colonize a gut, one thought to be sterile inside the womb. During birth and in the hours after, babies can swallow bacteria from the mother's birth canal, faeces and from whatever environment they arrive in.

No one knows yet whether bacteria move into a baby's spanking new innards in the same way they grab a foothold in a used, adult transplant. Much of what scientists know about the former process has been learned from a study of Relman's, in which he and his colleagues collected used nappies from 14 babies beginning with the first stool after birth and at regular intervals throughout the first year of life[1]. What they found mirrored some of the discoveries in the transplanted intestines: every baby's microbiota is unique but dynamic, with different populations of bacterial species shifting in abundance. And as in the transplant study, the babies showed a succession of colonization, with facultative bacteria settling in first, followed by a more complex and diverse population.

Nice and dirty

Whatever parallels may emerge from these studies, there is one obvious difference: the transplanted gut has already been soiled by the faeces, microbes and immune cells it hosted before. And, until recently, doctors did all they could to scour away the muck.

Paradoxically, the surgeons at Georgetown began to notice that the more antibiotics they used to keep the microbes to a minimum, the more intestinal infections they saw after the transplant. At first, the alternative seemed too fantastic to contemplate: taking an essentially infected organ and placing it in a body crippled by immunosuppressant drugs. But about a year ago, Georgetown and other centres began shifting their practice to do exactly that. Early evidence indicates that those who receive a gut replete with its native microbiota have fewer chaotic fluctuations.

That makes sense in retrospect, notes Eisen, because people who need transplants may do so precisely because they had trouble colonizing their bowels properly to begin with. In some patients — those with Crohn's disease or ulcerative colitis, for example — the native bacteria may turn on the body, inflaming the gut and scarring the intestinal walls. So the new intestine might be more likely to help if it comes with its own set of inhabitants. "To take out all the microbes seems completely inane," Eisen says.

The Georgetown researchers have now started to investigate how those bacterial fluctuations during colonization are controlled. They suspect that a gene called *NOD2*, which is expressed in some immune cells, is essential for keeping the chaos to a minimum. The NOD2 protein recognizes components of bacterial cell walls and controls the production of defensins, small proteins that kill particular bacterial species.

The team has found that about 35% of their transplant patients carry mutations in *NOD2*, regardless of the intestinal disorder, and that those with mutations are 100-fold more likely to have a failed transplant compared with controls[2]. The hypothesis is that this mutation somehow lowers production of defensins, so the immune system is unable to maintain the appropriate proportions of bacterial species. Perhaps patients with a mutant *NOD2* gene might benefit from doses of the bacteria that they are missing. In one patient with a *NOD2* mutation who later died, "the proportions of normalness were very, very off", Hartman says.

Quite what 'off' is, is hard to define. The team still has only a cursory understanding of what the microbiota looks like in healthy people, compared with their subjects. "It's like watching colonization of a disturbed ecosystem without knowing what was originally in the forest," says Eisen. That may be helped by the National Institutes of Health's Human Microbiome Project and other new research efforts that aim to catalogue the microbes in the human body (see page 578). "We need that field guide to microbes to understand when something is not normal," Eisen says.

"We live for poop like this."
—Stuart Kaufman

In the meantime, clinical signs are still the best predictors of a transplant's success. Two years after a new gut was slotted into Stephanie's body, she still has scars criss-crossing her abdomen but she is a healthy weight and eats whatever she wants. It is still not clear which microbes to thank: the donor's bugs that survived from before the transplantation, or new microbes that settled there afterwards. "Whoever's microbes have prevailed, they're probably good ones," says Fishbein, "because she's done exceptionally well." ■

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1. Palmer, C. *et al. PLoS Biol.* **5,** e177 (2007).
2. Fishbein, T. *et al. Gut* **57,** 323–330 (2008).

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