ILLUSTRATION BY GARY NEILL

Blood to blood

By splicing animals together, scientists have shown that young blood rejuvenates old tissues. Now, they are testing whether it works for humans.

BY MEGAN SCUDELLARI

Two mice perch side by side, nibbling a food pellet. As one turns to the left, it becomes clear that food is not all that they share — their front and back legs have been cinched together, and a neat row of sutures runs the length of their bodies, connecting their skin. Under the skin, however, the animals are joined in another, more profound way: they are pumping each other's blood.

Parabiosis is a 150-year-old surgical technique that unites the vasculature of two living animals. (The word comes from the

Greek *para*, meaning 'alongside', and *bios*, meaning 'life'.) It mimics natural instances of shared blood supply, such as in conjoined twins or animals that share a placenta in the womb.

In the lab, parabiosis presents a rare opportunity to test what circulating factors in the blood of one animal do when they enter another animal. Experiments with parabiotic rodent pairs have led to breakthroughs in endocrinology, tumour biology and immunology, but most of those discoveries occurred more than 35 years ago. For reasons that are not entirely clear, the technique fell out of favour after the 1970s.

In the past few years, however, a small number of labs have revived parabiosis, especially in the field of ageing research. By joining the circulatory system of an old mouse to that of a young mouse, scientists have produced some remarkable results. In the heart, brain, muscles and almost every other tissue examined, the blood of young mice seems to bring new life to ageing organs, making old mice stronger, smarter and healthier. It even makes their fur shinier. Now these labs have begun to identify the components of young blood that are responsible for these changes. And last September, a clinical trial in California became the first to start testing the benefits of young blood in older people with Alzheimer's disease.

"I think it is rejuvenation," says Tony Wyss-Coray, a neurologist at Stanford University in California who founded a company that is running the trial. "We are restarting the ageing clock."

Many of his colleagues are more cautious about making such claims. "We're not de-ageing animals," says Amy Wagers, a stem-cell researcher at Harvard University in Cambridge, Massachusetts, who has identified a muscle-rejuvenating factor in young mouse blood. Wagers argues that such factors are not turning old tissues into young ones, but are instead helping them to repair damage. "We're restoring function to tissues."

She emphasizes that no one has convincingly shown that young blood lengthens lives, and there is no promise that it will. Still, she says that young blood, or factors from it, may hold promise for helping elderly people to heal after surgery, or treating diseases of ageing.

"It's very provocative," says Mark Mattson, chief of the Laboratory of Neurosciences at the US National Institute on Aging in Bethesda, Maryland, who has not been involved in the parabiosis work. "It makes you think. Maybe I should bank some blood of my daughter's son, so if I start to have any cognitive problems, I'll have some help," he says, only half-joking.

THE POWER OF TWO

Physiologist Paul Bert performed the earliest recorded parabiosis experiment in 1864, when he removed a strip of skin from the flanks of two albino rats, then stitched the animals together in hopes of creating a shared circulatory system[1]. Biology did the rest: natural wound-healing processes joined the animals' circulatory systems as capillaries regrew at the intersection. Bert found that fluid injected into a vein of one rat passed easily into the other, work that won him an award from the French Academy of Sciences in 1866.

Since Bert's initial experiments, the procedure has not changed much. It has been performed on hydra — small freshwater invertebrates related to jellyfish — frogs and insects, but it works best on rodents, which recover well from the surgery. Up to the mid-twentieth century, scientists used parabiotic pairs of mice or rats to study a variety of phenomena. For example, one team ruled out the idea that dental cavities are the result of sugar in the blood by using a pair of parabiosed rats, of which only one was fed a daily diet of glucose. The rats had similar blood glucose levels owing to their shared circulation, yet only the rat that actually ate the sugar developed cavities[2].

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Clive McCay, a biochemist and gerontologist at Cornell University in Ithaca, New York, was the first to apply parabiosis to the study of ageing. In 1956, his team joined 69 pairs of rats, almost all of differing ages[3]. The linked rats included a 1.5-month-old paired with a 16-month-old — the equivalent of pairing a 5-year-old human with a 47-year-old. It was not a pretty experiment. "If two rats are not adjusted to each other, one will chew the head of the other until it is destroyed," the authors wrote in one description of their work[4]. And of the 69 pairs, 11 died from a mysterious condition termed parabiotic disease, which occurs approximately one to two weeks after partners are joined, and may be a form of tissue rejection.

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Today, parabiosis is performed carefully to reduce animal discomfort and mortality. "We observe the mice at length and have long discussions with our animal-care committee," says Thomas Rando, a Stanford neurologist who has used the procedure. "We don't take this lightly." Mice of the same sex and size are socialized with each other for two weeks before attachment, and the surgery itself is done in a sterile setting with anaesthesia, heating pads and antibiotics to prevent infection. Using inbred lab mice, genetically matched to one another, seems to reduce the risk of parabiotic disease. Joined mice eat, drink and behave normally — and they can be separated successfully.

In McCay's first parabiotic ageing experiment, after old and young rats were joined for 9–18 months, the older animals' bones became similar in weight and density to the bones of their younger counterparts[5]. More than 15 years later, in 1972, two researchers at the University of California studied the lifespans of old–young rat pairs. Older partners lived for four to five months longer than controls, suggesting for the first time that circulation of young blood might affect longevity[6].

Despite these intriguing findings, parabiosis fell out of use. Those who have studied the technique's history speculate that researchers thought they had learned all they could from it, or that the bar for getting institutional approval for parabiosis studies had become too high. Whatever the reason, the experiments stopped. That is, until a stem-cell biologist named Irving Weissman brought parabiosis back to life.

BACK TO THE SOURCE

Weissman learned to join mice together at the age of 16, under the supervision of a hospital pathologist in the small town of Great Falls, Montana, in 1955. His supervisor was studying transplantation antigens, proteins on the surface of transplanted cells or tissues that determine whether they are accepted or rejected by the host. Weissman remembers adding a fluorescent tracer to the blood of one mouse in a pair and watching it go back and forth between the animals. "It was really amazing," he says.

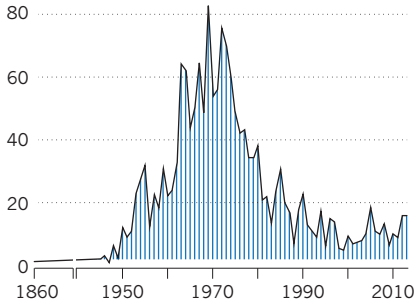
He went on to spend three decades studying stem cells and regeneration in natural parabionts, sea squirts of the species *Botryllus schlosseri*. In 1999, Wagers, then a new postdoctoral fellow in Weissman's Stanford lab, wanted to study the movement and fate of blood stem cells, so Weissman recommended that she use parabiotic mice and fluorescently label the cells she wanted

Share and share alike

Parabiotic experiments, in which two animals share a common bloodstream, were first attempted in the 1860s. By connecting animals with different qualities or conditions, scientists can investigate how blood factors, such as cells, proteins or hormones, influence health. In recent years, a few researchers have looked at heterochronic (old and young) mouse pairs to understand how young blood helps to repair many tissues.

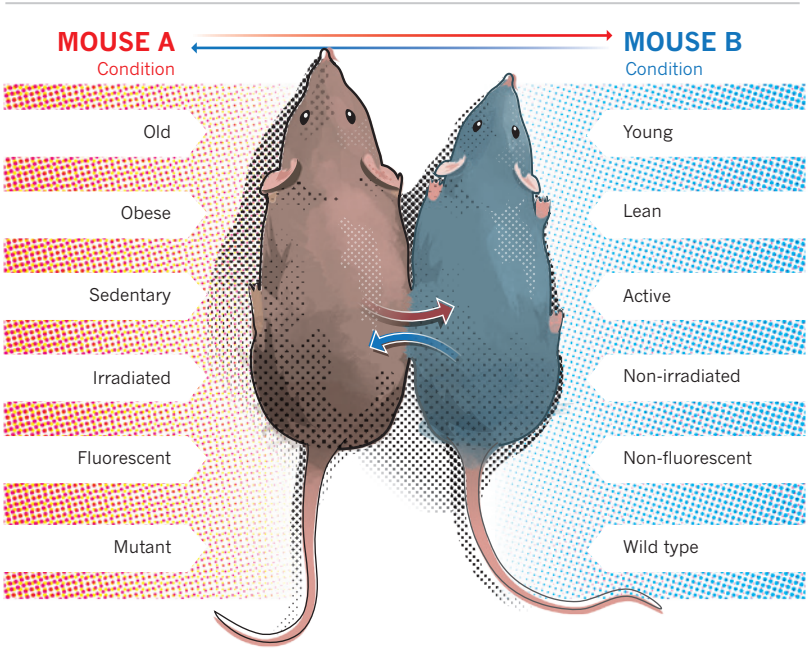
Publications on parabiosis

Parabiosis gained popularity during the 1960s and 1970s, but eventually fell out of wide practice.



A simple surgery

A veterinary surgeon will anaesthetize the animals, peel away a thin layer of skin along their sides and stitch or staple the exposed surfaces together. Wound-healing processes join the bloodstreams through a capillary network, and in one to two weeks, the animals are pumping each other's blood.



NIK SPENCER/*NATURE*; CHART DATA: A. EGGEL & T. WYSS-CORAY *SWISS MED. WKLY* **144**, W13914 (2014)

to track in one animal of a pair. Wagers' experiments led to two rapid-fire discoveries on the nature and migration of blood stem cells[7,8]. It also inspired her Stanford neighbours.

In 2002, Irina Conboy, a postdoctoral fellow in Rando's lab, presented one of Wagers' papers at a journal-club meeting. Michael Conboy, Irina's husband and a postdoc in the same lab, was dozing in the back of the meeting room.

The mention of stitching mice together jolted him awake. "We had been in discussion for years that ageing seems to be all cells in the body, that all tissues seem to go to hell in a handbasket together," says Michael. Yet they had been unable to think of a realistic experiment with which to investigate what coordinates ageing throughout the body.

"I thought, 'Hey wait, they're sharing blood,'" says Michael. "'This could answer that question we've been asking for years.'" At the end of the presentation, he ran up to Irina and Rando. He had not even finished his pitch before Rando said: "Let's do it."

The researchers teamed up with Wagers, who performed the old–young pairings for the experiment and taught Michael the technique (see 'Share and share alike'). Rando says that he did not expect the experiment to work, but it did. Within five weeks, the young blood restored muscle and liver cells in the older mice, notably by causing aged stem cells to start dividing again[9]. The team also found that young blood resulted in enhanced growth of brain cells in old mice, although the work was left out of their 2005 paper describing the results. All in all, the results suggested that blood contains the elusive factor or factors that coordinate ageing in different tissues.

After the team published its results, Rando's phone started ringing incessantly. Some of the calls were from men's health magazines looking for ways to build muscle; others were from people fascinated by the prospect of forestalling death. They wanted to know whether young blood extended lifespan. But despite the hints that this was true from the 1970s, no one has yet properly tested the idea. It would be an expensive, labour-intensive experiment.

Instead, members of the original research team branched out into separate efforts to determine what exactly in the blood is responsible for the rejuvenating effects. In 2008, Irina and Michael Conboy, by then at the University of California, Berkeley, linked[10] muscle rejuvenation to the activation of Notch signalling — which promotes cell division — or to the deactivation of the transforming growth factor (TGF)-β pathway, which blocks cell division. Then, in 2014, they identified[11] one of the age-defying factors circulating in the blood: oxytocin, a hormone best known for its involvement in childbirth and bonding, and already a drug approved by the US Food and Drug Administration for inducing labour in pregnant women. Oxytocin levels decline with age in both men and women, and when injected systemically into older mice, the hormone quickly — within a couple of weeks — regenerates muscles by activating muscle stem cells.

ALL THE ORGANS

Wagers was following up on the anti-ageing work at Harvard, where she had started her own lab in 2004. She recruited the help of experts in various organ systems to help her to evaluate the impact of young blood on their respective tissues. With neuroscientist Robin Franklin at the University of Cambridge, UK, her team showed[12] that young blood promotes repair of damaged spinal cords in older mice. With Harvard neuroscientist Lee Rubin, she found[13] that young blood sparks the formation of new neurons in the brain and olfactory system. And with cardiologist Richard Lee at Brigham and Women's Hospital in Boston, Massachusetts, she found[14] that it reverses age-related thickening of the walls of the heart.

With Lee, Wagers began screening for proteins that were particularly abundant in young blood but not old blood. One leapt out at them: growth differentiation factor 11, or GDF11. Wagers and Lee showed[14] that direct infusions of GDF11 alone were sufficient to physically increase the strength and stamina of muscles, as well as to reverse DNA damage inside muscle stem cells. No mouse

studies outside of Wagers lab have yet replicated the finding, but a similar protein in fruit flies extends lifespan and prevents muscular degeneration[15].

It is perhaps fitting that parabiosis' newfound popularity has spread among labs with close ties. Wyss-Coray, who worked in the room next to Rando's lab, had previously discovered prominent changes in levels of proteins and growth factors in the blood of ageing humans and people with Alzheimer's disease. Following up on Rando's unpublished brain results, he used old–young mouse pairs to show[16] that old mice exposed to young blood did indeed have increased neuron growth, and that young mice exposed to old blood had reduced growth. Plasma alone had the same effects. "We didn't have to exchange the whole blood," says Wyss-Coray. "It acts like a drug." Next, the team looked at overall changes in the brain, and found that young plasma activates brain plasticity and memory formation in older mice, and increases learning and memory. "We could not believe that this worked," says Wyss-Coray.

Neither could the reviewers. The first time Wyss-Coray submitted the work to a journal, it was rejected, he says, responding that it was too good to be true. So his team spent a year repeating the experiments at the University of California, San Francisco — a different facility with different staff, instruments and tools. The researchers got the same results. "After that, I was really reassured," says Wyss-Coray. "I'm convinced it works."

His research, published last May[17], caught the attention of a company in Hong Kong owned by a family with a history of Alzheimer's disease, which is characterized by neuron loss. One family member's condition had reportedly temporarily improved after they received a plasma transfusion. So the company put forward the initial funding to translate Wyss-Coray's approach to human clinical trials. Wyss-Coray formed a start-up company, Alkahest in Menlo Park, California, and in September 2014 it began a randomized, placebo-controlled, double-blind trial at Stanford, testing the safety and efficacy of using young plasma to treat Alzheimer's disease. Six out of a planned 18 people with Alzheimer's, all aged 50 or above, have already begun to receive plasma harvested from men aged 30 or younger. In addition to monitoring disease symptoms, the researchers are looking for changes in brain scans and blood biomarkers of the disease.

BAD BLOOD?

Wagers is eager to see the results, but she worries that a failure would be difficult to interpret and so could set the whole field back. Plasma from a 30-year-old donor may not contain factors beneficial to patients with Alzheimer's, for example. She, Rando and others would prefer to see testing for a specific blood factor or combination of known factors synthesized in the lab, for which the mechanism of action is fully understood.

There are also lingering concerns as to whether activating stem cells — which is what the young blood most often seems to do — over a long period of time would result in too much cell division. "My suspicion is that chronic treatments with anything — plasma, drugs — that rejuvenate cells in old animals is going to lead to an increase in cancer," says Rando. "Even if we learn how to make cells young, it's something we'll want to do judiciously."

Michael Conboy is concerned for another reason: he has seen enough paired mice die of parabiotic disease to be cautious about trying it in humans. "I would be leery" of any trial in which significant amounts of blood or plasma were transfused into an older person regularly, he says. Alkahest's chief executive, Karoly Nikolich, says that he understands the safety concerns, but he emphasizes that millions of blood and plasma transfusions have been carried out safely in humans.

The initial Alkahest study is expected to conclude by the end of this year, and the company plans to initiate further studies testing young plasma in the treatment of different types of dementia and age-related conditions.

All the caution over young blood is justified, given the history of dashed hopes in the anti-ageing field. In the past two decades, researchers have identified the anti-ageing properties of numerous treatments, including calorie-restricted diets; resveratrol, a chemical found in the skin of grapes; telomerase, an enzyme that protects the integrity of chromosomes (see Books & Arts, page 436); rapamycin, an immune-suppressing drug that extends lifespan in mice; and stem cells, which decline in function and number as people age.

> *"You often have these lucrative markets emerge on a slender foundation of credible work."*

Only two of these — caloric restriction and rapamycin — have been shown to reliably slow or reverse the effects of ageing across many mammalian tissue types, but neither has turned into an anti-ageing treatment. The former has produced conflicting results in primates; the latter has toxic side effects.

Young blood, by contrast, seems to turn back the effects of ageing, potentially with few known safety concerns in humans and, so far, with corroborated results from parabiotic ageing studies in multiple labs. But scientists and ethicists still worry about the treatment being tried in people outside approved clinical trials before evidence on its safety and effectiveness is in. Unlicensed stem-cell transplants are already a booming industry, warns Mattson, and unlicensed transfusion of young blood would be even easier.

"You often have these lucrative markets emerge on a slender foundation of credible work," says Leigh Turner, a bioethicist at the University of Minnesota in Minneapolis who has studied the anti-ageing field.

For now, any claims that young blood or plasma will extend lifespan are false: the data are just not there. An experiment to test such claims would take upwards of six years — first waiting for the mice to age, then for them to die naturally, then analysing the data. "If we had funding to do this, I'd do it. But we don't," says Michael Conboy. Still, he adds, "I hope that someone, somewhere is." ■

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