

BIOLOGY

# Germ-Free Guinea Pigs

## Animals and Plants for Laboratory Purposes Produced As "Bacteriological Blanks" by Young Scientist

By DR. FRANK THONE

**G**ERMS are the real cosmopolites, the true Citizens of Everywhere. There is no corner of creation where they are not found—except, perhaps, the molten lava lakes in the very throats of volcanoes. They float in the air, even up into the stratosphere. They swim in all waters, even the almost-boiling springs of Yellowstone Park. They swarm in the earth in unimaginable billions of billions. They cover all sticks and stones, all trees and lowly plants, all the works that the hands of men have wrought.

### Human Hosts

Most important of all, from our own egocentric viewpoint, they inhabit Us. They are on our skin and hair and teeth. They are in our noses and mouths and lungs. They are in our digestive tracts from end to end: before we get the good out of any meal we eat, our internal gardens of plant and animal microorganisms help themselves first. And what is true of ourselves is true of every animal in the world, from the mighty whale and the lumbering elephant to dainty humming bird and the humblest worm that creeps in the earth.

We have been too much in the habit, perhaps, of regarding germs or microbes

as always harmful. As a matter of fact, the great majority of them are probably harmless, and many kinds are known to be distinctly beneficial. But that is another story. Sufficient for present purposes is the regrettable fact that many germs are harmful, either by making us sick, or injuring our livestock and crops, or causing decay in our food and drink. And in spite of all the researches of the thousands of scientists who have worked on germ problems since the days of the mighty Pasteur, we are still lamentably ignorant of the nature of germs and of their ways of living at our expense.

A standard thing for bacteriologist or parasitologist to do, when he has a one-celled plant or animal suspected of mischievous tendencies, is to try it on a guinea pig. These docile little martyrs of science give up their lives in thousands every week, that our own lives may last the longer and be the more free from aches and ills.

### Unsterile

Yet whenever a scientist with a culture of germs decides to "put it through a pig," he is up against a dilemma. For his "pig" is already full of germs, in a most amazing variety, exercising effects so completely unknowable by present methods of research that the

scientist simply has to shut his eyes to them and pretend they are not there at all. A bacteriologist who would no more think of planting a culture of germs in a tube of broth or on a plate of gelatine not known to be completely sterile than an artist would think of matching colors on a slab of mud, will put his germs through a guinea pig, or rabbit, or other animal that is a regular walking universe of alien germs.

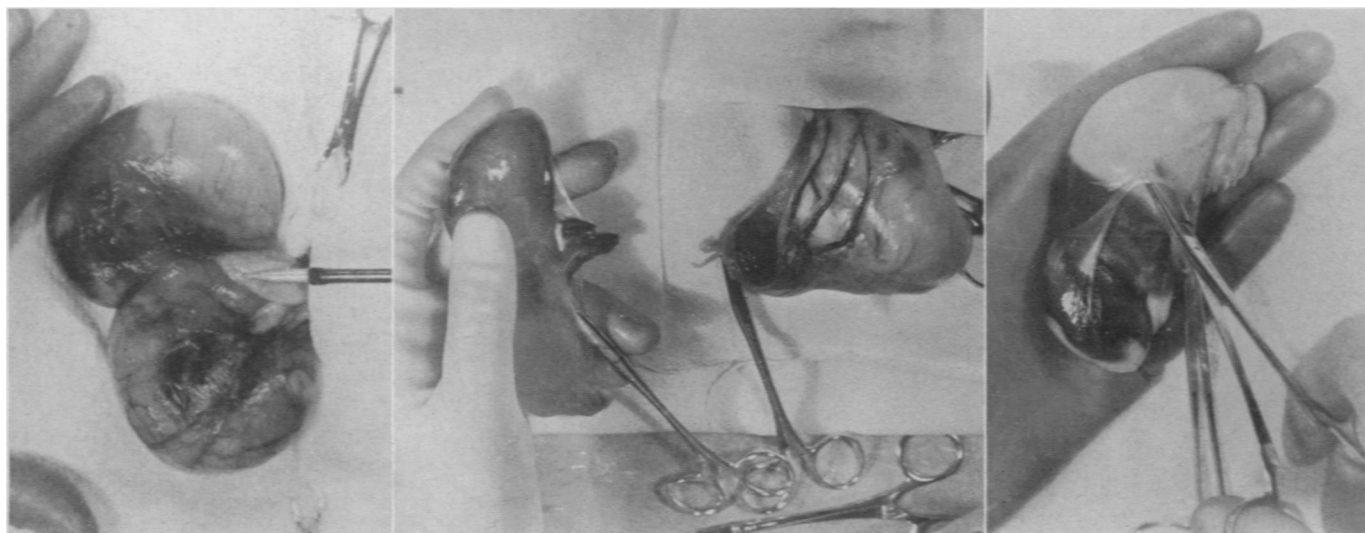
The only possible scientific defense of his paradoxical practice is that you can't get any guinea pigs that are as sterile as a well-steamed tube or plate of culture medium. If you steamed your "pigs" until they were sterile they would also be dead, and therefore useless for live-culture purposes. It is a case of a germly half-loaf being better than nicely sterile no-bread.

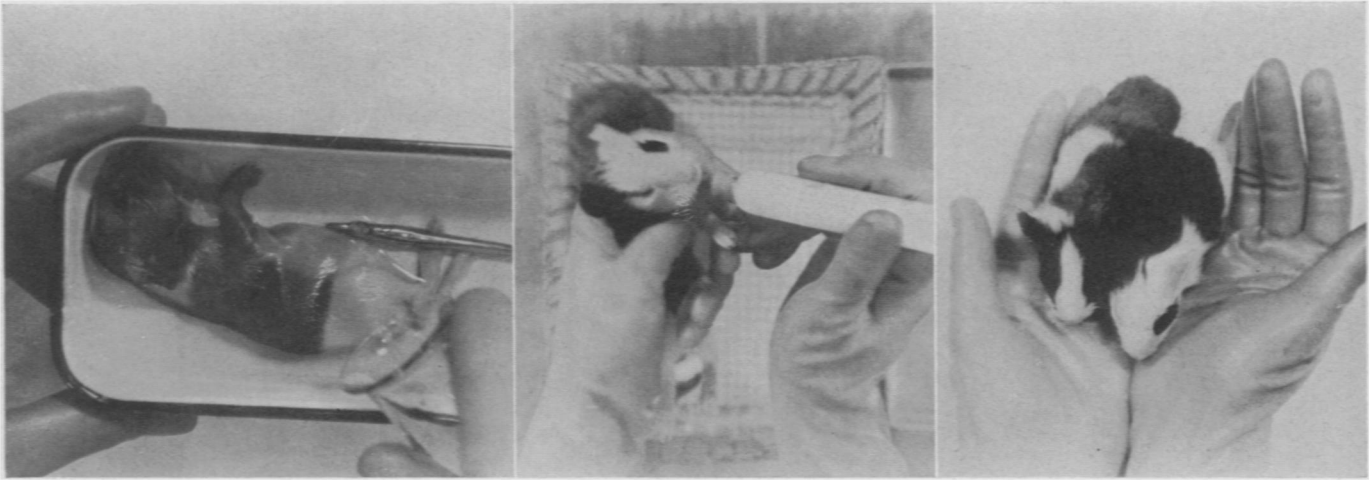
### Now Solved

But scientists are due to be relieved of this dilemma very soon. A young member of the Notre Dame faculty, Prof. James A. Reyniers, combining the mechanical planning of an engineer-inventor, the delicate-fingered skill of a surgeon, and the dogged determination that belongs to every true research worker, has developed a set of elaborate mechanisms, and perfected a technique

### CAESARIAN BIRTH

*Left: The uterus is brought into the germ-free air of the operating cage. Center: The umbilical cord is severed. Right: The membrane is quickly stripped from the baby animal.*





for their use, that will bring guinea pigs or any other experimental animals, within reason, into the world free from any taint of microbic contamination, will keep them germ-free through their whole lives, and will even permit germ-free parents to mate and bring forth germ-free offspring for as many generations as may be required. It is his ambition eventually to supply germ-free guinea pigs, or germ-free anything else that may be wanted, to biologists everywhere for research purposes.

### Unborn Are Free

Prof. Reyniers starts with the fact, long known to biologists, that unborn animals are usually germ-free. The chick in its shell, the developing mammalian fetus in its mother's womb, learn of the world's microbic wickedness only when they emerge into the light of day. They receive their initial contamination while they are being born, and with the first air they breathe, and in the first food they take. Bring them to birth in completely germ-free surroundings, and they will remain innocent of germs as long as you guard them well.

That sounds simple, but it is terrifically difficult to turn into accomplished fact. Yet Reyniers has turned the trick. Already he has reared, in his laboratory at the University of Notre Dame, over 2,000 germ-free guinea pigs, as well as germ-free chicks, rats, mice, rabbits, cats, insects, and several plant species. He works with guinea pigs mostly, because they are docile and easily handled. Cats are the hardest, he says; they fight, puncture rubber gloves with their claws, letting in germ-contaminated air and spoiling the whole job.

To get germ-free guinea pig infants from ordinary germ-infested mothers, you cannot let them be born in the

natural way. They would become contaminated at once. Therefore they must be brought into the world by means of the well-known Caesarian operation, performed under aseptic precautions that even human patients never experience. It requires an operating discipline of Prof. Reyniers and his assistants that combines the clock-like precision of a gun-crew with the surgeon's swiftness and sensitiveness.

First, a pregnant female guinea pig known to be within two or three days of delivery is selected. All her hair is removed with a depilatory, for hair is notorious as a lurking-place for contaminating germs. She is scrubbed clean, disinfected, sealed in a sterile Cellophane-lined envelope, placed on the operating board. All of this is done under a glass case, with careful aseptic precautions.

### Operating Room

Then the real operation begins. It takes place in a specially constructed metal cylinder, with an opening underneath through which the animal can be introduced. Cellophane covers this opening also. On opposite sides of the cylinder are pairs of arm-size openings, each with a pair of long surgeons' rubber gloves tightly gasketed in. This permits two operators to work, each of whom can watch through a glass-covered porthole on the upper side of the cylinder. The air inside is filtered free of germs, and the whole interior can be sterilized at any time by means of sprays admitted through permanently attached pipes. Everything is checked carefully for leaks before the operation starts, for a leak means contamination.

When the anesthetized guinea pig has been thrust up through the trap in the bottom, and lies under the stretched

### FIRST EXPERIENCES

*Left: The newborn animal gets a germicidal bath. Center: Sterilized milk is fed by hand every hour. Right: Ready to begin six weeks of germ-free existence.*

sheet of transparent cellulose, Dr. Reyniers makes his first incision. Instead of scalpels, electric needles are used for all cutting, because they automatically sterilize all tissue they separate, and also stop all bleeding. The edges of the protecting transparent sheet are sealed to the edges of the incision.

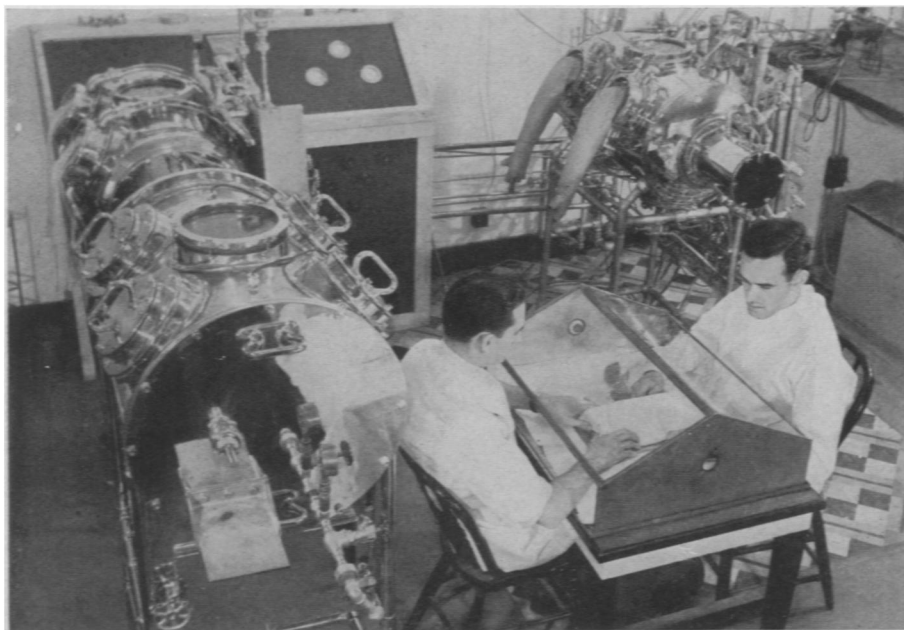
Swiftly the operators work. They lift out the uterus, containing the litter of unborn young. They open its side, removing the little animals one by one, each still enclosed in its protecting envelope, known to scientists as the amnion. In turn each amnion is opened, the little guinea pig slipped out, its umbilical cord clamped and severed, a germicidal bath administered, normal breathing stimulated if necessary. The little "pigs" have been born.

The mother may die during the operation, but this is not necessarily the case. Prof. Reyniers states that he has had female animals that produced second and third litters for him under his technique.

### Repeated Tests

At every stage during the operation, bacteriological tests are made: of the amnion as it is opened, of nose, mouth, all body openings of the young as they are brought forth. If signs of germ life appear at any stage, the whole laborious procedure is ruthlessly set down as unsuccessful, and the experimenters start over again.

After the whole litter, usually three or four little guinea pigs, has been born, a final drastic test for germlessness is



### THE OPERATION BEGINS

*Prof. Reyniers (right) and an assistant prepare a guinea pig mother for the Caesarian operation. At left, and in right background, are the germ-free, air-conditioned tanks where the little animals are born, live, and die in an aseptic world—all in the cause of scientific research.*

made. One of the new-born animals is instantaneously killed and its body reduced to hamburger in a meat-grinder attached to the inside of the operating machine. Samples of this guinea-pig hamburger are put into 22 tubes, each containing a different kind of germ food. If any germ is present anywhere in the animal, this test is calculated to show it up. If the "hamburgered" guinea pig passes this test, it is assumed that its brethren are all right also—at least at this stage of existence.

#### Sterile Food

Then the remainder of the litter is removed, through an opening in the end of the operating cylinder, into a second cylinder somewhat like it, but fitted out as a rearing cage. Again all air is made germ-free, and all water and food given to the young animals is kept rigidly sterile. They must be hand-fed, every hour, day and night, on a sterilized milk mixture, during the first five or six days of their lives. Every day a bacteriological test is made of every accessible body cavity. At the end of several weeks, another member of the litter is sacrificed on the altar of bacteriological precaution, via the meat grinder. If all these tests still show no germs, the remainder of the litter are removed into still another cylinder, the storage machine.

There, still on a regimen of absolutely

germ-free air, water, and food, the young guinea pigs grow up. At six months of age they are mature, ready for use in research experiments, or they can be mated for the production of germ-free young. Of course, if both parents are germ-free, there is no likelihood that the mother will cause the contamination of her own young at birth. The tedious Caesarian operation can therefore be dispensed with and the young born in the natural way, and suckled in the natural way afterwards. This should make for easier and more rapid production of experimental stock, once Prof. Reyniers is able to get on a quantity production basis. There appears to be no reason why germ-free animals should not be bred in indefinite numbers of generations, if due precautions are always maintained.

#### Expensive

The elaborate mechanical set-up necessary for the production of germ-free animals and plants has necessarily represented a great deal of labor and expense. The cost of one of the machines, on the present build-to-order basis, is equal to that of a rich man's automobile. However, if numbers could be ordered at the same time, the cost could be reduced to that of the average citizen's auto, Prof. Reyniers states. The governing officers of the University of Notre

Dame have been so impressed by his work and its possibilities that they have placed a new \$500,000 building at his disposal. Now he is looking hopefully about for the means to fill it with machinery for producing guaranteed germ-free guinea pigs.

There will be no lack of scientific work in the world for any number of germ-free animals he may be able to raise; he is confident of that. Research in filterable viruses, that cause such diseases as infantile paralysis, smallpox, and the common cold, can absorb thousands of such animals, for the viruses must have living hosts if they are to be grown successfully. And at present there are dozens of problems about them that cannot be solved with guinea pigs already loaded with other germs that confuse the results.

Research in immunology offers another large opportunity for the employment of germ-free animals. Again, the presence of unknown germ species in unknown numbers, infesting all possible experimental animals now available, makes it impossible to tell whether the production of a given antitoxin or other germ-fighting substance is a simple reaction to the invasion of the enemy germ, or whether the "resident" germs already present had something to do with it. If you can take an animal that has no germs at all, and inoculate it with just one kind of germ, then you have simplified the problem very greatly, and can hope for an intelligible answer.

#### Complex Causes

Allied to this second problem is a third, that still awaits solution. Are some diseases, perhaps, caused by several kinds of germs acting together, just as a cornfield may be ruined not by one species of weed but by several different species invading in succession? It has been suggested that the common cold is a disease of this kind. If you can start with a "blank" experimental animal, and give it only the kinds of germs you choose to let it have, there is a chance to get somewhere, out of the present confusion.

These are only sample research problems. Dozens more lift their heads over the horizon. If Prof. Reyniers is able to realize his ambition to be scientific godfather to thousands of germ-free guinea pigs, he need never be concerned lest his furry little protégés lack employment.

This article was edited from manuscript prepared by Science Service for use in illustrated newspaper magazines. Copyright, 1937, by Every-Week Magazine and Science Service.

Science News Letter, March 20, 1937