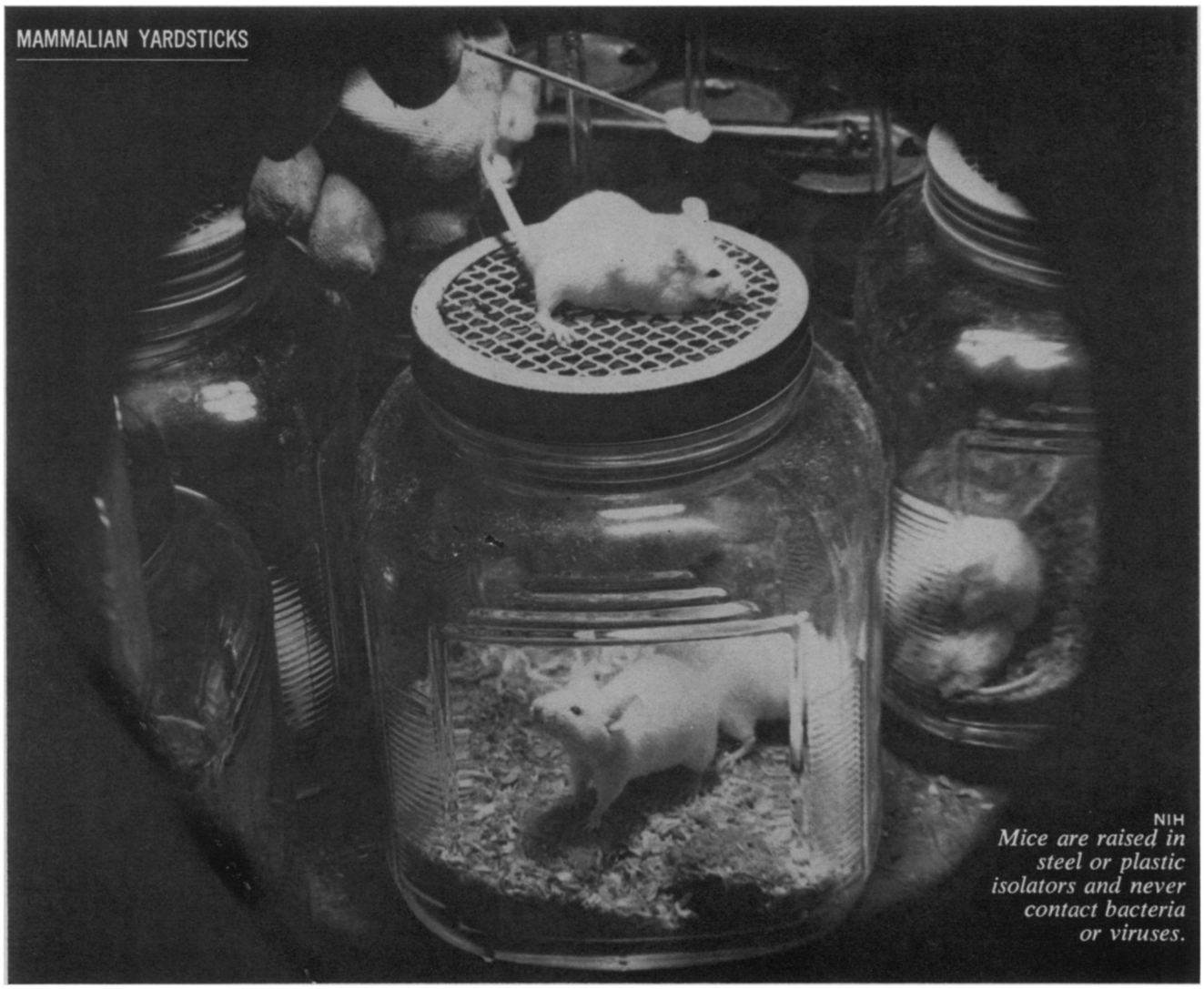

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Mice are raised in steel or plastic isolators and never contact bacteria or viruses.

Is clean living bad for mice?

Controversy has been stirred by a report that some germ-free animals, considered the ultimate in experimental controls, are malnourished

by Christopher Weathersbee

Reports by a Pennsylvania State University pathologist that germ-free mice, a trusted experimental tool, may occasionally be undernourished or downright starving (SN: 4/6 p. 334) have caused consternation among biologists.

It appeared after his announcement that much of the most advanced work in biology and medicine might be founded on faulty data. The mice are used frequently as the ultimate in mammalian measuring sticks; if the stick is off so is the measurement.

As the air clears, however, it seems that the potential impact of Dr. Richard L. Naeye's findings is less than it might have been. It appears also that Dr. Naeye is finding few colleagues working with germ-free animals who accept his results.

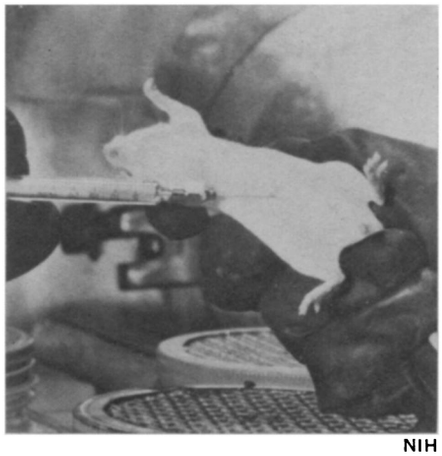
Germ-free animals have had as many biological variables eliminated as possible. They are born and raised entirely out of contact with any microorganisms, harmful or otherwise. And they are of a pure strain, inbred to make inherited traits as similar as possible. It is their monotonous sameness that makes them so valuable as standards or controls.

An experimenter is supposed to rest assured that when he injects a particular germ or performs some other test on a germ-free animal, the observed effect is due entirely to the test, not to organisms already present and certainly not to an abnormality of the animal such as malnutrition.

Germ-free mice are often used, for instance, in studies of immunity. Yet it is known that malnutrition affects the basic nature of the immune response. If Dr. Naeye's findings were to reflect a widespread occurrence, undersized mice might have been finding their way into—and invalidating—crucial experiments in immunology, in such critical fields as cancer research, transplant surgery and virology, to name a few.

It turns out, however, that much of the germ-free-animal research is done in large centers like the National Institutes of Health in Bethesda, Md. Such centers can support their own animal rearing programs and thus have resident experts able to spot any malnutrition and correct it. Neither NIH researchers nor NIH animal breeders have encountered undersized mice in recent years.

Nor has the University of Notre Dame's Lobund Laboratory run into



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Mouse gets injection in isolation.

any difficulties. Lobund pioneered germ-free research; at first undersized animals were a problem, but improved diet licked it. Dr. Morris Pollard, director of the laboratory, says some 12,000 germ-free animals are produced there annually, with no sign of malnutrition. He doubts that Dr. Naeye's mice are representative of germ-free animals generally.

Several researchers using large numbers of animals produced on commercial farms likewise say they have seen no trend toward malnutrition or small size in the germ-free mice they buy. They say that diet at the breeding farm might have a lot to do with the quality of the mice.

However, these researchers have to buy their animals from one of only three or four commercial breeders. One worker reports he was supplied by the same breeder that supplied Dr. Naeye, yet he found no malnourished animals.

A spokesman for the breeder says he cannot account for Dr. Naeye's findings. He says his firm has just completed a Government study of the condition of the germ-free mice produced there and has found them almost indistinguishable from normal mice in size, weight and organ condition.

Nobody could be found, in fact, who has run into the difficulties Dr. Naeye reports; several said they doubted the rigor of his methods and his experience with germ-free animals.

Dr. Naeye sticks by his guns, however. He freely agrees that germ-free research is only incidental to his main line of work. But he points out that all the animals were sacrificed within 24 hours of their having left the breeding farm, so experience in feeding and handling them at his end would not be of consequence.

He reports that he received 136 germ-free mice and 136 normal mice for comparison, all of the same inbred strain. They arrived in four batches. Two-day-old mice were 87 percent of normal weight: at 16 days old they were 67 percent of normal; at 28 days old 55 percent, and at 60 days old, 66 percent.

Furthermore, he says, these aren't isolated incidents. He says the literature on germ-free research, when it shows weights at all, often records abnormally low weights. He says one commercial producer told him that most of that farm's germ-free animals were undersized.

A possible cause of the trouble, he says, is the fact that diets for germ-free animals have to be sterilized. This is usually done by autoclaving the food. Heat, however, destroys several types of nutrients, notably some vitamins. For good growth these have to be replaced after the autoclaving is done.

Institutional breeders, rearing the animals for their own use, sterilize heat-sensitive vitamins by filtration and add them to the ration after the sterile food is in the isolator. This procedure is uneconomical for a commercial operation.

One breeder instead predicts how much of each heat-sensitive vitamin will be destroyed, then adds excess vitamin to the ration before autoclaving so that enough survives the heat for the animals' needs. It is conceivable that variables in autoclaving procedures, for instance, might occasionally result in a batch of vitamin-poor food.

Another suggestion is that some autoclaved diets might not be suitable for very young mice with immature teeth. Two-day-old mice would be unaffected by this variable because they still are suckling. The 28-day-old mice would recently have been weaned and would be struggling with a tough-to-bite diet.

Some improvement is noted in the 60-day-old mice, which might be expected to have better developed teeth and thus deal with the diet better.

Dr. Naeye, who ordered most of his mice young, notes that tissue analysis and inspection of internal organs of the germ-free mice showed a condition closely resembling the abnormalities found in humans and animals which are undernourished during their normal period of rapid growth. This observation, plus the fact that the mice tend to improve with age, lends some credence to the teeth hypothesis.