

Mack Fulwyler in His Own Words

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PROLOGUE

I met Mack Fulwyler only once, in 1999. We spent a week together in May at Hortobágy-EPONA, Hungary, during the Hungarian Society for Cytometry meeting run by Janos Szollosi. During that time, I had a wonderful opportunity to talk with Mack about the history of flow cytometry and why he had developed the things that he had invented. He told me many stories and I actually took notes! I also found out that the Smithsonian had made a historic video in 1991, in which Mack had been interviewed. I purchased copies of the eight videos made by Ramunas Kondratas and started to wade through them. I never made it to the end (14 h), but for some reason, just prior to Christmas in 2004, I came across the videos in my office and started watching them again. After wading through 10 h of tape, I came to the interview with Mack Fulwyler, which was conducted in the laboratory of Dr. Boris Rotman, at Brown University. What struck me was that Dr. Rotman had one of Mack's original instruments in his laboratory and it was right there in the video. At about 4 AM on the day before Christmas, I plugged "Boris Rotman" into Google and came up with a paper published in 2003, in which Dr. Rotman's email address was included. I emailed him immediately, and amazingly, he replied a couple of days later. It turned out that he was in the process of closing his laboratory and disposing of the instrumentation, which included an original Fulwyler sorter! He generously offered to donate it to us to preserve this valuable instrument.

All interviews (mentioned later) were carried out on June 28, 1991, by Ramunas A. Kondratas, in the laboratory of Dr. Boris Rotman, Professor of Medical Science, Brown University (currently Emeritus Professor, Brown University). The text is taken directly from the video and reproduced later.

BACKGROUND

In 1964, Marvin Van Dilla sent a letter to Boris Rotman because he was interested in work with fluorescent cells and he wanted to use living organisms in the instrument that he was building in Los Alamos (1). At that time, Dr. Rotman was working at the Syntex Institute for Molecular Biology at Stanford University, and had published a

paper (2) on the use of 6-hydroxyfluoran-13-D-galactopyranoside (2), a deoxy derivative of fluorescein. At that time, Dr. Rotman was working in Dr. Lederberg's laboratory. This was the first paper to describe the use of fluorogenic substrates to measure cellular properties. Marvin Van Dilla was particularly interested in this technique. Subsequent to the interactions between Dr. Rotman and Dr. Van Dilla, Dr. Rotman received the instrument shown in this paper [Fig 1], in 1967, when he paid \$5,000 plus the electronics that were purchased independently. The instrument included the cell sorting modules (which were never used in Dr. Rotman's lab) and the fluorescence detection module, UV light source, and associated electronics. In early 2005, Dr. Rotman donated the entire original instrument to Purdue University, where it is being held with a view to development of a major historic display.

In Mack Fulwyler's own words...

WHY DID YOU INVENT THE CELL SORTER?

"...It arose from less admirable motivation... When I first went to Los Alamos to work under Marvin Van Dilla, our primary interest was in monitoring the fallout from atmospheric nuclear weapons testing, that fallout appearing in meat, milk, and other food products."

"With the arrival of the atmospheric nuclear test ban treaty, the appearance of radioactive fallout in the diet and in humans diminished, so we had much less to do in terms of monitoring fallout. So our group, which consisted of four individuals, three physicists, and a physical chemist, looked around to see what we could do to assist the biologists who were the other 60-some people of the group. Marvin and I then became interested in exploring at first the Coulter counter, a device currently used in hospitals... A Coulter counter uses a small orifice in a glass tube. The cells are suspended on one side of that orifice

Based on material developed by Ramunas A. Kondratas, Curator, Division of Science, Medicine, and Society, The Smithsonian Videohistory Collection.

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FIG. 1. The Fulwyler instrument as installed in Dr. Boris Rotman's Laboratory in Brown University, immediately prior to disassembly in March 2005. The instrument had not been altered or moved since installation in 1967, except for the addition of a laser instead of the UV lamp.

and then sucked through the orifice via a vacuum. An electric current is also passed through that small orifice at the same time the cells are passing through and the voltage across the orifice is measured—as the cell passes through the orifice it obstructs the flow of electrical current and you measure the electrical signal that results from that—the presence of the signal says that a cell is going through, the amplitude of that electrical signal tell you the size, the volume of the cell going through. So the device allows you to run a sample with cells through it and to obtain a distribution histogram that represents the range of sizes of cells in that population.”

WHAT WAS THE DRIVING BIOLOGICAL QUESTION AT THAT TIME?

“We had a pathologist in our group who was using that device to analyze blood and he would adjust the aperture current and some other characteristics of the machine so that he could cause a small subpopulation of the red blood cell distribution to move away from the main distribution of red blood cells, and the pathologist thought that this represented immature RBC that had just been produced. Well, Marvin and I did not believe that that was the case, and so we set out to try to convince him that he was incorrectly using the device, and we were not successful in doing that.”

“It occurred to me that if I could just pick out what was thought to be this abnormal population, just physically isolate those cells, run them back through the Coulter counter, get the same distribution, it would demonstrate that this was an artifact that he was misusing the machine. So my motivation in trying to come up with trying to sort cells was to disprove this fellow's interpretation of data.”

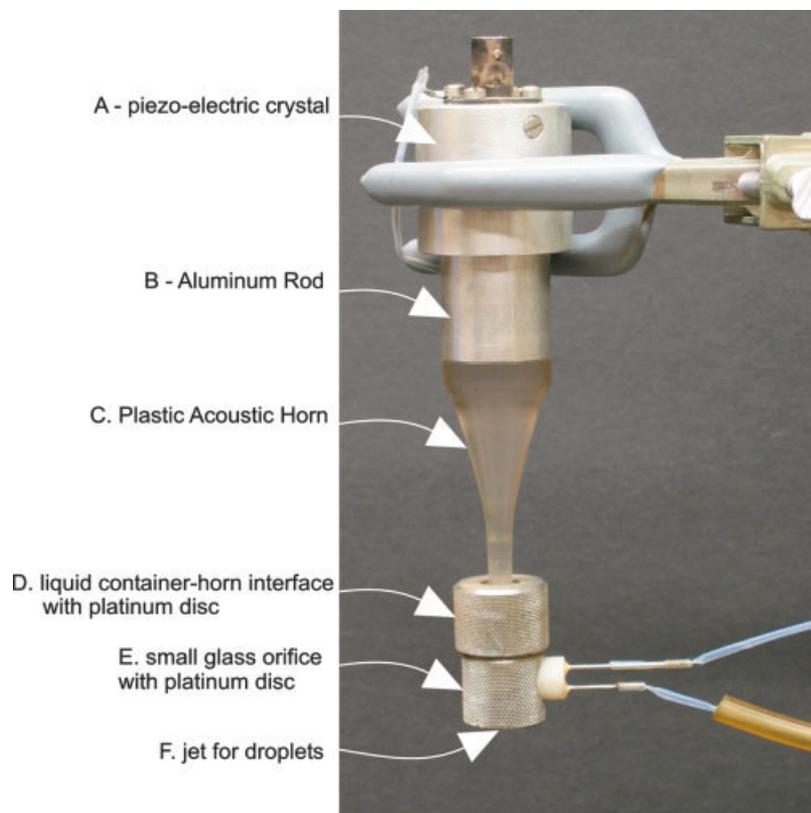
“At that stage, I set out to look for ways to physically isolated cells based on some electrically generated signal, and I started out looking at valves—mechanical valves that could be electrically switched and move a flowing stream of liquid from one channel to another channel and back, but when you look at that it's a very slow process and you could not process cells very rapidly.”

“Then I saw a paper by Dick Sweet, to develop an ink-writing oscillograph, a device to produces a jet of ink in the air. The jet is vibrated so that the inkjet breaks down into droplets. The droplets can be charged according to an electronic signal that is coming from something you want to analyze. . . The charged droplets then entered a deflection system where they were deflected, and then they impacted on a moving strip of paper that passed beneath the system. So these moving streams would ride out the signature of the electrical signal coming through the device.”

“I saw that as a way to move small amounts of liquid very rapidly, so what I put together initially was the idea of using a Coulter volume sensor that generates an electronic signal proportional to the size of the object, and this ink-writing oscillograph, which partitions the stream of liquid into small droplets which could then be charged and individually deflected. So I saw these small droplets as really just carriers for a cell, . . . as a means of moving a cell around. So that was the initial idea.”

“I put out a letter describing the process to a number of scientists in the laboratory (Los Alamos Scientific Laboratory) and I have forgotten the exact number, but I think it was about eight people I asked for their opinions as to whether the methods would work, and the majority came back saying no it probably wouldn't work, for various reasons. But under the encouragement of my boss, Wright Langham, and Marvin Van Dilla, I went ahead and tried it,

FIG. 2. This is a photo of the sorting chamber designed for Mack Fulwyler's instrument sent to Dr. Boris Rotman's laboratory at Brown University in 1967.



and made a trip out to visit Dick Sweet, . . .who was very cooperative, very supportive, thought the idea would work, and loaned me some of his devices for making ink jets.

"This device is the 2nd or 3rd version of my efforts to make a flow cell which would both measure Coulter volume and produce droplets. . . . One of the big problems was to isolate from the electrical measurements the effect of the acoustic energy vibrations in the system. And this is one of the ones that worked!"

"There is a piezo-electric crystal mounted in this part of the system [Refer Fig 2A], it's 1 inch in diameter and half an inch thick, and it's driven by the sine-wave generator and the amplifier. The vibrational energy produced by the crystal is coupled into an aluminum rod here [Refer Fig 2B] which rests right up against the crystal, and from the aluminum rod into this plastic horn [Refer Fig 2C], this is called an acoustic horn, it's a catenoidal shape, and the reason for that, is that . . .the range of motion resulting from the piezo-electric crystal is very small. So that this shape of an acoustic horn is like a transformer in that it increases the amplitude of the motions, even though a smaller amount of mass is moving. Within this portion, the end of the plastic rod is in direct contact with the liquid, so that you are coupling the acoustic energy right into the cell suspension, which then jets out a small orifice in a platinum disc down here [Refer Fig 2F]. The electrodes for the system using Coulter counter principle, inside of this is a large piece of platinum which is in contact with the solution and then there is a small glass ori-

fice, which is the volume-sensing orifice, then there is a platinum disc [Refer Fig 1D] in the end which establishes contact on the downstream side of the liquid jet. Coming out the platinum disc [Refer Fig 2E], then through a small drilled orifice of about 60 microns, you have a liquid jet which travels down for perhaps a centimeter or so, and then, because of the action of the piezo-electric vibration, it breaks into droplets [Refer Fig 2F]. This is all shielded, so that all of this metal here [Refer 2A, 2B, 2D, and 2E] serves the purpose of shielding the sensitive electrode from electromagnetic influences surrounding it." (Comment: Missing on the earlier mentioned system are the deflection plates and collection system.)

[The key papers published on this technology were initially in Science (3) and other sources (4-6).]

THE FULWYLER PATENT

"The patent which issued in 1965 covered the concept of analyzing a cell by any electrically convertible means and it specifically mentioned Coulter volume, fluorescence, radioactivity, means of sensing cellular characteristics, light scatter was one of them. . . .so the basic principle said any means of sorting objects, biological and non-biological, according to an electronically extractable measurement."

"At that time I worked for the University of California, which was under contract with the Atomic Energy Commission. Under that contract. . .the commission had first rights to all patents coming out of the laboratory, the uni-

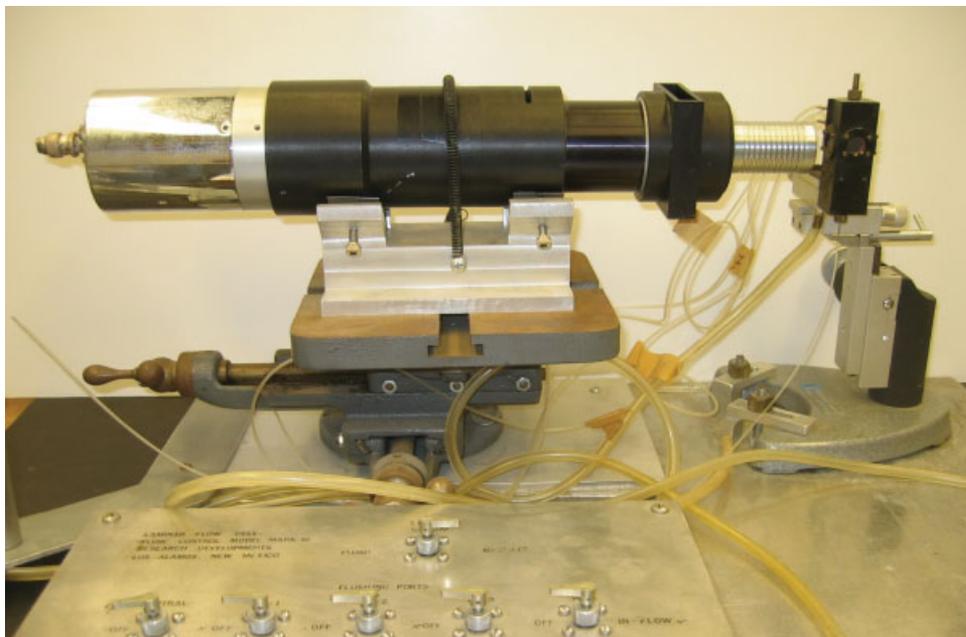


FIG. 3. The fluorescence detection unit which was used by Fulwyler and Van Dilla to collect cellular fluorescence signals in 1967. Top center is the single PMT, on the right is the flow cell. Below is shown the fluidics distribution unit. Not shown is the UV lamp unit that would have been positioned on the right side of the image.

versity had second rights to all patents coming out of the technology, The Atomic Energy Commission chose to take this patent, and at that point it becomes property of the US government, and any American citizen can get the rights to this patent at no cost. . .” (Editorial comment: Becton Dickenson licensed this patent.)

“Even though we were in a biological group at Los Alamos, we had a hard time in getting the biologists to really accept the idea of quantifying cell populations. The technologies, the sorting and the analysis, bring in the capability of analyzing whole populations of cells, hundreds and thousands, millions of cells, analyzing each of those cells, recording the measurements of each of those cells then describing the whole population in terms of percentages of each of those characteristics, and that idea was new to biologists at that time so it was difficult to get them to think in those terms. Normally, they think in terms of taking a flask full of cells and grinding them up and measuring an average value of some characteristics, so the idea of population studies was new.”

FIRST USES OF THE TECHNOLOGY

“Marvin Van Dilla had a heavy interest in DNA analysis, so our group and other supporting groups at Los Alamos began to look at DNA properties in malignant and non malignant cells. I had a National Cancer Institute project to apply the technology to the detection of cancer cells and to replace the Pap test. The first biological group who could clearly see the uses of the method were the Herzenbergs in the Stanford group, who could see the application of fluorescent sensing and the use of antibodies in the characterization of the immunological and surface properties of cells. So these were the first two heavy uses, DNA analysis and immunological staining.”

MARVIN VAN DILLA IN HIS OWN WORDS

“Really our group leader should get a lot of credit in all of this, because he not only got these two diverse types of people together but was very supportive and encouraging through the whole development, although the way he encouraged it was to come down to the lab and look at what you were doing and get you to plan it in detail and then shake his head and say, ‘That will never work,’ and that made you so mad that you would be damn sure to get it working and this was his style of management and it was great, although at the time it seemed irksome.”

USE OF FLUORESCENCE

“We originally started using cell volume or cell size that we were extracting from the Coulter idea, . . .and those experiments produced very illuminating results to a couple of the cell biologists around the lab, and a couple of them got very intrigued by the possibilities of high-speed measurements of cell distributions, and we got to thinking about this and came to the conclusion that if measuring the distribution of cell size was important and useful in cell biology that measuring other more important properties of cells, . . .for example DNA content in cells is a fundamental biological property of cells, and if we could measure the DNA content which would have to be done in a different way than using the Coulter principle, . . .if we could measure other properties, other cellular properties, like the cellular RNA, or cellular surface molecules, or cellular enzymes, it would be more important than just measuring the size of the cell, and so we got to thinking about all of this and came up with the idea that we should imitate the techniques that had been developed for fluorescence microscopy, in which you used fluorescence stains for cells on a microscope stage to light up a cell [Refer Fig. 3], to make a cell fluoresce under stimulation under blue or

April 30, 1968

M. J. FULWYLER

3,380,584

PARTICLE SEPARATOR

Filed June 4, 1965

5 Sheets-Sheet 1

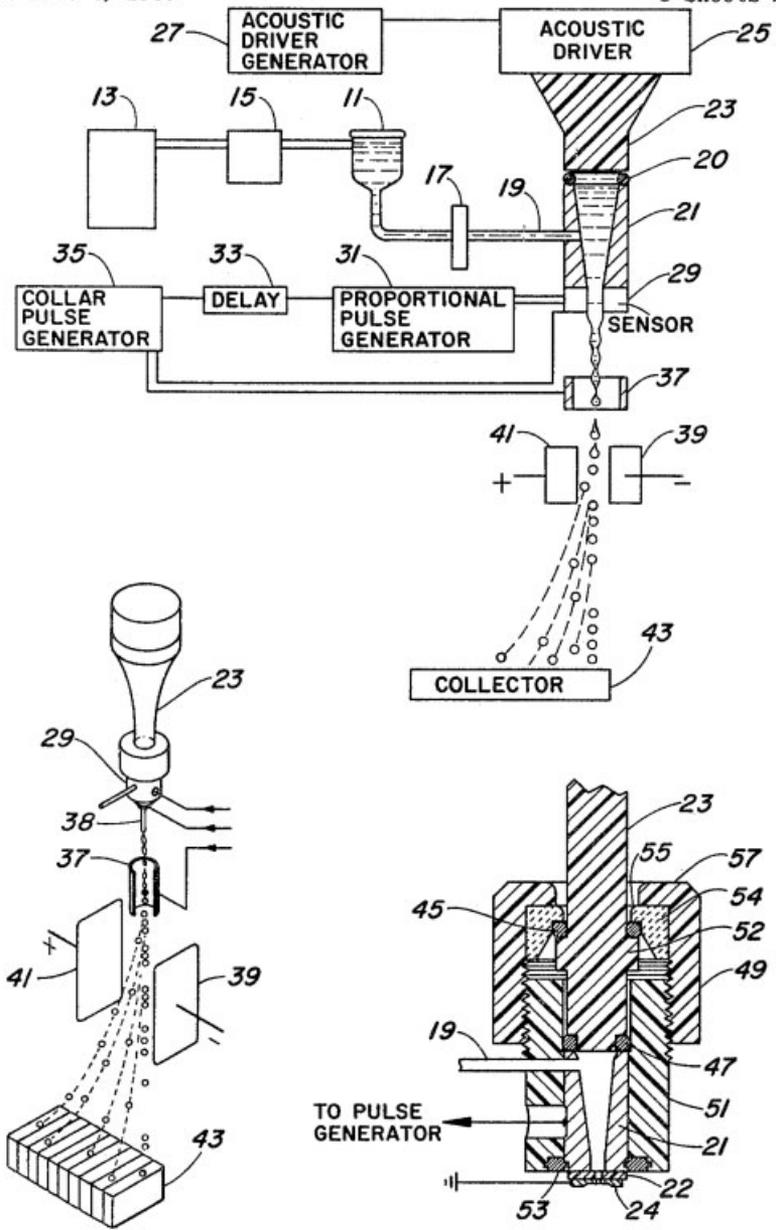


FIG. 4. A page from Fulwyler's patent on the cell separation technology patent #3,380,584 showing the fundamental components of the invention of the cell sorter.

INVENTOR,
Mack J. Fulwyler

BY
Robert A. Anderson
Attorney

Table 1
Mack Fulwyler Patents

	Patent no.	Date filed	Title
1	6,818,184	April 17, 2003	Capillary array and related methods
2	6,610,499	Aug 31, 2000	Capillary array and related methods
3	4,717,655	Oct 15, 1984	Method and apparatus for distinguishing multiple subpopulations of cells
4	4,526,276	Apr 28, 1983	Apparatus and method for sorting particles by gas actuation
5	4,499,052	Apr 30, 1982	Apparatus for distinguishing multiple subpopulations of cells
6	4,302,166	Mar 15, 1979	Droplet forming apparatus for use in producing uniform particles
7	4,230,558	Oct 2, 1978	Single drop separator
8	4,162,282	Apr 22, 1976	Method for producing uniform particles
9	4,148,718	Oct 19, 1977	Single drop separator
10	4,095,898	Jun 10, 1976	Particle analysis system with photochromic filter
11	3,989,381	May 5, 1975	Optical chamber with spherical reflective portion and apparatus employing same
12	3,380,584	June 4, 1965	Particle Separator [see Fig. 4]
13	3,710,933	Dec 23, 1971	Multisensor Particle Separator

UV light, and use fluorescence stains that give you information about important cellular components, and one of the important stains that had been developed for fluorescence microscopy over the years was called the Feulgen technique, in which you could make the DNA light up and fluoresce and give a nice optical signal, so we realized that optical sensing of cells was important to develop and so we set out to see if we could adapt the fluorescence staining of cells that had been developed for microscopy to this flow system. We decided to move on from the Coulter principle which was the initiation of all of this to optical measurements of cells, both the fluorescence of stained cells and the light that was scattered off the cells. So we got interested in both the light scatter and the fluorescence emission from stains that bind to important biochemical components inside the cell—DNA was the one we focused on initially.”

FULWYLER ON WALLACE COULTER

(In 1971, Wallace Coulter established a company in Los Alamos called Particle Technologies, which Mack Fulwyler ran from 1971 to 1976, when Coulter moved the company to Hialeah, Florida.)

“Wallace Coulter runs his company in a very different way from what most public companies are run, in that his motivation while on the one hand is to make money, he also runs the company with another objective which is intellectual satisfaction for himself. He likes to pursue ideas, he likes to try new things, and he would explore new ideas and new technologies much more adventurously than a publicly funded company where you have to justify to a board of directors or to a group of shareholders how you are spending their money, so it was unusual that I was able to do a fairly wide variety of projects.”

FULWYLER ON BECTON DICKINSON

“I then took a job as a technical director of B-D, a small group that was run by Bernie Shoor.

I left B-D in 1982 and took a position as professor in the Department of Laboratory Medicine at UCSE, with the hope that I would be able to sell some things to the funding agencies that could not be funded in industry.”

FULWYLER ON NIH FUNDING

“I found it much more difficult to get funding than I anticipated. Harder, in that my skills and capabilities that were really in innovating new technologies and new approaches didn’t fit very well with what NIH saw as suitable for funding. From my perspective, NIH was most interested in funding projects that were directed against a specific disease state, for example, or a specific area of basic research, and they were not particularly interested in funding a device or development of a technology, and I found it very difficult to get NIH funding for my research.”

FULWYLER ON FLOW CYTOMETRY AND QUANTITATION

“It’s bringing to those fields quantification that was not present before, and that quantification is a necessary step to convert it from an observational science to a quantitative science so it’s a step toward converting biology from a soft science to a firmer science, so I think that’s a major influence.”

“The development of quantitative methods of analyzing optical and physical properties of cells allows you a much greater range of things to ask. You can ask questions like ‘What is the fluidity of the membrane? What is the proximity of two binding sites on a cell membrane?’ You can ask questions like ‘How quickly does a substrate come through the cell membrane to be acted on inside the cells?’

“I see the future as development of more specific probes, probes for different cellular properties, probes that will analyze physiological functions, . . . more to seeing what the cell does, rather than how it looks.”

FULWYLER ON SPACE RESEARCH

“On the space project, here I have an opinion and that is that we shouldn’t put people in space, we should put robots, and save a lot of money, I don’t favor big expensive physics experiments like the Superconducting Collider—I strongly believe that that much money appropriately directed into a biological research program would yield much more social benefit earlier.”

FULWYLER ON NEW EMERGING TECHNOLOGIES

“I have a comment—there is a new technology called confocal imaging emerging. What makes that especially interesting now is that there are some emerging developing dyes that are specific for metabolites or ions. . . . With that technology you can look down inside a living cell, you can stimulate that cell with a compound or light or other external influence, and watch how that cell responds in terms of opening the cell membrane to the passage of calcium. . . . This type of technology can allow you to watch the interactions among cells within a tissue. . . . Now we should begin to talk about combinations of cells, communities of cells, and this is a much higher perspective and I think that this is an emerging area.”

POSTSCRIPT

Mack Fulwyler was truly one of the innovators in the field of cell analysis [Refer Table. 1]. I count it a privilege to have met Mack, albeit only once. Mack designed a tech-

nology that was not being demanded by others at the time, because it was not even thought of. Mack Fulwyler created a new technology and transferred a technology from the electrical engineering field into the field of biology. It was one of Mack's great desires to contribute to the quantification of biology. This he surely did.

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