

3-B. DISCUSSION AND INTERPRETATION OF THE CHANGES PROVOKED BY ZERO GRAVITY ON THE OTOLITH UNIT OF FROGS

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GERATHEWOHL: The next speaker is Dr. T. Gualtierotti, who is a professor at the Medical School at the University of Milan, Italy. He has been affiliated with Dr. Margaria, who is the head of the neurophysiology department there. Dr. Gualtierotti has been instrumental in experiments on aviation and space medicine for quite a while. I knew his name from the literature before we met, after he came to this country. Every time I have to introduce him, I do not know what more I should say in order to tell about his competence and his qualifications. I would like to point out, however, that he is going to report an experiment which is also unique. Dr. Montgomery's experiment is unique, insofar as the Russians have not attempted a microscopic experiment in a satellite. This is one where we are going to have a first in the biological sciences.

The Russians also have not attempted as yet to do an experiment of the caliber that Dr. Gualtierotti is going to do; namely, record action potentials from the vestibular nerve of a living being. Dr. Gualtierotti has studied with Adrian in England, and he has been instrumental in some of the vestibular experiments that Adrian has been doing, and he has developed ultramicroelectrodes for actual insertion in single vestibular nerve cells to record action potentials.

Now, I do not have to point out that the vestibular organ as one of the gravity receptors has been thought to be instrumental in orientation in space, also, of course, for orientation under nongravity or weightless conditions. Dr. Gualtierotti is really the first one to prepare an experiment of this sort to be conducted in an Apollo spacecraft. He is at the present time senior research scientist at the Ames Research Center, where he is particularly involved in preparing this type of research.

GUALTIEROTTI: I would like first of all to thank you, Dr. Montgomery, because you spared me the necessity of speaking further about the importance of gravity. I do not think anyone can add anything, either historically or physically, to the particular problem.

I would like to thank Dr. Gerathewohl, because he spared me the necessity of defending the experiment so far as importance is concerned.

I would like to add only one point: that it is obvious that as soon as we left gravity, or we left weight, the otolith part of the inner ear, which is directly concerned in measuring gravity, is the obvious organ which has to be studied first. It is like trying to study a completely dark environment and forgetting about the eye. So I do not think it was a very brilliant idea to start with that.

Now, I would like to make some comments about the stages and the problems that such an experiment had. The first very difficult problem was technical. Whoever is accustomed to working with microelectrodes, and recording from single fibers or single cells in a living human being, and especially in an unanesthetized living being, knows that it is very difficult. It is very difficult even when you deal with perfect laboratory conditions in a shielded room with a perfectly nonvibratory support, and so forth. So we had to revise our technique to be able to record from single units after the vibration and high acceleration of the takeoff, and we had to find a way of maintaining the recording for a long time. We are planning to record for 3 days, which is also a major achievement. I do not think that anyone up to now has ever been able to record from a single unit more than 6 or 7 hours, or 10 hours at the most.

I have reported on this technique several times in the literature, so I would like only to make a very brief visual summary of how it works.

The main point is the electrodes we used (fig. 1). They are ordinary tungsten micro-electrodes, with a tip about or below 1 micron used according to the usual technique. It is fixed by a screw to a piece of polyethylene tubing, which contains enough air to assure buoyancy, and to bring the entire system to the same density as the nerve tissue. This is the main point, that it has the same density as the nerve tissue and the environment. It will not be displaced by vibration or acceleration. It has exactly the same density; even at very high acceleration on the order of 100 G or 1000 G, it will not move. But we have proximity enough to stand up to 14 G in acceleration, and up to about 8 G vibratory acceleration.

Another point is that it is counterbalanced by a weight which brings the center of gravity in the center of the mass, so that it does not have any torque or momentum during vibration.

Another problem was that the electrode has to be floating. If it is attached to something, it will start vibrating or the structure underneath will move out from it, so it has to be floating, and the point was how do we place and leave the electrode in place after it has been

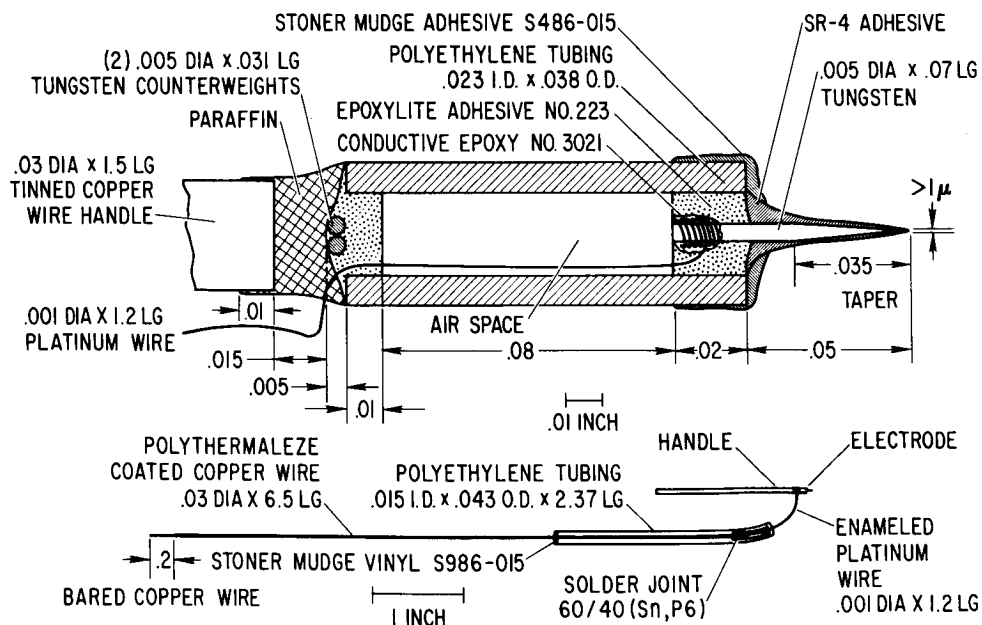


Figure 1. —Schematic of the electrode having the following characteristics: (1) same density as environment; (2) no vibration, the electrode being short and floating; (3) release from holder without displacement through dissolving the connecting paraffin drop.

stereotactically planted. How do we detach it from the stereotactic apparatus without moving it? We tried all kinds of different techniques, and finally found there was a very simple way. The main electrode is attached to the handle by a very small amount of paraffin. So when it is fixed in place, we just warm up the handle with a coil, without touching it, and the paraffin will dissolve, and the electrode will stay there without being displaced.

The electrical potential through the electrode is recorded through a very thin wire (about 1 mil) enameled platinum wire. It does not exert any practical pull or restraint on the electrode. So when the wire is in place, the electrode is completely floating in an environment of the same density.

Figure 2 gives an idea of the dimension of the electrode. We have to say also that there was another difficulty which I did not mention before when I made the previous presentation, but it is very important. The real reason why we could not get a long recording from a single unit, aside from injury or mechanical lesions, is that the actual current density on the tip of the electrode is very high, even with a very-low-current input in the high-input pre-amplifier that we use. For instance, we found out that a current of less than 10^{-13} ampere was enough to spoil our substrate after about 48 hours. So, of course, in a completely automated environment, we cannot adjust it as it is normally done in acute experimentation in the laboratory. We have to rely on something that has current just low enough to avoid this kind of destruction by current flow.

So we developed a solid-state preamplifier which has an input impedance of about 1000 megohms and has a current near to 10^{-15} ampere. Even in this case, the average duration of a preparation is about 3 or 4 days.

Figure 3 is the vestibular nerve of a frog; and it is very easy to get to it in the frog. We used the frog because it can stay in water, and immersion in water further damped vibration and helped the electrode to stay in place. It is very easy to reach the vestibular nerve, and we implant the electrode in the vestibular nerve to record single fiber activity.

Then we attach the frog to a tilting table (fig. 4), for otolith response, namely, response to tilting, to be sure that we are dealing with gravity receptors, gravity sensors. The gravity receptors in the otolith are divided into two groups. One group is more responsive to transient acceleration, and the other is sensitive to steady-state acceleration; namely, to the

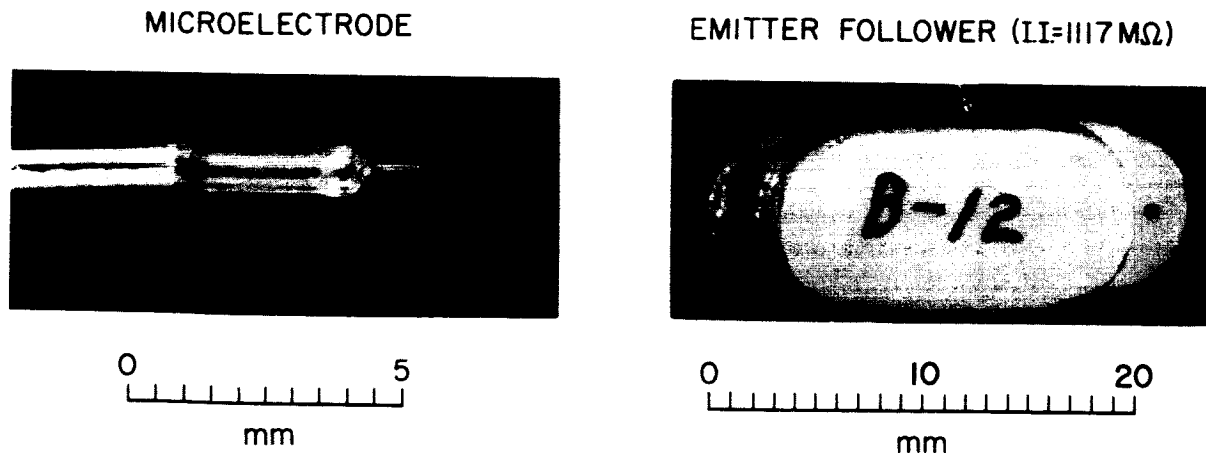


Figure 2. —The electrode and emitter-follower with the dimensions shown. The electrode is implanted stereotactically in the vestibular nerve and tested for otolith-type response on a tilting and rotating table instrumented with three-directional accelerometers.



Figure 3. —Surgical preparation of the vestibular nerve in the frog.

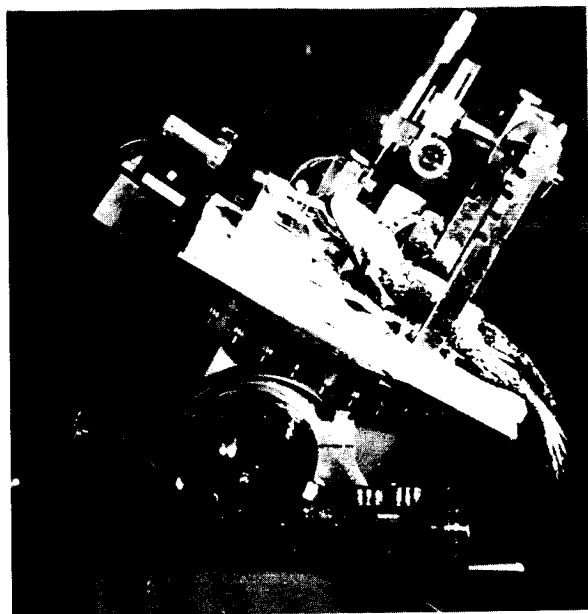


Figure 4. —The tilting table. Agar is poured in the hole, the microelectrode is released, the bone is reconstructed with dental cement, and wiring is connected to the emitter-follower fixed to the frog's jaw. The frog is then placed in a life-support system. The frog shown in figure 5, more simplified, was used for plane parabolic flight experiments.

gravity vector. So, I have been working mainly with these last ones. We will see an example of the two later on. In this way we can find out if we are dealing with an otolith gravity receptor, gravity sensor, and in which direction it will respond.

Once this is achieved, the hole is completely cut out, the bone is reconstructed with tensile cement, and the animal is placed in a special life-supporting system. Now, I have performed already a series of experiments on zero gravity or weightlessness during parabolic flight in a jet plane; and for that we had a more simple life-supporting system than the one used for the space flight.

Figure 5 is the package for the flight experiment, and for the experiment in aircraft it is completely filled with water in which the frog is immersed. We just supplied oxygen, flowing oxygen, without any means to take the CO_2 out, because the duration of the experiment was only about 2 or 3 hours.

Figure 5 shows the frog, the old model preamplifier, the one which is implanted in the frog; and in this case, the head of the frog is fixed on a holder. Leads for the EKG can be seen coming out and going into the preamplifier.

Now, for the space experiment, such a package would not do. First of all, we have a piggyback experiment and we are practically dealing with vacuum conditions. So we had to build a real life-supporting system more like a small satellite.

Then we find out that for the high acceleration and wide vibration range that we have on the liftoff, the fact that the head was fixed in a holder, fixed on the metal part of the life-supporting system, was not good enough. Then, the frog has to be really floating, although any angular displacement had to be avoided.

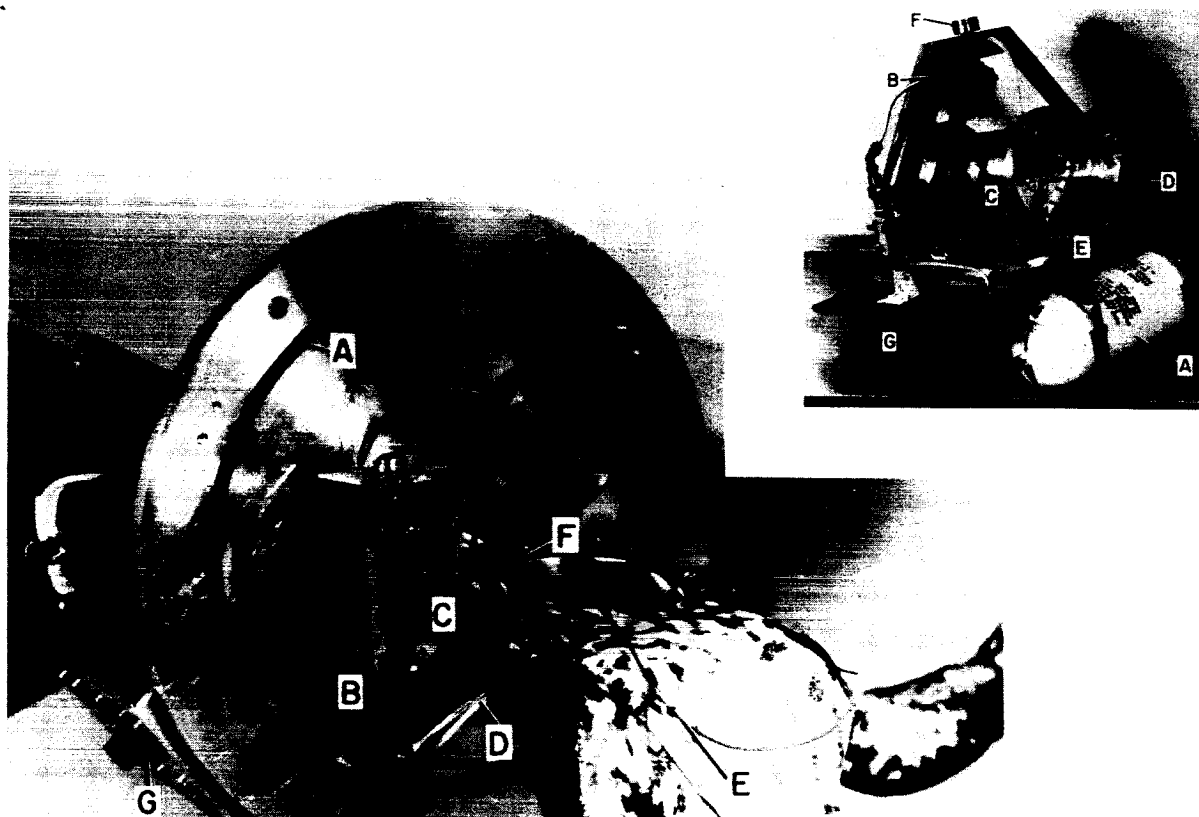


Figure 5. —Frog life-support system. For the experiment now in preparation, involving 3 days of space flight, a more elaborate package has been studied. Its main features are shown in figures 6-9.

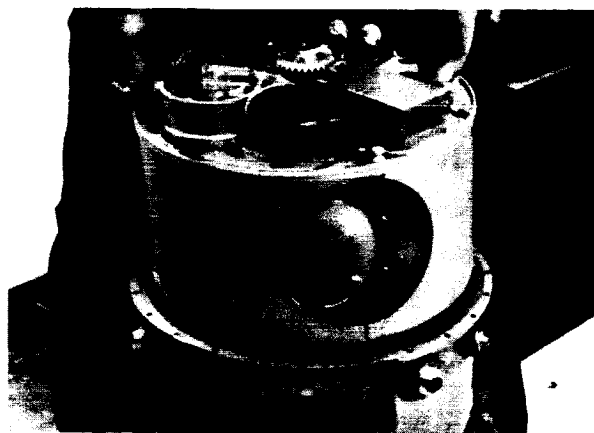


Figure 6. —Orbital otolith experiment package.

The package in figure 6 has been built by the Applied Physics Laboratory at Johns Hopkins University, and we have already completed the engineering model which is now undergoing tests.

Figure 6 is the outside or the outline of the engineering model. It weighed 86 pounds, and it is completely covered and self-contained.

We developed also a tester (fig. 7) for checking all of the parameters of this life-supporting system while it is already placed in the spacecraft. So with this, we can check one by one all of the different systems that will be illustrated later.

Figure 8 is the main schematic of the life-supporting system. We are planning to study not only the effect of weightlessness by itself on the spontaneous activity of the single unit of the otolith organ, but

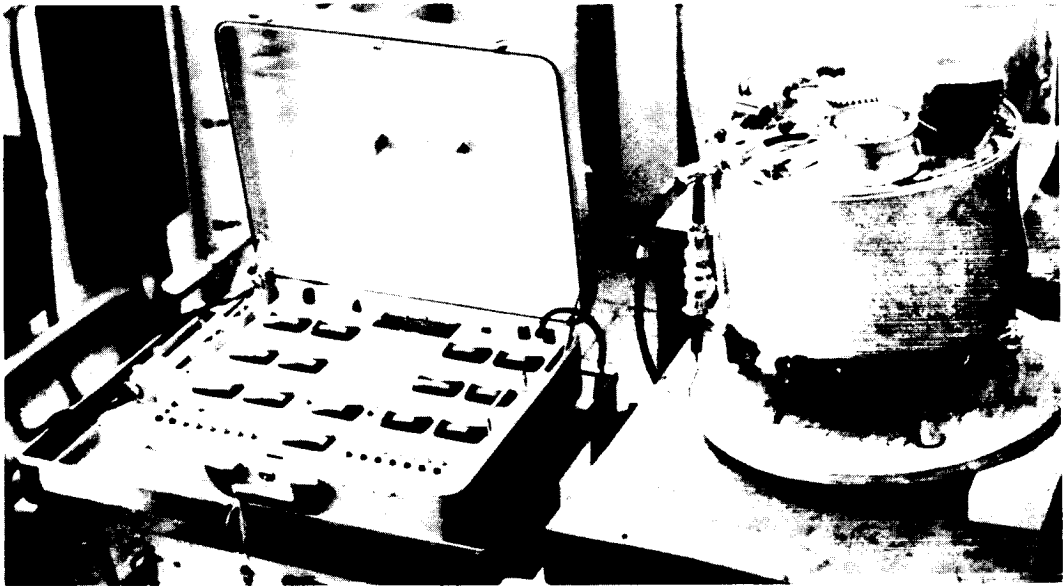


Figure 7.—Portable tester (left) and life-support system (right).

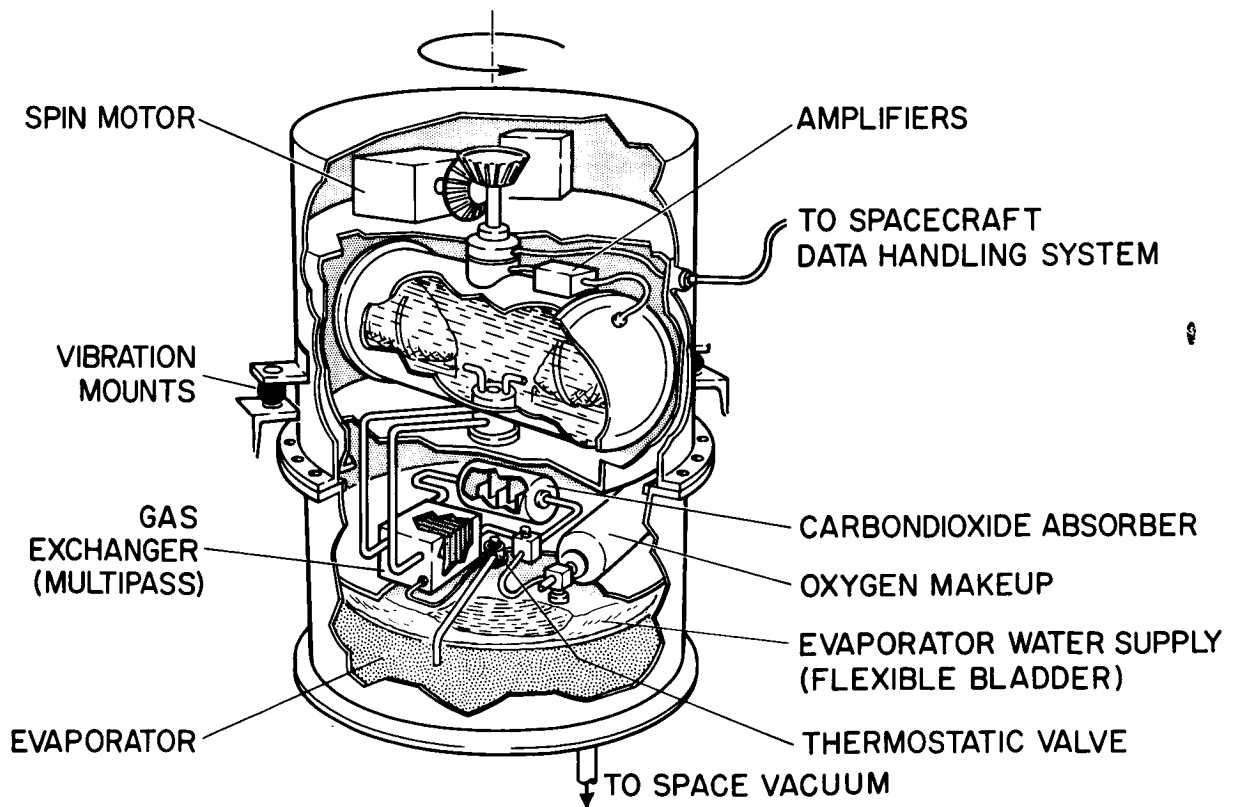


Figure 8.—Life-support system assembly with centrifuge, lung, and cooling system.

also how it responds to acceleration, both in the weightless state and as a result of the high acceleration during takeoff, and whatever further acceleration and vibration would be applied during maneuvers in orbit.

Therefore the package has a centrifuge-like device which is operated by the mechanism which allows a maximum acceleration of about 0.5 G, which is, for a single unit, just outside the maximum range. The content is shown in figure 9. It is filled with water, and a net sac which will contain two frogs. The electronics, the support for the main amplifier, is also shown in figure 9.

The water is kept oxygenated by means of an artificial lung which has been developed by GE. It consists of a very large number of very thin layers of silicon rubber which is permeable, selectively to CO_2 especially, and to O_2 , and it is a very good device. It absorbs oxygen from the air phase, the gas phase, and takes CO_2 out of the water phase. It was tested a number of times, and it seems to work very well.

The water is pumped through the system, and then the CO_2 is absorbed by the CO_2 absorber, and then is sent again into the chamber.

Temperature control was very important, not so much for survival, but to keep the response steady. A frog can survive at a proper partial pressure of oxygen at a temperature between 50° and 100° F. Since the frog is sensitive to temperature and acquires the same temperature as the environment, we get a change in the response of the nerve, or any mechanism in the body, as a function of the temperature. To keep the response steady, we had to keep the system at a fixed temperature, with a maximum range of plus or minus 3° or 4° F.

We found that the main problem was not so much the heating, but the cooling. So we have a cooling device which works on evaporation, with a store of water which is evaporated in vacuum. We had quite a problem before we developed the chamber, because in vacuum the water tended to become ice and clog the output. So now we have a chamber which assures pressure a little higher than the vacuum, the outside one, and then the vapor is discharged.

The entire device is mounted on shock mountings because there is a critical range in vibration between 250 and 400 per second, which probably is the resonance frequency of the electrode, which has to be avoided. So the shock mount is not there to absorb the acceleration as such, but to absorb that particular range of vibratory acceleration.

Figure 9 shows how the frog is placed in the container, floating completely free. The net is shown for illustration purposes only, but it is really all around the frog, and is attached at several points, so that it can move sideways, and it is fixed on the frog, too. The frog is eventually likely to rotate on his own axis, which does not matter much, because the otolith units are responding only to acceleration in this direction, and rotation would not change things much, but even that we tried to avoid as much as possible.

Figure 9 also shows the preamplifier, the input stage, fixed directly on the jaw of the frog, and on both sides we are planning to implant two microelectrodes each in one nerve, for each frog, so we will have four units just for safety purposes, and possibly for comparison, if we are lucky enough to have all four working.

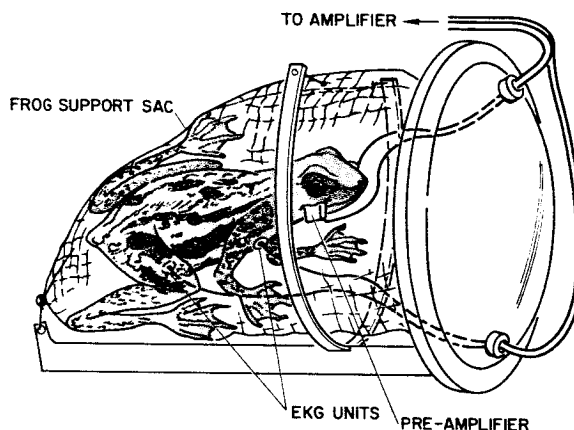


Figure 9. —Frog in net in proper position inside centrifuge.

The electrodes go to the main amplifier, and we have EKG leads for the control.

This solved nearly all of our technical problems, but we still had more problems. First of all, the physiology of the labyrinth is not very well known. This means we had to do basic biological work to find out what we have to look for as the response of the unit. By the way, I want to mention the fact that we had to deal with a single unit because it allowed us to play with numbers. I believe in numbers. So we can really elaborate the response as an equational function, or something like that, because in dealing with a single unit, we are really having a numerical output.

But what is the important, significant information of this output is still under discussion, so we have worked more than 400 of these units to find out just that.

I will summarize very briefly the basework under laboratory conditions, and then if we have time, I can discuss the results obtained during 25 plane flights, each of which had several 25-second periods of weightlessness, and see how they compare as baseline work for the space experiment.

The information in figure 10 has already been shown a number of times. It shows a typical vestibular unit response. This is what happens when the vestibular unit is not stimulated, namely, in a horizontal position, as far as the unit is concerned. Now, the horizontal position is only as far as the unit is concerned. The animal may not be horizontal at all, but the important thing is how the functional axis of the unit is directed. But as far as this unit is concerned, it shows continual activity, and then during tilting, there is an increased rate of firing. It is maintained for a certain period of time, although we do have some accommodation here.

There are two problems. First, as several other people have reported, in unrestrained, waking animals, as in this case, even in the vestibular nuclei, the discharge is very irregular. There is a certain irregularity. Therefore, the basic theory that the information may be some sort of frequency modulation system, or something like that, is really under discussion.

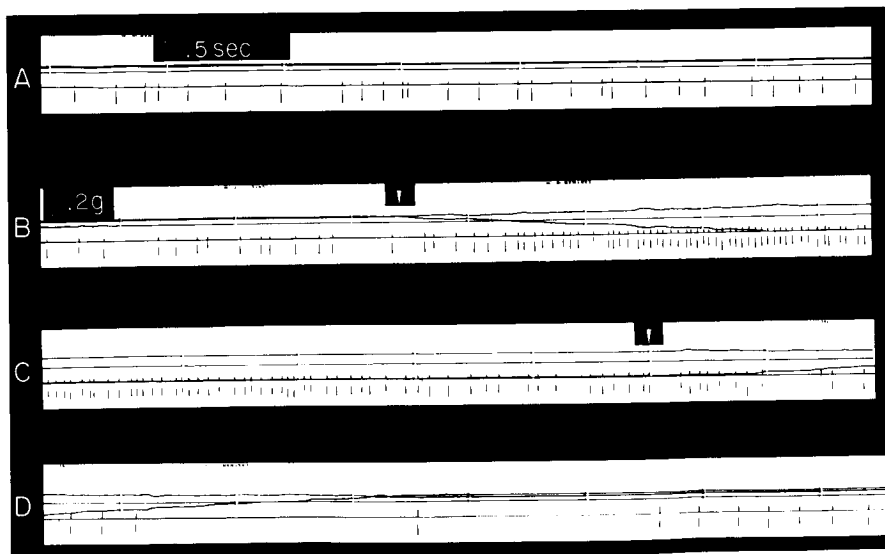


Figure 10.—Typical otolith response to tilting. Relevant information is time interval between consecutive pulses. Test of microelectrode stability indicates that preparation can be subjected to experimental routine shocks (plane flight or space flight).

By the way, the significant information in figure 10 is the interval between the different pulses. The pulses are all exactly the same in the same unit. So amplitude or duration of a pulse does not mean anything. The information is certainly provided by the time interval, by the duration of the interval between one pulse and the following one. Now, when these are as irregular as that, there is quite a problem, although it is obviously the kind of response that occurs.

Another problem is this. In this particular setup, we have quite a basic noise, which is provoked partially by the high impedance of contact, and partially by the fact that the electrode has a minute point. So the amplitude of the spike is not a good way to determine whether we are dealing with single units or more.

Another problem develops during a plane experiment. For instance, the entire half-hour flight would correspond to some 250 000 intervals, and 250 000 spikes, and I cannot go around looking for amplitude in each one of those, because I would still be here 10 years from now. But as I say, the amplitude is not a good index in this particular case, so we worked out a computer program.

Figure 11 is a vibration test, in an otolith unit. The vibration in this particular case is about 70 per second. We went all the way from 2 per second to 600 per second. But we did not have a shaker that could go high enough so that we could explore all of the vibration frequencies. But even at 4.5 G, in this particular case, we were actually able to record without too much noise and interference. At this high vibration level, even at this high frequency, this is valuable physiological information. We get a high response, and the unit is nearly sideways, although normally otolith units do not respond to vibration. But when you reach a particular high intensity of stimulation, you get quite a discharge from the unit.

After that we tested it again for 2 days, for normal response, and there was no alteration in the response as an aftereffect of this high-G vibration. To solve the first point, a histogram is made of a large number of events.

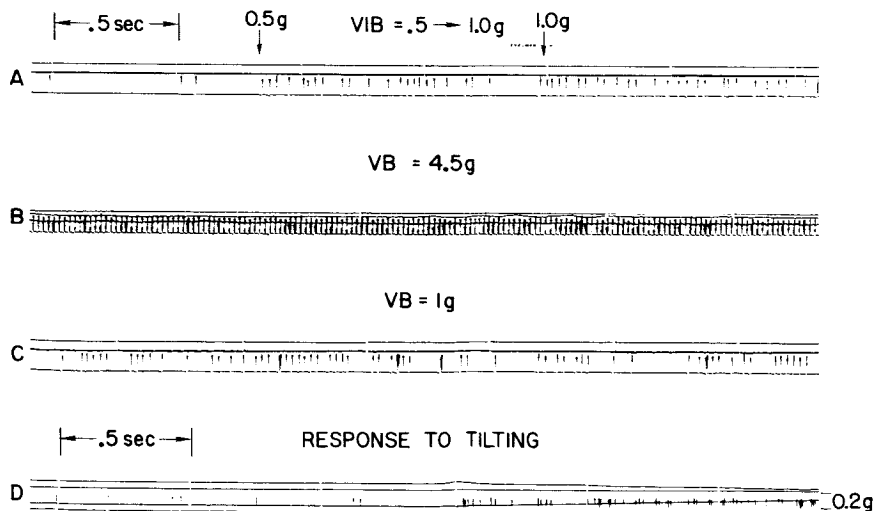


Figure 11.—Vibration tests. (1) It is not possible to use all the spike amplitude to determine whether a single unit is involved, owing to base noise artifact. (2) If a single unit is involved, discharge rate is very irregular. Sensory information cannot be conveyed through a pulse-interval modulation mechanism.

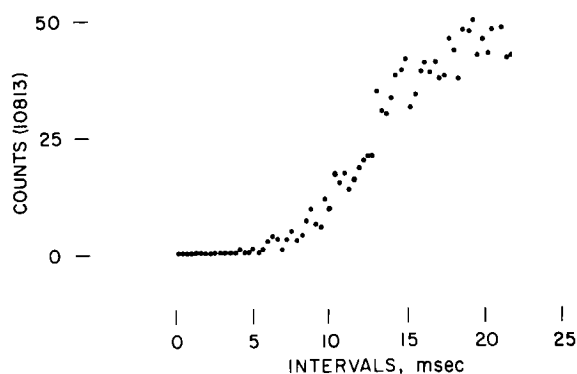


Figure 12.—If minimum interval is observed, a single unit is involved; even two units would cover all intervals from synchronization on.

The second point is a well-known factor in vestibular response. In alert animals, Bizzi, Pompeiano, et al. (*Science*, 145, 1964, 414-415), recording from the Deiters nuclei in the unrestrained, unanesthetized cat, found that the units discharged irregularly. Computer

analysis of response confirms this fact. On the centrifuge, we have something similar. Up to 10 G there is a fully separated response.

Now, how do we know this is a single unit and not a number of units, two, three, or more?

Figure 12 provides data on a very short time base for nearly 11 000 intervals. We worked all the way in the entire experiment in sections. In this way the intervals were classified in groups, by their value, and the onset of the histogram itself can be seen. If we had any minimum interval with such a high count, we were sure that we were dealing with one single unit. Also, if you have two units, the data would go all the way in a large enough number of times from complete synchronization to complete asynchronization. So it was possible to run the 200 000 intervals of the entire series, and find out that we were dealing with a single unit.

If we see some noise here, we discard that part of the record which shows that there is either noise or an additional unit coming in. For instance, in this case, this particular count, which corresponds to about three parabolic paths, shows that we did not have any second unit coming in or noise coming in.

This, of course, is due to the refractory period or to the time constant of the single unit. A single unit needs a certain time to recover before it is ready to fire again, and therefore this is the equivalent time. It is about 5, 6, 7—it varies from 5 to 12 milliseconds which, of course, corresponds to what is known on the time base of the unit.

Next, we measured directly the intervals and the acceleration. The intervals in figure 13 are measured as consecutive intervals, as the distance between the zero baseline, and each one of the dots. So there is the same scattering of data that is shown in the records, and this is increased activity during the acceleration. What happens mainly is that there is more concentration of data and all of the longer intervals are eliminated. There is the maximum interval. The maximum duration interval comes down to this level, while during spontaneous activity it goes up to this level. You can see some accommodation; namely, at the steady-state acceleration for this particular unit, it tends to increase its frequency. This is accommodation only. It is slowly accommodating, not as fast as this, but it shows accommodation. Then when you go back, you obtain the same scattering.

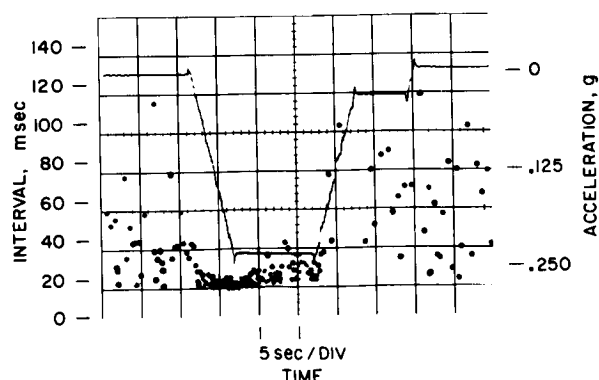


Figure 13.—Acceleration and time interval as function of time in a relatively fast accommodating otolith.

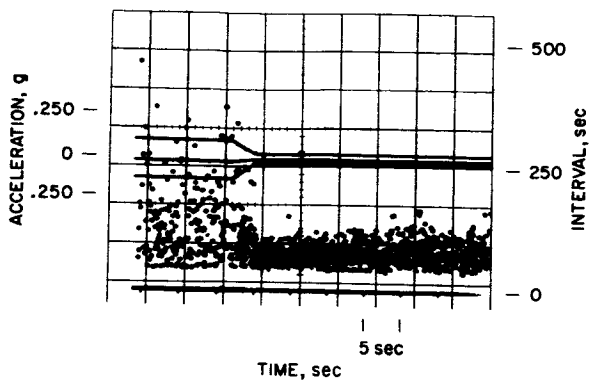


Figure 14.—Acceleration and time interval as a function of time in nonaccommodating otoliths.

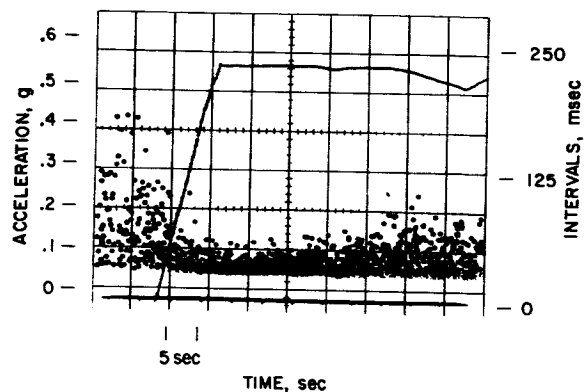


Figure 15.—Nonaccommodating otolith function sensitive to slight changes of acceleration.

Figure 14 shows three examples of a nonaccommodating unit, a unit that really responded. The unit in figure 14 responded to tilting in a direction which is midway between the two accelerometers. That is why there are two accelerometer changes, with a frequency of 5-second intervals. The frequencies tend to remain the same, or the change tends to remain the same all through the steady acceleration or gravity component that is supplied.

Figure 15 is a response to a different action of tilting with the same effect. It is sensitive. As soon as the acceleration changes slightly, immediately there is a corresponding variation in the character of the response.

UNIDENTIFIED: Is that interval plotted on your ordinate, as well as acceleration?

GUALTIEROTTI: Yes. Acceleration is here, and this is intervals. So this would respond to this curve, and this would correspond to this, to the intervals.

I think it is clear enough. Each one of the intervals corresponds to the distance from the baseline to each dot. We are dealing with a single unit, just by looking at this, because there is no firing below the line. When you have more than one unit, you have all sorts of intermediate intervals; except this is a limited number of intervals, even though there are quite a few.

At this point we can plot directly on the interval the changes provoked by the acceleration.

Figure 16 shows the relation between acceleration and interval. This is the range of spontaneous firing, and this is how it goes down during increased acceleration, until it reaches saturation roughly at this point. So this unit, for instance, has a range from zero to about 0.1, 0.15 G.

If we take the envelope of the two-dimensional figure, and we plot the curve, we have a nearly perfect biorhythmic relationship; namely, as far as the range is concerned, this unit seems to respond to this decrease of acceleration; i.e., it is in proportion to the amount of the stimulus. So it seems that the relevant information is more statistical than just based on a possible duration. Our theory now is that this unit works on a statistical basis by changing the range of the intervals that are present in the activity.

Now I would like to show that there are otoliths of different ranges. This one, for instance, I would say it is about 0.15 G. There are some units that are extremely sensitive.

Figure 17 is $G/100$ on the scale—this otolith responds to a fraction of a hundredth of a G ; although we have still the same function, we have a sensitivity in the entire range covered by less than $0.01 G$ on the order of $0.005 G$, or something like that. We have quite a few of those, although most of them are on the order of $0.2 G$, and some are of a little longer range going up to $0.7 G$. I have not been able to find in the 400 units which I have studied up to now a unit that would respond to the entire range from zero to $1 G$, although there are some units which we have seen during the plane flight where they have responded above $1 G$, from $1 G$ to 1.2 , or 1.2 to 1.4 . This sounds strange, but is probably not so strange because, after all, sometimes the impact of landing after a flight would cause it.

So this is the basic work that has been done for finding out how to treat the data, and what is the basic information.

All of this has been made for transients or change in acceleration. We did similar work for steady-state acceleration. Figure 18 shows this.

During steady-state acceleration, there is a steady change, an alteration that remains at the different levels of excitation. To study that, we make histograms of a sufficiently large number of data at each different level of excitation. For instance, figure 18 shows the accelerometer output, the main change in this one. Figure 19 shows the histograms during no excitation, with a very large pattern. There is also an intermediate value of excitation, and a maximum or supramaximum value of excitation.

Now, there are two main changes. First of all, the tail of the curve disappears. There are no intervals above this point. Included are interval duration, interval time, and the number of events. I have chosen roughly the same number so that we can compare it directly with the histograms. The change at steady state is a change in the tails; namely, the longer

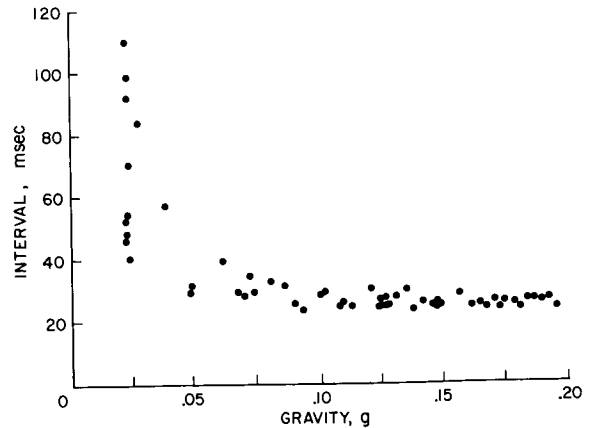


Figure 16.—Time interval as a function of acceleration in an otolith of average sensitivity indicates logarithmic function.

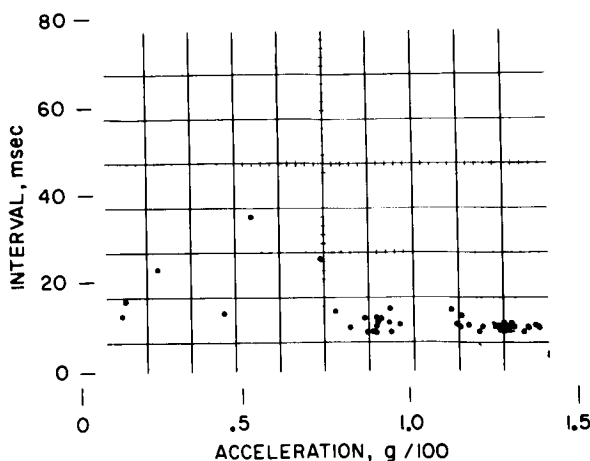


Figure 17.—Time interval as a function of acceleration in an ultrasensitive otolith. Given marked irregularity, the response consists of a progressive elimination of the longer intervals. The envelope in figure 16 and this graph indicates a logarithmic response to stimulus. Function according to the Weber-Fechner law. The otoliths which show nearly no accommodation are to be considered true gravitoceptors, namely, they respond to steady-state acceleration. For study purposes, histograms at different levels of excitation may be used.

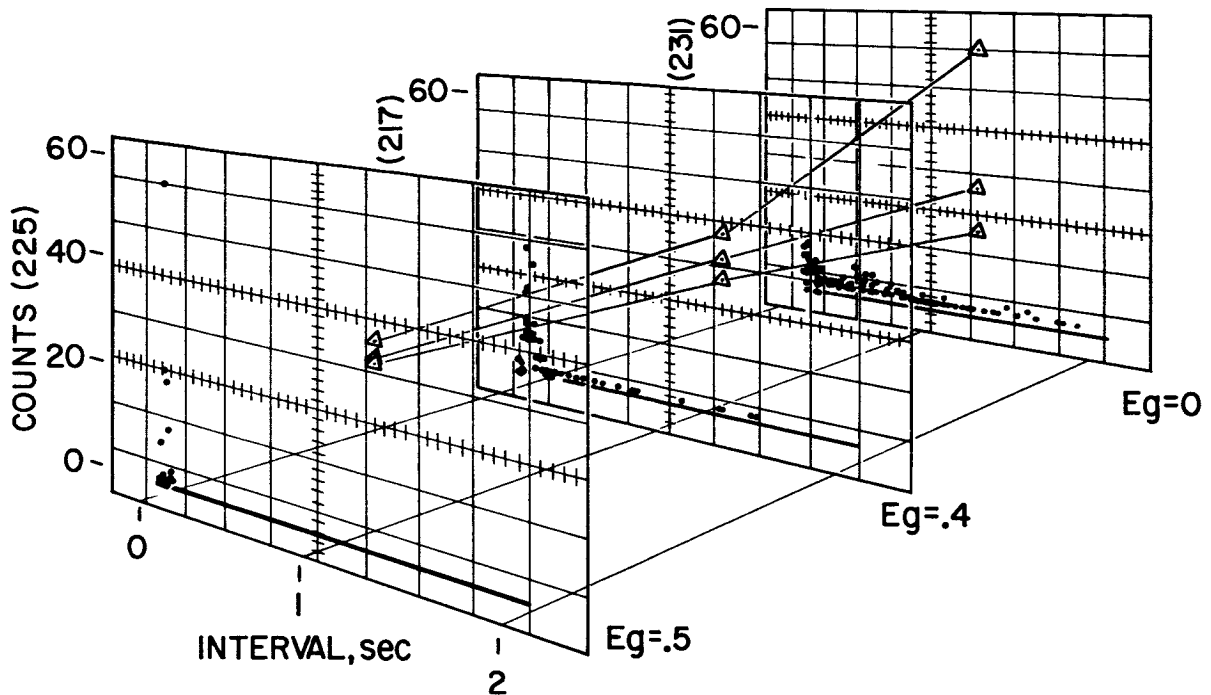
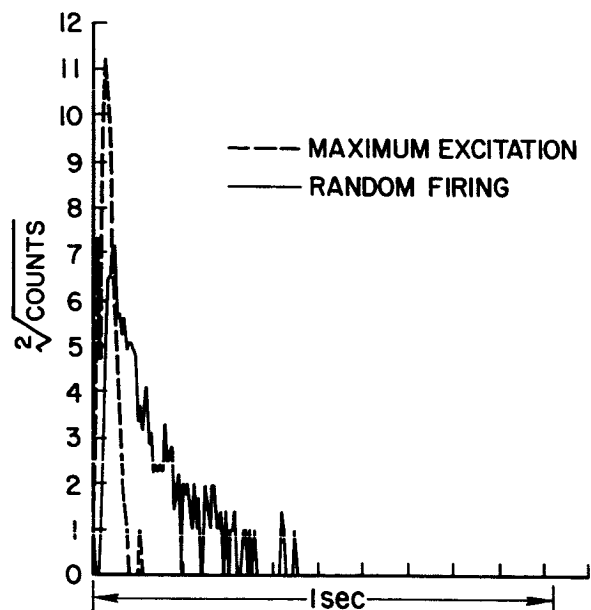


Figure 18.—Accelerometer output at steady-state acceleration. The results shown permit a discussion about which is the information as far as the centers are concerned.

Figure 19.—Two extremes of steady state: no excitation (spontaneous firing) and maximum excitation. Two characters only have evidently changed significantly, as can be seen from the shortening of the tails and the concentration of data, indicating that the organ seems to work in a statistical fashion.



interval disappears. Second, we have a high concentration later. In this case, for instance, most of the data, nearly 60 percent, or more than 55 percent of the data, are at this particular value, whereas in this case, for the same number of counts, you have a more even distribution.

Now, we are working on what kind of distribution it is. We still do not know yet.

The point is this: I wanted to go into detail at this particular point, because that is really the basic study that was necessary for preparing the space experiment. So now we have developed the technique, we know what to look for, we are still studying on some particular points of this interpretation, and I have no time to tell you what happens during the plane flight, but I can say that we have definite changes during the plane flight.

There are at least two major changes. First of all, the sensitivity of the same unit is higher. We compared the response to the same test acceleration during level flight as we did immediately after the parabolic flight. We have some sort of inhibitory effect during 0 G that lasts throughout the 0 G period. Of course, we have to distinguish two main different units. The one that responds at 90° from gravity, which is not directly affected from the lack of weight, and the other which, on the contrary, responds in the direction of gravity. We studied both in a significant manner.

I think I will report a complete discussion of this at the seminar we will have at Ames in January.

DISCUSSION

GERATHEWOHL: Are there any questions in conjunction with the Gemini bioscience experiments?

LEVIN: In the experiment in which the bacteria or other life forms were exposed to the space environment to check survival, and so forth, what measures were taken to prevent these organisms from escaping into space? How could you expose them to space without risking contamination?

GERATHEWOHL: I am sorry I cannot answer this question, because I am not familiar with Dr. Hemenway's technique. The only thing that I can say is that Dr. Hemenway has already done similar experiments in conjunction with the Air Force program on high-altitude rockets, and he has published some of his results. I think, in his publication, he describes his technique, and this would answer your question. I do not know how he puts them in any kind of box and prevents them from escaping other than by either embedding them in a certain type of medium which carries them, or by putting something on top of it, perhaps Plexiglas. As a matter of fact, Dr. Hemenway and his associate who does the biological experiment have already published the results; and they found there were some micro-organisms which they had exposed which survived. Just to review his results very briefly, there were very few specimens of the micro-organisms or spores which survived. That is one result. The second is the most deadly means for killing any life under these conditions is ultraviolet light. They found this in the laboratory and also again under the conditions of space. I think you can find this in Dr. Hemenway's publication.

MARTON: Dr. Montgomery, I understand that the primary thing you are looking at is the effect of weightlessness on human cells' response, and if I understand the Apollo mission correctly, there will be extensive navigational midcourse correction as well as thrusting from the circumlunar to the other orbit, as well as the acceleration for rendezvous, and the accelerations for the three men moving around in their cabin, and this would probably create G-vectors that are not only of significant value, but of perhaps significant duration, so I wonder if you are taking any sort of procedures to at least assess when they occur, how frequently, and for how long, so that you can equate these to possible differentials in your analysis.

MONTGOMERY: I think it is evident that in any satellite flight you are not going to have exactly weightless conditions at all times during the flight. We expect to get flight-profile characteristics back as nearly as they can be provided for us, and these we can put into our control experiments on the ground. Other than that, since this is a piggyback experiment, there is not any way we have to control those features. But this Apollo mission is not oriented toward the Moon, and we hope to find out what 0 G does to human cells during an Earth-orbital flight.

LAWTON: Dr. Gualtierotti's data suggest that the most sensitive otolith single cells that he has identified are sensitive to about 5×10^{-2} G.

GUALTIEROTTI: Yes.

LAWTON: 0.05 G?

GUALTIEROTTI: Not 0.05; 5×10^{-3} .

LAWTON: 10^{-3} ?

GUALTIEROTTI: Yes. There are very few of them, and they are being confused, I think, in the literature with in-and-out units. It has been described by Löwenstein and other people that there are two kinds of vestibular units, one that responds gradually to acceleration, and that is to respond as a function of the amount of displacement of the head, and the other one just responds to going in or out from the basic position. Now, I do not agree with that on the basis of my results, because the two units respond to the same characteristics. The only difference is the range. We have all the intermediate ranges, really, if you explore a large enough number of units in the same nerve. I have been able to explore up to 40 units in the same nerve, and have been able occasionally to find a unit responding to that high sensitivity, and a unit responding to intermediate value up to 0.2 G.

Another point is this: that all the experiments that were done up to now in biology have been done on acute preparations with anesthetized animals. Only recently they started using so-called chronic preparation, and really chronic preparation. They found out that it takes about 12 to 15 hours, and sometimes even 20 hours before you get a steady response. The response changes because the nerve first goes up, and then it becomes normal again, so there is some injury effect that disappears, provoking a constant response after about at least 15 hours.

So all of this, of course, shows that we might just have different values in the sensitivity of the system. We have to know that for our plane flight, because I am planning to use a long-range unit, and not a short-range unit, because as the sensitivity increases with 0 G, it would just become impossible to study it.

SEADAH: Dr. Gualtierotti, I wonder if you would comment on the vestibular physiology as it relates to radiation and, more specifically, on changes possibly in all of its functions as a result of the radiation.

GUALTIEROTTI: There is some interesting work from which it seems that radiation might directly destroy part of the otolith receptors nearly specifically. I do not remember the authors, but it has been reported in several papers that that might be one of the effects. The otolith cells are sensitive to a number of things. For instance, they are partially destroyed even by antibiotics. They are destroyed by Aureomycin, and different things. So even a mild radiation might destroy selectively the cells before any other structure. We have had a problem in the Apollo mission, because we have been advised that very unluckily our package is near a tank which contains a radioactive component just for measuring the level of the liquid in the tank. So we had about 20 millicuries, and we had to make tests to find out if this would impair the frog. But we found out that the limit to get some alteration is about 500 millicuries, so we are very fortunate.

DUSEK: Dr. Montgomery, there are many controls in your experiments. How about the lighting effects. We know that light does affect various types of animal cells. Is this going to be a particular feature here, or will every time the astronaut decides to take an observation, is he going to flash a light on the cell population?

MONTGOMERY: Every time that he wants to focus the microscope, yes, he will have to illuminate. Six times a minute during the time the camera is operating, he will flash a light. It just happens that the intensity of the light which is falling on the specimen is not as great as the intensity of light which is used ordinarily in the laboratory for the time-lapse motion picture photography. If there is an effect of such light, over this length of time, it is not known to us. It is certainly true cells can be killed with visible light by taking time-lapse

motion pictures at too high an intensity of light, but these intensities are not within that range. These cells also do not have any chromatophores in them. For example, they do not contain melanin. They are not pigmented cells. I think those effects would be minimal.

GUALTIEROTTI: I would like to ask one question myself, Dr. Montgomery. What happens to this culture during the 14-day period as a norm? Do they change much?

MONTGOMERY: It depends on the strain of cells that you use. We plan to use two strains of cells. One is Chang liver cells, which are a strain of fetal embryonic human liver, started by Dr. Chang, which are now widely available and used in a great many laboratories. The reason for using those is that they were started from a normal strain of cells, although I doubt that they are perfectly normal cells any more. There are a great deal of data available on their behavior, both in our laboratory and others. They are hardy. They are not contaminated by PPLO organisms as are Chang liver cells, or as far as we know, ambient viruses and other things that wander through your tissue cultures.

The second strain that we hope to use is the normal diploid strain of L human embryonic lung cells, which cannot be maintained indefinitely in tissue culture for the reason that they are diploid cells. Normal diploid cells will not grow indefinitely in tissue culture. The diploid cells will form a monolayer, and stay as a monolayer during this period of time; but the Chang liver cells, once they form a monolayer, will then begin to form small cellular aggregates. They will not exceed, however, in some way the capacity of the chamber to contain the number of cells, so that after they reach a certain number of cells in a given amount of media, apparently their growth rates begin to slow down over this 21-day period.

GUALTIEROTTI: They grow, of course, so you will have a different kind of cells during this space experiment. How long does one cell last?

MONTGOMERY: Well, one cell lasts until it divides, and in Chang liver cells, this takes about 45 to 60 minutes, and occurs roughly somewhere between the neighborhood of 9 to 12 hours.

GUALTIEROTTI: There you are studying the effects of zero gravity on a changing population.

MONTGOMERY: That is right. These cells will grow, divide, and will not be the original cells you started out with.

PRESTON: I have two questions. You mentioned that you anticipated exposing the film for a fraction of a millisecond. I am interested in the kind of film that you use.

The second question is, do you know when during the life cycle of this cell DNA synthesis occurs, since you have indicated you plan to use tritiated thymadine, which is not a precursor?

MONTGOMERY: Well, DNA synthesis occurs in the same period in the life cycle of these cells as it does in any other. There is a period when it has got to synthesize DNA before it can divide. It has got to synthesize DNA in the S-phase. Now, if you are asking me if I can tell by looking at a cell what phase the cell is in, the answer, of course, is no. The only way you can do that is to have synchronous cultures, and producing synchronous cultures is a fairly technical stunt. It is not a complication that we hope to get into. Really, this is where numbers will come into it. If you have a reasonable population of cells on which you have ground-control data, unless the variation in the uptake of the label is very large, it probably will not mean anything. It is still something that I think is worth doing as a beginning experiment. The number of experiments you can do on cells in satellites is at the moment remarkably small. I would be the first to admit that they are not the kind of experiments that I would design sitting in my laboratory, but I am not going to be sitting in my laboratory.

I am sorry, I missed the first question.

PRESTON: What is the type of sensitive film that you plan to use?

MONTGOMERY: The film that we use is the same film that they use to photograph football games, and the reason for that is that you can get it developed in about 2 hours on any single day that you want it under very highly controlled conditions. It is Eastman Kodak. I think it is S-100, but I will have to look that up and tell you. It is not an unusually sensitive film. It is film that is readily commercially available.

PITTS: As a layman, I am still a little bit worried about the possibility of light sensitivity of your cells. You may feel quite comfortable about this, but from my standpoint of ignorance, I would ask whether or not you are going to have a record of the duration and time pattern of lighting of that culture, so that you can reproduce this on your ground-based controls?

MONTGOMERY: Well, in the biosatellite, of course, we will have a very exact record, because the film will have a little marker on it which tells us exactly what day and at what time of the day, and on which day the camera either photographed or did not photograph, so on the biosatellite we will have a very standardized and reproducible thing.

In the Apollo flight, we will have to depend on the log that the astronaut prepares, saying that he turned the light on at this and that time. Again, as a practical matter, in 15 years of looking at cells, this does not seem to be a very big factor. I am not saying that light is not important, because light is important, but under the conditions that it is used in tissue culture laboratories all over the world, I do not think it is a major factor.