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RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

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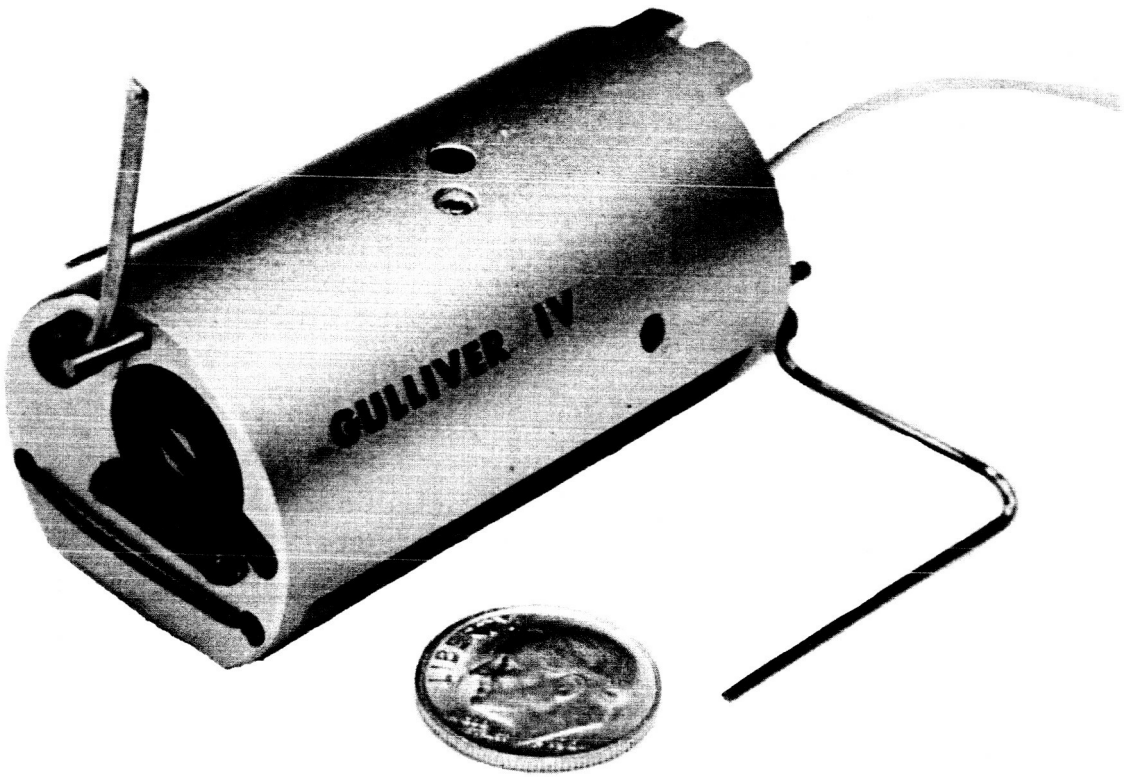

GULLIVER IV, IN SITU MODEL

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I. SUMMARY

During the past year, the Mark IV, in situ, model of Gulliver was designed, fabricated and tested. This feasibility model, shown in the Frontispiece, is a truncated cylinder measuring 1.187 inches in diameter and 2.250 inches in length. The weight of the unit is 81 grams excluding the umbilical cord connecting it to the main capsule. Concluding the year's effort, a field test of the in situ instrument was performed at Reston, Virginia. A highly favorable, early response was obtained.

Despite the good results of the field test, the in situ concept was demonstrated to possess a sensitivity exceeding that of the current in situ instrument by approximately 2-1/2 orders of magnitude. An account is given for this loss of sensitivity which is a trade-off for various advantages in reliability, simplicity and weight-saving aspects of the instrument.

A statistical summary for the entire five-year Gulliver program is presented. The results obtained from all tests of all types, except those in which mechanical failure or contamination of the controls occurred, are included in the summary. This represents a highly conservative overview of the program in that the data obtained during the developmental stages of the media and results obtained with difficultly culturable organisms are incorporated into the summary numbers. Of a total of 1,414 tests made in planchets, with the automatic recorder, in the anaerobic

cabinet, in the in situ instrument and in actual field tests, 1198, or approximately 85%, produced test responses exceeding the respective controls by greater than a factor of 4. In more than half of the total tests performed, the test level exceeded the control level by a factor of 3, or greater, within the first hour of the experiment.

A number of recommendations concerning the Gulliver experiment and instruments are made. These recommendations are based on the total experience of the Gulliver program. It is hoped they will facilitate further development in whatever manner the planetary exploration program evolves.

II. BIOLOGICAL INVESTIGATION

A. IN SITU SUPPORT

A large portion of the total program effort this year was devoted to the development of the in situ Gulliver instrument, Frontispiece. While the instrument was being developed and fabricated, the data upon which it had earlier been predicated were re-examined and investigated in greater depth. Previous reports (1,2) had established the fact that the direct application of labeled medium to soil in the field produced large and rapid responses. When such soil was removed to the laboratory and sterilized, planchet tests produced responses at the level of medium controls.

In situ tests were repeated in local field soils at Reston, Virginia. In a typical test, several drops of M11-C^{14} medium (Table No. II-1) were pipetted onto the undisturbed soil surface. A one-inch-diameter planchet, into which had been inserted a paper fiber pad moistened with saturated solution of barium hydroxide, was immediately inverted over the spot. Fifteen minutes later, the planchet containing the pad was removed and the pad, still in the planchet, was dried under an infra-red lamp. The pad was transferred to a fresh planchet and its radioactivity measured in an internal flow counter. During the first 15 minutes after inoculation, radioactivity levels of up to 250,000 cpm accumulated on the pads.

These high responses again raised the question of possible nonmetabolic production of the labeled gas by physico-chemical reactions between the soil and the medium. In previous tests of this possibility (3),

Table II-1 - Composition of basal media

INGREDIENT	MEDIUM			
	M9	M5	M10	M11
K_2HPO_4 (g/L)	1.000	1.000	0.063	1.000
NH_4NO_3 (g/L)	0.200	-	-	0.188
KNO_3 (g/L)	-	0.500	0.031	0.031
$MgSO_4 \cdot 7H_2O$ (g/L)	0.200	0.200	0.013	0.200
NaCl (g/L)	0.100	0.100	0.006	0.100
Malt extract (g/L)	-	3.000	0.188	0.188
Beef extract (g/L)	-	3.000	0.188	0.188
Yeast extract (g/L)	-	13.000	0.813	0.813
Ascorbic acid (g/L)	-	0.200	0.013	0.013
L-cystine (g/L)	-	0.700	0.044	0.044
Bacto casamino acid (g/L)	-	4.000	0.250	0.250
Proteose peptone #3 (g/L)	-	20.000	1.250	1.250
Soil extract* (ml/L)	100.00	250.000	15.600	15.600
pH	7.2	7.0	7.0	6.9

* Soil extract is prepared by suspending 500 g. of air-dried soil in 300 ml. H_2O. The mixture is autoclaved for one hour, filtered, and liquid loss is made up to 1000 ml. with water.

heat sterilization of the soil and subsequent radioactive testing had given evidence that the reaction was metabolic. However, it seemed conceivable that the severe heating cycles may have altered the character of the soil. Accordingly, this time another approach to investigate whether or not the gas was of metabolic origin was made.

In this series of tests, the geometry of the in situ model was simulated so that the experiments might serve the further use of offering a comparison between the in situ instrument and the planchet collection method. Accordingly, instead of one-inch-diameter planchets as previously used, one-half-inch-diameter planchets were used and fitted with one-half-inch-diameter paper fiber pads for gas collection. As a further simulation of the instrument, the collection planchets were elevated one inch above the ground surface on one-half-inch-diameter hollow cylinders. An undisturbed soil patch at Reston, Virginia was selected as the test site. Onto one spot, 1.0 ml. of Bard-Parker antimetabolite was pipetted. The liquid was permitted to percolate and air-dry for 15 minutes. Then 0.25 ml. of Mll-C^{14} was placed on the same spot. A second spot was immediately inoculated with 0.25 ml. of Mll-C^{14}. The one-inch-high, one-half-inch-diameter, hollow cylinders were then placed over the two inoculated spots and the top of the cylinders immediately capped with inverted one-half-inch-diameter planchets containing barium hydroxide getter pads. After an elapsed time of only one minute, each collection planchet was replaced with a fresh one. Subsequent gas collections were made at total elapsed times of 15, 30, 45, 60, 120 and 180 minutes. These were

15-minute collections beginning at the indicated times, except for the 15-minute observation for which the collection period was 14 minutes. The results are compared in Figure No. II-1. Sufficient gas was deposited on the planchet pad to produce a radioactivity count of 1978 cpm within the first minute of the experiment. In the same minute, the soil which had been pretreated with the antimetabolite produced only 20 cpm. The results clearly confirm the metabolic nature of the response and again demonstrate the rapidity and sensitivity of the in situ approach. The maximum rate of gas production occurred within the first minute of the experiment and it is of considerable scientific interest that positive evidence for microbial life in soil had been established in this short period.

The production of $C^{14}O_2$ was severely curtailed after 30 minutes. Substrate limitation might have caused this. One reason for the limitation of available substrate might be its physical removal by percolation through the soil. Perhaps a more viscous medium would overcome this problem. Methyl cellulose was selected to increase viscosity of the medium. Medium M9-C^{14} was supplemented with 0.0, 0.1, 0.5 and 1.0% Dow Methocel (standard grade, methyl cellulose, type MC, 4000 cts viscosity). In situ and planchet determinations were made using 0.5 ml. of each medium preparation with Washington, D. C., soil and with Escherichia coli, respectively. In the in situ tests, somewhat enhanced responses were obtained from the medium preparations containing the Methocel as seen in Table No. II-2. However, it is not believed that the advantage gained is

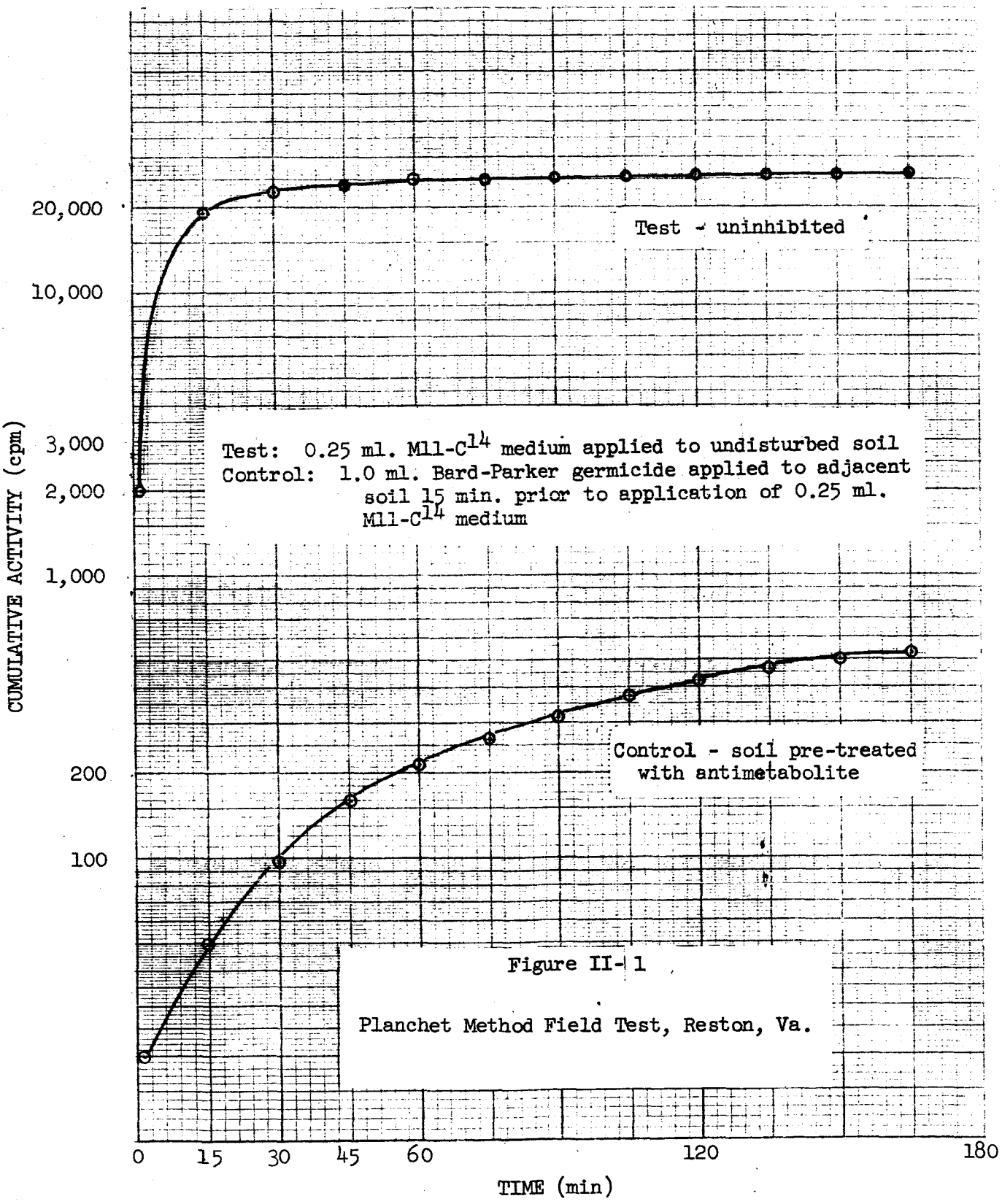


Figure II-1

Planchet Method Field Test, Reston, Va.

Table II-2 - Influence of methyl cellulose on *in situ* and planchet determinations

INOCULUM	TYPE OF DETERMINATION	METHYL CELLULOSE (% w/v)	NET RADIOACTIVITY (CPM) Successive Incubation Periods (hr)		
			1	2	3
None	Planchet	0.0	68	41	53
		0.1	67	35	52
		0.5	43	41	44
		1.0	48	38	50
Escherichia coli	Planchet	0.0	92611	32491	15448
		0.1	95572	30451	13040
		1.0	80259	23904	10009
Washington, D. C. Soil 100 mg.	Planchet	0.0	32627	38028	44920
		0.1	27045	33236	42367
		0.5	31527	28906	31566
		1.0	26384	30810	32019
Washington, D. C. Soil	*In situ*	0.0	41114	11182	6429
		0.1	55812	13876	10039
		0.5	59499	14601	9980
		1.0	68614	15506	9815

sufficient to warrant confining the medium to the surface of the soil in view of the speculation that the highest populations of microorganisms on Mars might lie beneath the surface.

B. MEDIUM DEVELOPMENT

1. Basal Media

The Fourth Annual Progress Report (1) discussed the development of the medium designated as M10 for use in the Gulliver experiment. This medium was a 16-fold dilution of an organically complex medium, M5, also developed during this program. In comparative tests, data obtained with M10 and the various test species and soils were generally as good or better than results obtained with the simple medium (plus soil extract) designated M9. The M10 apparently supplied the complex organic requirements of some species without imposing these compounds in concentrations high enough to inhibit other organisms. The net effect was an enhancement of general response.

During this year, further improvement in response was sought. Water had been used as the diluent in preparing M10 from M5. This achieved the desired reduction in the concentrations of the organic compounds, but it was felt that this might have reduced the ion activity of the medium to the point where the microorganisms might suffer osmotic shock. Accordingly, a series of experiments was performed in which a modified M10 was prepared by diluting M5 with M9, the simple medium, rather than with water. The resulting medium was designated M11. The

compositions of M9, M5, M10 and M11 are shown in Table No. II-1. Table No. II-3 presents the C^{14} substrates in the amounts added to the media for the test purposes.

The results of tests comparing M5, M10 and M11 in planchet determinations are given in Table No. II-4. E. coli and the microbial populations of Blacksburg, Virginia and El Centro, California soils produced the greatest initial responses with the M11 medium. In each case, peak gas production occurred earliest with the M11, demonstrating that its labeled substrates were most readily metabolized.

Having improved sensitivity through the use of M11, further efforts with it were made in an attempt to shorten the normal lag period preceding exponential growth of soil populations. Tests similar to, but not as extensive as, those, conducted in the development of the earlier media were performed to determine the influence of substrate concentration on response. The soil selected for these tests was from the Blacksburg, Virginia sample which, on a previous test, had demonstrated early metabolic activity but a prolonged lag period. Standard planchet determinations (1) were performed using M11 medium with the C^{14} substrates shown in Table No. II-3 except that the concentration of labeled glucose was varied from one-half normal to twice normal. The data obtained, Figure No. II-2, revealed a five-hour lag period except for the one-half normal glucose medium in which lag persisted for 12 hours. Since the normal and twice normal glucose concentrations resulted in the same lag, it does not seem that increased substrate concentration can reduce the

Table II-3 - C^{14} substrates used in Gulliver media development.

C^{14} SUBSTRATES	SP. ACT. (mc/mM)	mM	uc/ml	% (w/v)
Sodium formate	20.0	0.25	5.0	0.002
D-glucose-U.L.	3.6	0.36	1.3	0.006
DL-sodium lactate-1	5.5	0.24	1.3	0.002
Glycine-1	5.1	0.22	1.3	0.002

Table II-4 - Comparison of Media M5, M10, and M11

PLANCHET DETERMINATIONS

INOCULA	SUCCESSIVE INCUBATION PERIODS* (hours)	NET RADIOACTIVITY - CPM C^{14} Media** (0.5 ml.)		
		M5	M10	M11
Escherichia coli	1	9522	16881	17425
	3	29499	41549	49607
	5	79596	54154	57820
2×10^6 cells	24	3421	363	308
Blacksburg, Virginia Soil	1	806	1539	1858
	3	947	1816	2187
100 mg.	5	855	2171	2238
	24	7271	3420	1870
El Centro, California Soil	1	6449	8007	15737
	3	8147	13369	18128
100 mg.	5	12843	24411	32179
	24	4717	1544	1168

* $C^{14}O_2$ collection time - 15 minutes following each incubation period

** C^{14} substrates - (formate, glucose, lactate, glycine)-C^{14}

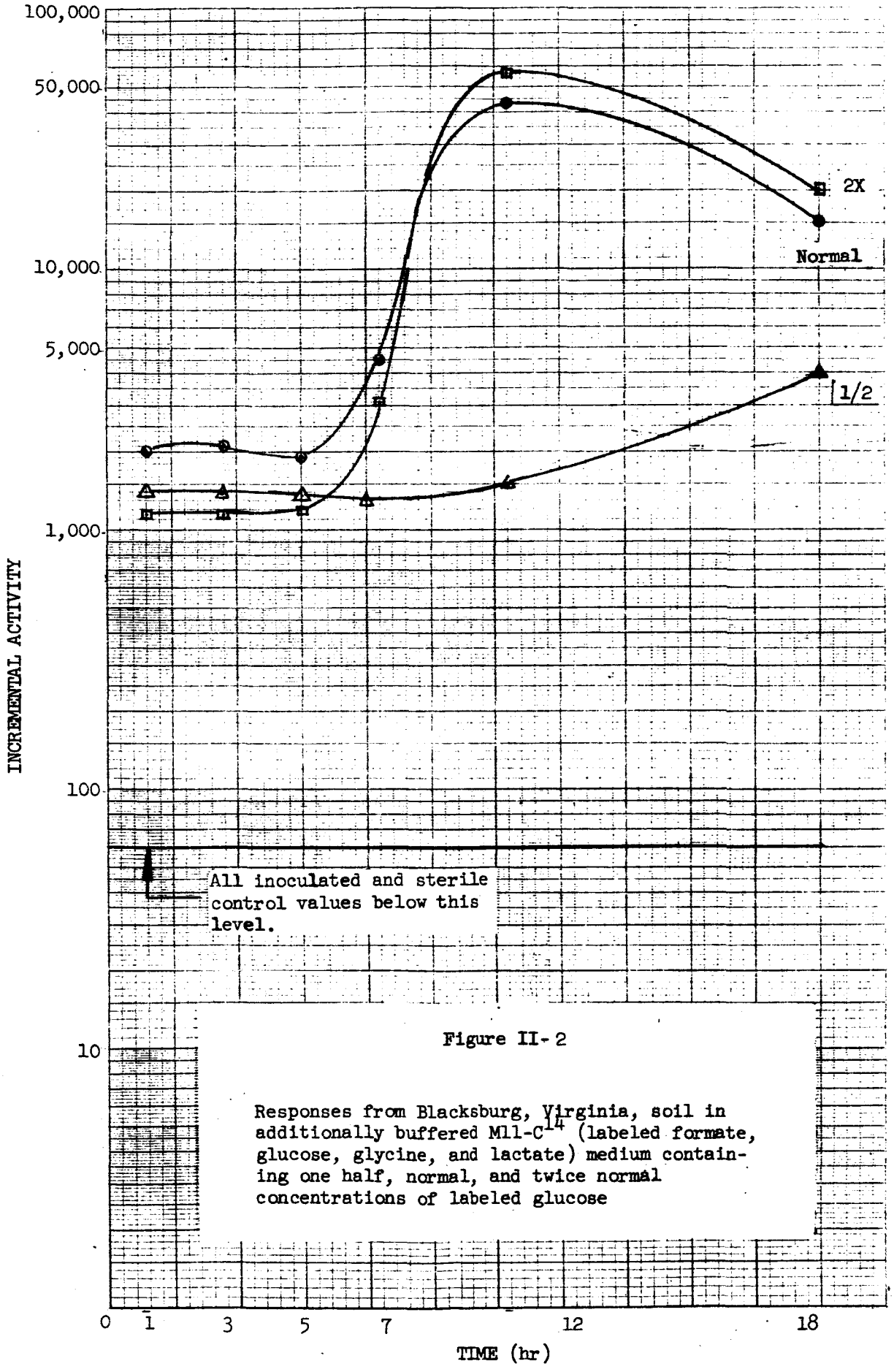


Figure II-2

Responses from Blacksburg, Virginia, soil in additionally buffered M11-C^{14} (labeled formate, glucose, glycine, and lactate) medium containing one half, normal, and twice normal concentrations of labeled glucose

lag period to less than five hours with this soil. However, within one hour the metabolic response from all three concentrations of substrate was more than 10 times the inhibited control levels.

This experiment also confirmed that the normal glucose concentration determined previously in the program was also optimum for M11. Accordingly, no further studies were made to reconfirm the optimum levels of formate, glycine and lactate in the M11.

Stotzky and Norman (4-6) investigated substrate concentration, nitrogen, phosphorus and sulfur as limiting factors in the production of CO_2 from soil. However, their methods did not permit investigation of the very early kinetics of CO_2 production. It was decided to study the early effects of varying the nitrate and phosphate concentrations in the Gulliver experiment. The following complements of nitrate and phosphate were added to normal M11-C^{14} medium:

	1	2	3	4	5
K_2HPO_4 (g/L)	1.000	2.000	2.000	1.000	0
NH_4NO_3 (g/L)	1.190	0.380	0.190	0.380	0
KNO_3 (g/L)	0.031	0.062	0.031	0.062	0

In the event that the added nutrients would induce the organisms to change their demand for substrate, each of the above media was prepared with one-half normal, normal and twice normal glucose C^{14} concentrations. Routine planchet determinations were performed with

Blacksburg, Virginia soil. No advantage was found for any of these media above the normal Mll-C^{14} responses. Exponential growth occurred in five hours in all of the test cultures.

Calcium sulfate, calcium nitrate, and manganese sulfate, not previously incorporated in the Mll medium, were also investigated as additives. The following concentrations were placed in Mll-C^{14} media:

(1)	$CaSO_4$,	100 mg/L
(2)	$CaSO_4$,	5000 mg/L
(3)	$Ca(NO_3)_2$,	100 mg/L
(4)	$Ca(NO_3)_2$,	5000 mg/L
(5)	$MnSO_4$,	10 mg/L
(6)	$MnSO_4$,	100 mg/L
(7)	Control,	-

Planchet determinations were made using 0.5 ml. of each of the media and 100 mg. of Blacksburg soil inocula. No reduction in lag time was derived from the addition of these compounds to the medium.

2. Nonmetabolic $C^{14}O_2$

As previously reported (7), a few of the many samples of soil tested from different locations throughout the nation produced some $C^{14}O_2$ even after having been sterilized. At that time, it was found that the production of nonmetabolic $C^{14}O_2$ from sterile soils placed in the Gulliver media occurred in soils of low pH, although all low pH soils

tested did not produce nonmetabolic $C^{14}O_2$. Thus, it seems that some factor other than low pH was indicated. During this year, in an attempt to find this factor, a series of experiments was performed to test the possible implication of various components in the Gulliver reaction: the labeled substrates, the planchet material, the antimetabolite, and the possibility that spores in the soil survive the repetitive autoclaving process. In essence, the results confirmed those of the previous report (7) without shedding much additional light on the subject: 1. buffering of the medium eliminated or considerably reduced the nonmetabolic evolution of $C^{14}O_2$, 2. the formate and glycine were the primary sources of the nonmetabolic $C^{14}O_2$, 3. the planchet material did not seem to contribute significantly to the problem although the results obtained with aluminum planchets showed slightly higher levels of nonmetabolic $C^{14}O_2$ evolved than those obtained when plastic planchets were used.

During the course of the Gulliver program, approximately 50 different soils have been tested. The pH's of many, but not all, were determined. Seven were of pH 5.5 or less. Three of these seven, Alaska, Barrow-1; Alaska, Barrow-2; and New Jersey, Metuchen, produced troublesome levels of nonmetabolic $C^{14}O_2$ during the test. Another of the low pH soils, Orange, Virginia, produced lower, but detectable, levels of nonmetabolic $C^{14}O_2$.

Even when buffer is not added to the medium to overcome this problem, the differences between results from the test and control portions have been significant. Still, a better understanding of this phenomenon is desirable.

3. Preflight Sterilization of Media

The National Aeronautics and Space Administration has prescribed dry heat at 145° C. for three 36-hour cycles for preflight sterilization. The effect of such a sterilization process on the Gulliver media must be known. Four media, M9, M5, M10 and M11, each containing the formate-glucose-lactate-glycine-C^{14} substrates, were freshly prepared. One-milliliter aliquots of each were placed in glass vials, heat sealed, subjected to the prescribed heat cycles, and placed in storage. At such time as may be desired, the contents can be tested.

C. TEST MICROORGANISMS AND RESPONSES

1. Test Collection

All of the stock cultures obtained and maintained during the course of the space program were examined, sub-cultured, and catalogued. The soil collection was also checked and placed in storage.

2. Anaerobic Determinations

Because of the possible importance of anaerobic organisms on Mars, 38 anaerobic determinations were made this year on Clostridium perfringens, Clostridium tetanomorphum, Clostridium sporogenes and Desulfovibrio desulfuricans in Gulliver C^{14} media. All four organisms responsed in M9 and M11. Growth occurred in 17 tests; metabolism in 11 tests. The remaining 10 were invalid due to mechanical malfunctioning of the automated anaerobic chamber, or because of contamination of the controls. Typical growth responses are presented in Table No. II-5.

Table II-5 - Results of anaerobic tests

ORGANISM	C. PERFRINGENS		C. TETANOMORPHUM	C. SPOROGENES		D. DESULFURICANS
Cells/test	1×10^6	1.8×10^5	1.2×10^5	5×10^6	1.6×10^6	1.2×10^3
C^{14} medium	M9	M10	M10	M9	Difco AC	buff. M11
SUCCESSIVE INCUBATION PERIODS (hours)	NET RADIOACTIVITY (CPM)					
1	10	115	26	10	58	740
4	378	400	220	76	1,052	2,250
8	-	500	550	-	20,640	3,500
20	22,000	17,000	6,000	3,340	27,000	5,200

3. Spores

Rapid chemical germination of spores has been reported (8). An effort to induce such early germination by adding n-Dodecylamine [$CH_3(CH_2)_{10}CH_2NH_2$] to spore suspensions of Bacillus subtilis var. globigii was made. The spores were exposed to 0.00006 M Dodecylamine for periods of one, two, three, and five minutes, filtered, washed with unlabeled Mll medium, and suspended in Mll-C^{14} for planchet determinations. No apparent benefit was derived from the use of the Dodecylamine.

D. FIELD TEST OF IN SITU INSTRUMENT

Because of electronics problems encountered, completion of the feasibility model of the in situ instrument took longer than anticipated. The year's effort was concluded with a field test of the instrument on a patch of bare, undisturbed earth near the Life Systems Division laboratory at Reston. The results of this test are presented in Figure No. II-3. The test was begun at approximately 10 a.m. during a slight drizzle. The air temperature was 21° C. Within one hour, the response exceeded 2000 cpm and rose to 5000 cpm at the fifth hour. As shown on the curve, numerous readings were taken. The smooth shape of the curve testifies to the stability of the instrument's counting system during the test period. Inasmuch as only one instrument was completed, no control was run with the test.

Section II, A, reports on in situ type tests using one-inch planchets with pads inserted for gas collection. They elicited

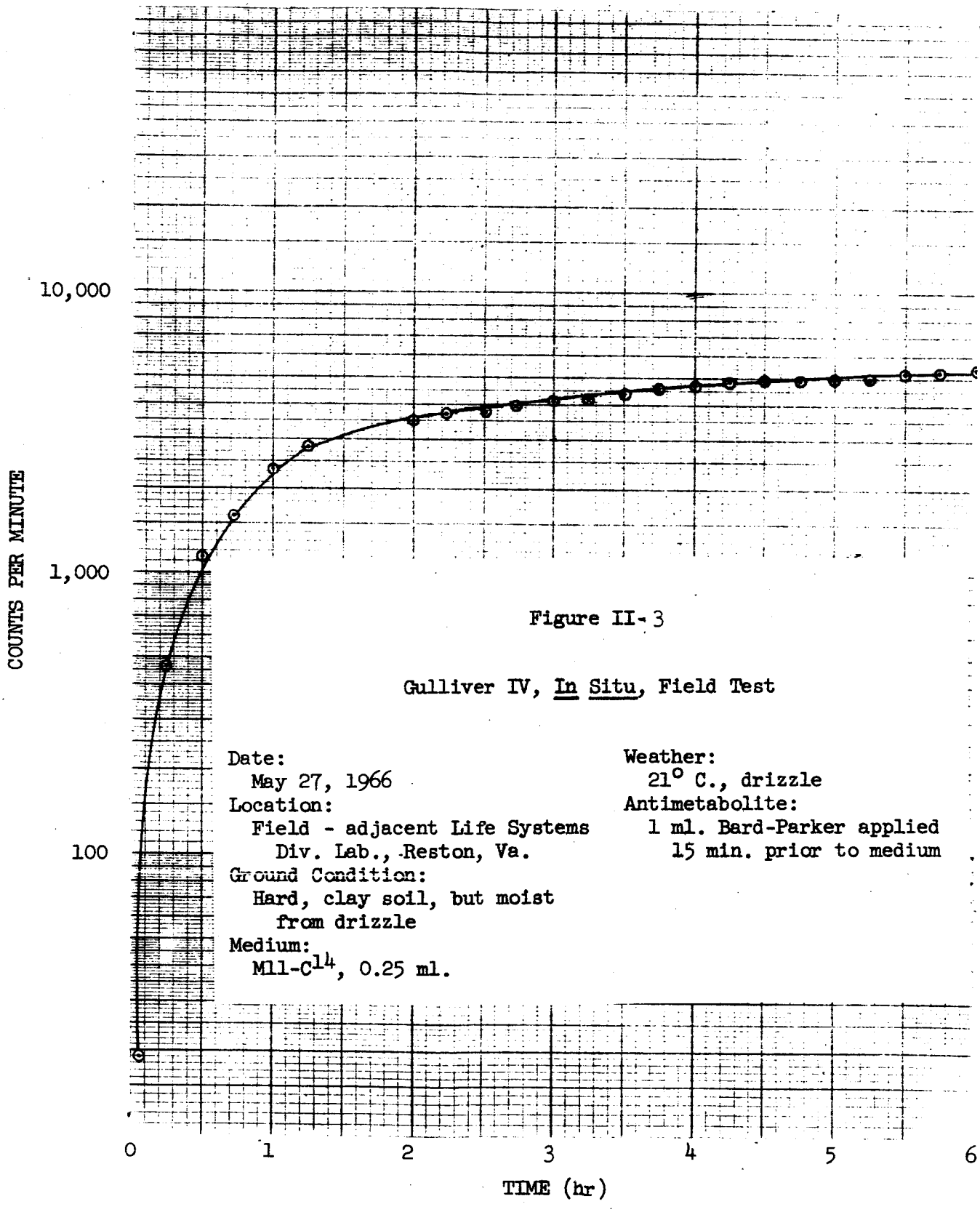


Figure II-3

Gulliver IV, In Situ, Field Test

Date:
May 27, 1966
Location:
Field - adjacent Life Systems Div. Lab., Reston, Va.
Ground Condition:
Hard, clay soil, but moist from drizzle
Medium:
M11-C^{14}, 0.25 ml.

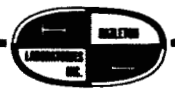
Weather:
21° C., drizzle
Antimetabolite:
1 ml. Bard-Parker applied 15 min. prior to medium

considerably higher responses than did this field test of the instrument. The typical example reported resulted in a radioactivity level on the barium hydroxide pad of 250,000 cpm only 15 minutes after the medium had been deposited on the ground. This, compared to the approximately 450 cpm detected by the instrument after the first 15 minutes of the experiment, shows an advantage of 556-fold for the planchet experiment. While these two experiments were not run simultaneously or on adjacent spots of ground, similar advantage factors for the planchet tests were obtained in the early periods of incomplete tests in which both systems were operated comparatively.

This forfeit in sensitivity can readily be accounted for by known factors. The one-inch planchet has four times more getter area and diffusion path cross section area than the instrument. The geometry factor between the one-half-inch-diameter planchets and the instrument was determined to be a factor of two, due to the longer diffusion path length in the instrument in which the geiger tube sits higher above the ground than does the gas collection pad in the planchet. Measurements comparing the *in situ* counting system (reading the C^{14} through the getter and the mica window) to the Nuclear-Chicago flow counter in which the planchets were read showed an advantage of 40-fold for the latter. Finally, measurements made in which the instrument type baffle was interposed between the soil and the getter in one-half-inch planchets showed a further reduction of 2.5-fold. These factors can account for a total difference in sensitivity between the planchet method and the *in situ* instrument of $4 \times 2 \times 40 \times 2.5 = 800$.

The planchet tests demonstrate that the sensitivity and rapidity of microbial detection offered by the Gulliver method is extraordinarily, probably uniquely, high. Because of "trade-offs" made to accommodate incorporation of the procedure into an automated spacecraft laboratory, the sensitivity has been reduced approximately two and one-half orders of magnitude. Is this an excessive sacrifice? At this time, the experimenters believe it is not. The sacrifice permits the device to be made smaller and hence makes possible the delivery of a greater number of units. By counting through the getter and geiger counter window, the necessities for a mechanical getter transport system and an automatic gas valving system to operate an internal flow counter are eliminated, resulting in considerably increased reliability for the instrument. Finally, in evaluating the "trade-offs," it must be borne in mind that the sensitivity retained by the instrument is still very high.

No direct comparisons of sensitivities were made between the Mark III Gulliver and the in situ Gulliver instruments because time did not permit reactivation of the Gulliver III unit for simultaneous field tests with the in situ model. However, the approximately 2300 cpm achieved by the in situ instrument in the first hour of its field test exceeds the one-hour values obtained by Gulliver III in any of the eight local tests and four extreme natural environment field tests reported (2) for the intensive Mark III test period and also exceeded the one-hour result obtained in the single Gulliver III test performed last year (1). The entire six-hour record of the in situ field test



exceeded, at all points, the first six hours of record of nine of the previously cited 13 field tests of the Mark III instrument.

While the present "trade-off" position seems satisfactory, it may not remain so. As the state of the art of the appropriate instrumentation and spacecraft parameters improves, it should become possible to add to the sensitivity of the in situ instrument.

III. STATISTICAL SUMMARY OF GULLIVER TESTS

The Gulliver program now concludes its fifth year of development. During that period many changes have occurred in methodology, media, and instrumentation. As had been initially proposed, a great many microbial species in pure cultures and in mixed populations occurring in natural soil have been challenged by the test. In all, approximately 50 pure cultures of microorganisms of known species, approximately 65 unidentified pure cultures isolated from soils and the mixed populations of micro-organisms occurring in 50 natural soils obtained from many points in the United States and Alaska were tested.

The known species of microorganisms have included heterotrophs, chemotrophs, phototrophs, halophiles, aerobes, facultative anaerobes, anaerobes, spore formers, non-spore formers, thermophiles, mesophiles, psychrophiles and halophiles. These have been representative of bacteria, streptomycetes, fungi, protozoa and algae. Field-obtained lichens have also been tested. None of these pure or mixed populations has failed to give a positive response in M9 or M10 with labeled substrated. The Gulliver method was modified to detect photosynthesis, not only with algae, but a photosynthetic response was obtained from Rhodospirillum rubrum when grown anaerobically. The only failure in eliciting response occurred in an attempt to detect metabolism of pleuro-pneumonia-like organisms (PPLO's) as reported previously (9). However, despite several attempts at maintaining these organisms in culture, growth in subcultures of the

original stock was not demonstrable by conventional means. Hence, there is no way of knowing whether or not the PPLO's in the Gulliver-tested cultures were viable or not.

Table No. III-1 presents a statistical summary of all Gulliver tests run either in the field or in the laboratory in all stages of development during the entire program. A total of 1414 "tests" were made. A "test" consisted of a set of two to three (most commonly three) replicates of a given culture and an equal number of sterile control and/or inhibited control replicates made with the test medium in the cases of the planchet and anaerobic cabinet determinations. The in situ and field tests are not as easily categorized in that, in some instances, antimetabolite was applied to the control at the time of inoculation of the test unit whereas, in others, the antimetabolite was applied to the control unit after growth became evident in that unit. Hence, the right-hand side of the tabulation is not applicable for the in situ and field tests.

Bearing in mind the fact that the statistical summary includes all tests made, those preparatory to the finally designed experiments as well as the experiments themselves, it is remarkable that only in 128 instances did the average response of the test replicates fail to exceed twice the average response of their controls. (Of these 128 instances, nearly all are attributed to preliminary tests with six species of microorganisms.) Ninety-one percent of all the tests exceeded their controls by a factor of two or more. Furthermore, 85% of all tests exceeded their controls by a factor greater than four.

Table III-1 - Statistical summary of Gulliver tests

TEST	RATIO OF TEST/CONTROL AT MAXIMUM DIFFERENCE						TIME FOR TEST TO EXCEED 3X CORRESPONDING CONTROL LEVEL (HR)								
	Total	0-1	1-2	2-3	3-4	>4	1	2	3	4	5	6	7	12	24
Planchets															
Soil	406	6	9	4	2	385	339		31		13		2		6
Bacteria	546	50	39	30	26	401	163	8	251		2				33
Recorder															
Soil	181			3	4	174	148	7		3				20	3
Bacteria	162	4	13	13	3	129	58	38	11	8	6	5	3	2	14
Anaerobic Cabinet	73	4	3	1	2	63	54	4	3	2	1				2
Sub-total	1368	64	64	51	37	1152	762	57	296	13	22	5	5	22	58
In Situ	23					23	not applicable								
Field Tests*	23					23	not applicable								
Total	1414	64	64	51	37	1198	-	-	-	-	-	-	-	-	-

* Instrument

The right-hand side of Table No. III-1 offers some indication of the time required for the determinations. For each of the types of tests there is shown the number of hours required for the test cultures to exceed their respective controls by three-fold. In more than 50% of all the tests performed, this activity level was attained within one hour. In addition, the reason that the three-hour responses are so numerous is that, in the majority of the tests reported in that column, one- or two-hour collections were not made. In approximately only 4% of the tests was it necessary to wait longer than five hours to attain three times the control level. The field tests and in situ tests are excluded from the latter percentage in that there are no data on the times at which the test units exceeded three-fold the control levels for the reasons previously cited. However, of those tests in the latter two categories which were conducted with simultaneously inoculated test and control units, and which were read at appropriate times, almost all qualified within the five-hour period.

The numbers of cells per inoculum producing the results varied widely. In the soil determinations, the ambient populations present produced the responses. A number of total bacteria counts were made on the soils and these ranged from 100 cells to 1000 cells per mg. of soil. Where soil tests were run in planchets, the amounts of soil used as inocula ranged from 1.0 mg. to 100 mg. In the tests made with pure cultures, the inocula were varied between several hundred cells and several million cells per test. Details on many of the specific

tests have been presented in the various Quarterly and Annual Progress Reports. In theory, the Gulliver technique is capable of detecting a single cell given sufficient time. In practice, it has detected gas produced from a calculated inoculum of 12 cells (as determined by replicate pour plates) after four hours of incubation.

IV. INSTRUMENTATION

A. IN SITU CONCEPT

The Fourth Annual Progress Report (1) discussed advantages of an in situ life detection experiment over an experiment requiring sample collection: (1) utilization of an undisturbed section of a planetary surface as a sample; (2) the availability of a significantly larger volume of sample than would be collected by the sticky-string sample retrieving method of Gulliver III; (3) extending the sample cross section below the immediate surface layer which may be subject to intense ultraviolet irradiation; (4) the possibility of the planetary soil acting as a chromatographic bed to effect composition and concentration gradients of the applied microbiological medium, thereby increasing the opportunity for the organism to find some favorable formulation; and (5) a significant decrease in the real time required to complete the experiment. These advantages, along with the reduced size thought possible for the in situ as compared with other Gulliver models, warranted an investigation of the applicable design parameters for the new concept. A potential disadvantage for the in situ type instrument should be pointed out. At the low atmospheric pressure believed to prevail on Mars, the liquid medium might evaporate very rapidly (since the temperature will be maintained above freezing) if the instrument-to-surface seal is not adequate.

This section of the report presents the important design criteria for an in situ version of Gulliver, an assessment of their effects, and a design approach which utilizes a compromise of the requirements to evolve a practical instrument for the conduct of the experiment. The design, fabrication and testing of the instrument are reported.

B. PHYSICAL AND ENVIRONMENTAL FACTORS

The controlling design factors in an instrument developed for an extraterrestrial experiment are not necessarily those imposed by the experiment to be performed. Limitations of size, weight, reliability, power consumption and data processing requirements, which are secondary to the experiment, are defined by the restrictions of payload weight, power availability, cost, and telemetry associated with the spacecraft. The following discussion pertains to the ultimate development of the in situ instrument.

1. Size and Mass

The size and weight objective set for the in situ version of Gulliver is an instrument package, cylindrical in shape, 65 mm. long (2.250 inches), 30 mm. in diameter (1.187 inches), weighing a maximum of 81 grams. With the exception of the primary power supply, this package contains all of the components required for the experiment including preliminary data processing of the electrical signals.

2. Sterilization

Before launch, the instrument must be sterilized (13) by dry heat and an ethylene oxide-Freon-12 moisture mix gas treatment. The

prototype temperature sterilization requirements for qualification are three cycles, each of 36 hours duration, at 145° C. in an inert atmosphere.

To achieve a reliable sterilization of flight hardware by processing with the gas, the gas must contact the surfaces to be sterilized. Therefore, the design of the instrument package should be such that it is possible to assemble sterilized components under clean room conditions into a package which contains a minimum number of obstructed chambers, grooves, mechanical joints such as screw threads or porous materials.

From a mechanical point of view, the high temperature requirement will influence the sealing techniques and the electronic components on the signal processing circuitry.

It is assumed that the sterilization specified will be the only intentional heat cycling employed and that sterilization by heat exposure while in solar space will not be attempted. Sterilization by irradiation at levels of 10^7 rads or more would adversely affect materials and components currently available.

3. Launch Environment

The rocket booster and secondary rocket motors used to launch or orient the space capsule will produce intervals of shock and vibration to all instrumentation components. The complete instrument package, designed to be launched from the planetary lander-capsule itself, must be constrained in a mounting in such a manner that destructive resonances do not occur.

The instrument package proper should be designed to withstand vibration environments of 25 G at frequencies from 2 cps to 2000 cps and impact loading of up to 1000 G with duration of up to 3 milliseconds.

4. Space Environment

a. Vacuum

The absolute pressure of solar space, not under the influence of a planet, is 10^{-12} to 10^{-16} TORR (14). For all practical purposes sealed enclosures are pressurized at 1 atm (760 TORR) if sealed in an Earth environment. The metal components of an instrument may be adversely affected by exposure to vacuum, organics will evaporate, and, of course, liquids must be confined. Dry lubricants will be required on movable parts to prevent cold welding due to volatilization of surface films. Leakage rates for proven seal designs, stability of organics and adhesive compounds are available to make a selection best suited for the application.

b. Radiation

A large amount of published data exists on radiation environments in solar space. The nature of the proposed instrument design is such that it is not anticipated that it will be exposed directly to the space radiation environment and, hence, will not be seriously disturbed by high energy electron radiation dosages occurring on space capsule surfaces. The amount of shielding proposed is a minimum of 1 g/cm^2 which will limit radiation effects to protons and bremsstrahlung radiation intensities of less than 10^3 rads during the periods of solar flare activity. This radiation level is not significant in terms of materials damage, but

selection of solid-state electronic components must be made with this hazard as a consideration including the anticipated effects upon detector operation of the absence of Van Allen belts around Mars.

c. Meteoritic Particles

The proposed instrument, not being exposed directly to the space environment, is not considered vulnerable to the effects of micro-meteorites. Furthermore, the use of multiple instruments reduces the hazard of catastrophic failure of the experiment since the mechanical damage would be localized and would have a low probability of affecting all instruments simultaneously.

d. Equilibrium Temperature of the Spacecraft

The equilibrium temperature of the spacecraft depends on its absorption coefficient for solar radiation and its distance from the sun. For spherical vehicle configurations, this equilibrium temperature can range from 110° K to 500° K in the Earth-Mars path (15). It is to be assumed that internal temperatures of the spacecraft will be maintained above freezing levels for electrolytes in internal batteries used for power. The Gulliver experiment requires a liquid metabolite and the storage stability of this medium is enhanced at reduced temperatures (280° K).

5. Planetary Environment

a. Landing

The landing profile environment is not expected to exceed the launch environment in mechanical aspects, and the low density of the

Martian atmosphere should limit any aerodynamic heating to acceptable levels within the lander capsule.

b. Operating Environment

The mean surface temperature of Mars, 250° K and an anticipated low temperature of 203° K, are well below the freezing point of the liquid media and thus provisions must be made for liquefying the material and maintaining the liquid state during the experiment. Improved information on Mars surface pressure and temperatures will permit better design to insure maintenance of the liquid phase during the experiment.

The Martian atmosphere is known to contain an appreciable quantity of carbon dioxide. The Gulliver experiment depends on the collection of carbon dioxide evolved from organisms which have metabolized radioactively tagged nutrients. The carbon dioxide collection is achieved by chemical gettering and natural atmospheric carbon dioxide present will also be gettered. Better data on Mars atmospheric CO_2 content will permit suitable design of the required capacity for the getter system. Current treatment of this problem is described later.

C. DESIGN AND FABRICATION OF IN SITU INSTRUMENT, MECHANICAL COMPONENTS

1. Canister

The objective of minimum weight for the instrument can best be met by selecting a housing material with a low density that will satisfy all of the environmental criteria. The use of magnesium adequately protected by anodic coating in the exposed areas plus the addition of

solid dry lubricants in all areas where moving parts are located seems to be a logical choice for a final flight model.

For the feasibility model made during this program, aluminum alloy 6061-T6 was used. No provisions for protection were made inasmuch as no exposure to the weather was expected. Moving parts were, however, protected during the tests with dry molybdenum disulfide (MoS_2).

The first design for an in situ model that was proposed at the end of last year's program consisted of a double-ended canister 42 mm. in diameter, 95 mm. long. An ejectable detection module was located at each end of the canister. The canister was to be ejected from the lander capsule by using compressed gases or pyrotechnic devices. Upon leaving the launch tube in the lander, four folding legs in each end would extend to keep the canister a certain distance from the ground and provide sufficient room for the detector modules to gravity orient themselves to a vertical position after ejection from the canister. A model was made of this device, Figure No. IV-1, in order to test the orienting features of the modules. After a number of tests were performed using different springs and leg lengths, it was found that the operation of this device lacked the reliability required for the experiment. It was very difficult to obtain vertical positioning and the low weight of the detector module made sealing to form a metabolic chamber on a rough surface very questionable.

Other approaches were then considered. It is obvious that there are three critical areas in the mechanical design of an in situ instrument:

Figure IV-1 - In Situ
Prototype Instrument

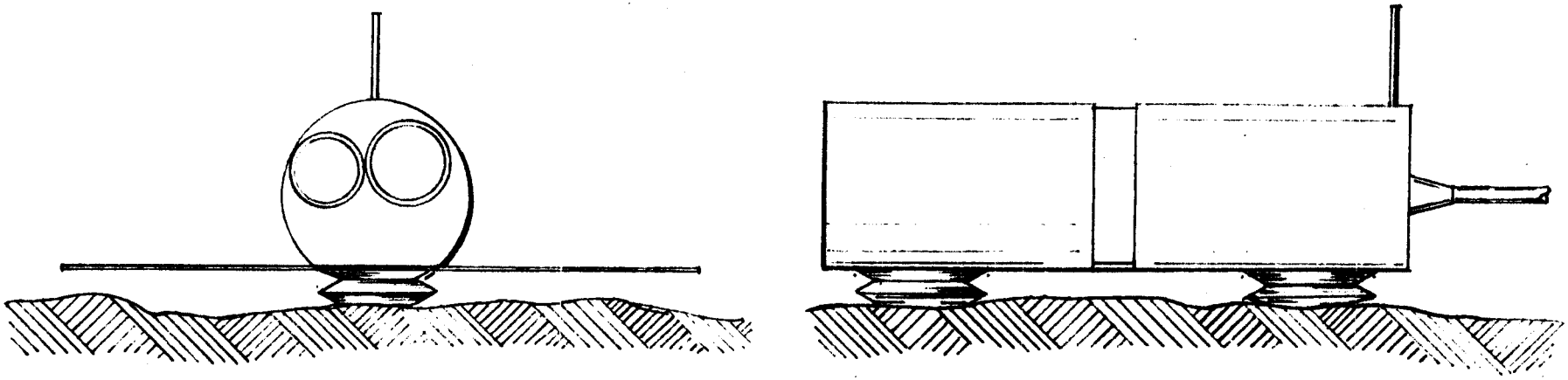
a. attitude with regard to the ground

b. adequate seal between the instrument and the ground to form a metabolic chamber

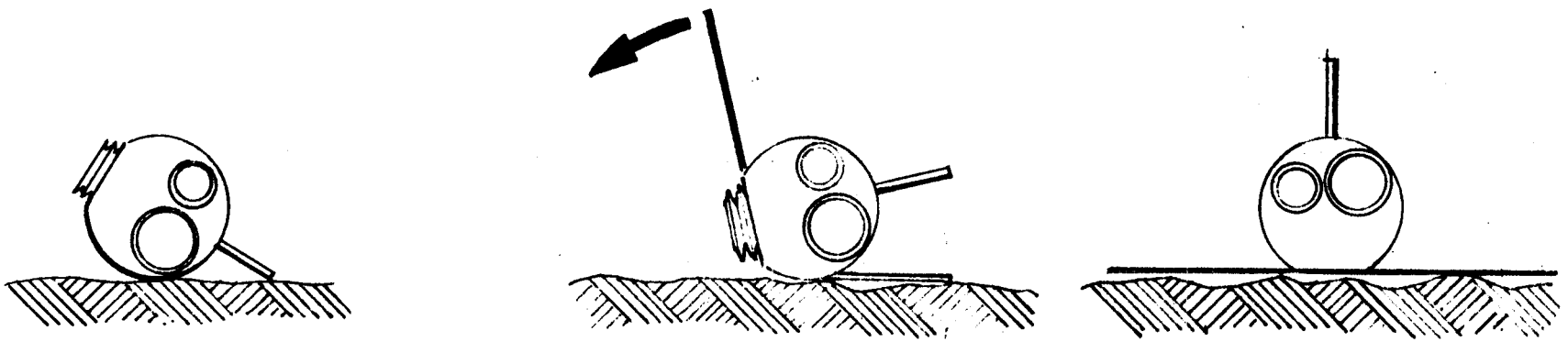
c. ruggedness and simplicity

Various configurations were considered in order to achieve these goals. It was finally decided that it would be easier and safer to maintain the integrity of a canister without any deployable device. Means of orienting the canister after landing on the ground would then have to be provided in order to effect a seal with the ground. This became a very simple operation when compared with the task of unfolding legs and releasing modules in the previous design. Another gain was that total weight of the canister became available to effect a sealing pressure against the ground. As initially conceived, the new, double-ended device is shown in Figure No. IV-2 where is also shown a sequence of operation of the self-orienting action. As can be seen, the detector chambers are part of the body of the instrument instead of units ejected from the ends of the body.

If a double-ended unit were used, some savings in weight could be obtained by making some of the components perform a double function. An example would be ampul breakers, baffle actuators, and a single orienting mechanism. This was compared with the possibility of employing a single-chamber instrument. Although a slight weight penalty was involved in having a system composed of a number of single chambers, it was felt that the gain in reliability and simplicity warranted this approach. Furthermore, the



SELF-ORIENTING MODULE



ORIENTATION SEQUENCE

Figure IV-2

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single-chamber unit imposed many of the same design challenges of the double unit. Thus, the experience gained could later be applied to the design of a double-ended unit if desired.

2. Single Chamber Module

The concept followed in the design of the single chamber, self-orienting module was one of maximum utilization of volume in order to obtain a small, rugged unit that would withstand the high impact forces encountered during ejection from the capsule and landing on the surface of the planet. To this effect, several layouts were made. That chosen was a truncated cylinder 1.187 inches in diameter by 2.250 inches long, with an estimated total weight of 81 grams of which 39 grams correspond to the main body of the module, 10 grams to the electronics, and 32 grams to the mechanical components. A reduction in weight, however, will be possible by appropriate substitution of materials in the final model. Substitution of the aluminum main body with magnesium and some of the stainless steel mechanical components with anodized aluminum could reduce the weight by 10 to 15 grams. This weight does not include the weight of the umbilical cord, which obviously will vary with the length chosen. Figures No. IV-3 and No. IV-4 show the module configuration.

The orienting pin to pre-position the unit at a convenient attitude so that the legs will be effective when they unfold is a spring-loaded rotating arm. It extends as soon as the in situ unit leaves the ejecting pipe in the lander capsule. It is located in the forward end of the module and its final desired position is established by a positive mechanical stop.

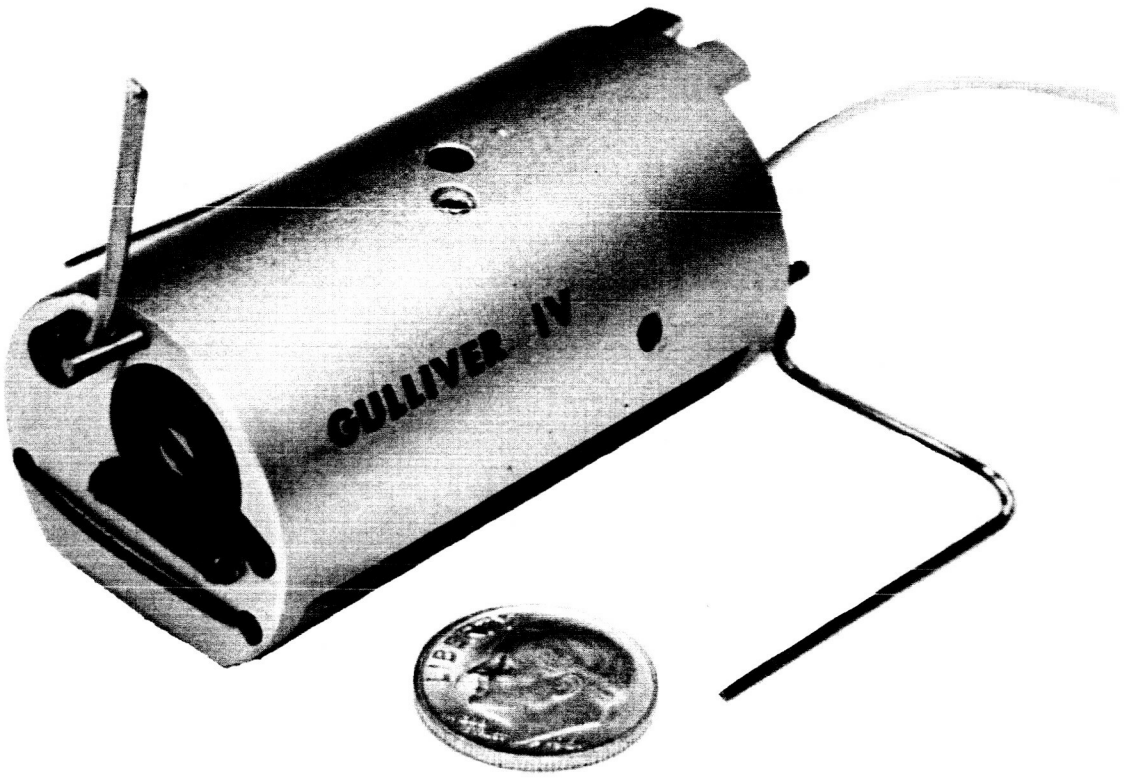

Figure IV-3 - Gulliver IV, In Situ Model

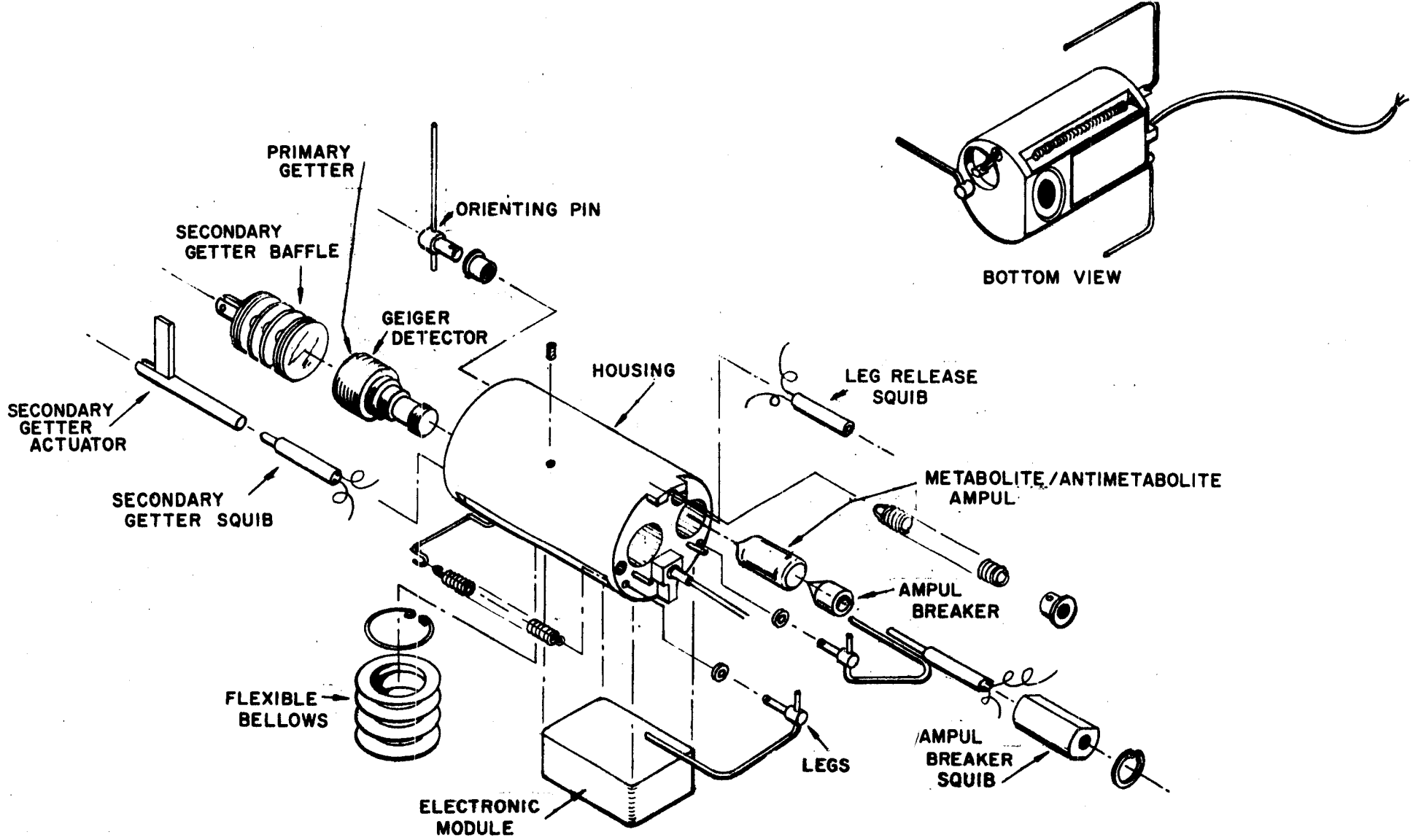

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Figure IV-4 - Gulliver IV In Situ - Single Chamber Module

After the instrument lands on the surface, an electrical signal fires a squib piston actuator (Atlas 1 MT 114). The plunger of the actuator releases two semi-rigid legs from a retaining boss. These legs are spring-loaded in a fashion similar to the orienting pin and, once released, they generate a rotational force that rolls the module into the operating position. Different types of uprighting means were tested such as thin leaf springs, music wire, and coil springs, but none of these was as effective; the actions were too violent or did not have enough power to rotate the module. Release of springs was also unreliable. The semi-rigid legs have proved to be highly dependable in their operation. The rotational force is easy to adjust by either one of two means, changing the wire size of the spring or changing the amount of torsion applied to the spring.

The module in contact with the surface encloses a volume. The area of the surface is approximately one cm^2. The height of the chamber is approximately 1.5 cm. and the volume, therefore, is approximately 1.5 cc. If the effective depth of the area sampled during the experiment is 0.8 mm. (1/32 of an inch) the total sample volume is at least 0.08 cc. If we assume a surface density for Mars similar to that of the Earth, then the total sample mass will be (0.08 cc. x 2.67 g/cc) approximately 0.21 grams of material, as weighed on Earth.

3. Temperature Control

One of the more important requirements of the experiment is the temperature range in which it must be conducted. The liquid medium applied to the surface must remain liquid. Martian temperatures range

well below the freezing temperature of the proposed metabolic medium and some provision is needed to maintain or insure the liquid state for the conduct of the experiment. A very low level of metabolic activity (inferred from Earth conditions) is to be expected at temperatures near or below freezing. Hence the experiment must survive as long as possible.

On the other hand, temperatures higher than several degrees C. may impose evaporation problems at the newly estimated Mars atmospheric pressure of 7 mb. (16). Midday high temperatures at the Martian equator of up to 30° C. can be expected. Thus a control or sensing system is required to insure temperature control or that the experiment does not start until the paper ambient temperature arrives.

The conditions outlined will be very difficult to maintain unless heating is provided in the area of the ground where the metabolic chamber is formed. A small radiant heater can be incorporated directly inside the metabolic chamber. The heat energy required is dependent on the thermal conductivity of the surface. At the present time it is possible only to estimate such a factor.

If it is assumed that the Martian surface is homogenous, then the heat loss through one square centimeter, with a temperature differential of 30° C. across a surface layer 0.25 cm. thick, in one second will be given in calories, Q, by the equation:

$$Q = K \frac{(t_2 - t_1)\, aT}{d}$$

where K is the specific heat conductivity; t_2 and t_1 are the temperatures in degrees C. of the two surfaces; a, the area of the surface in square cm.; T, the time in seconds; and d, the thickness of the layer in cm.

The constant K ranges from 0.0052 for basalt rock to 0.00013 for diatomaceous earth. The average value of K for the Earth's crust is 0.004. For basalt rock the heat loss is 0.62 calories per second, per square cm. To maintain the temperature difference of 30° C. on a basalt surface, an energy input of 2.6 watts is required (17). For a material similar to the average Earth crust, the required power is 2.1 watts, and for diatomaceous earth the power required is 0.067 watts.

For an experiment conducted for a period of two hours, the total power required to maintain the surface 30° C. above the ambient temperature at a depth of 0.25 cm. below the surface would range from 150 milliwatt hours to 5.2 watt hours. At an electrical potential of 20 volts, this power requirement could be supplied by a current of 100 milliamperes to the heating element, assuming all of the power dissipated was used to heat the surface.

This model of the Martian surface can be considered only an approximation. The heater in the instrument might be mounted so that it will irradiate the surface enclosed by the instrument. The heater current could be regulated by a proportional controller whose input signal could be derived from two thermistor elements. In view of the range of input power required for heating, a controller would be necessary and could be a series current regulator type.

4. Photosynthetic Experiment Capability

In order to provide for the conduct of an experiment to determine whether or not photosynthetic activity is present, a light source which will provide illumination from an incandescent filament and can be programmed on and off is required. In such a unit, two Sylvania ML-202A miniature incandescent lamps might be mounted so that they both illuminate the sample surface. The two bulbs provide redundancy even though they are designed to withstand shock and vibration. The illumination level at the sampling surface from a single bulb would be approximately 20 millilumens.

5. Chemical Gettering of Carbon Dioxide

The biochemical experiment is designed to detect the radioactive carbon dioxide evolved by organisms which have metabolized labeled nutrients. In order to maintain a reasonable detection efficiency for small quantities of gas, the gas is collected on the detector face by a chemical getter. The getter is lithium hydroxide evaporated by vacuum deposition onto a clean surface in a layer averaging 10^{-3} cm. in depth. This material has a high affinity for carbon dioxide and is temperature and pressure stable.

Carbon dioxide in the ambient Martian atmosphere plus any gaseous dissociation products evolved during storage of the medium as well as any biologically produced carbon dioxide will be collected by an exposed getter surface. If the quantity of the non-biologically evolved gas is large, it may saturate the getter surface and limit the experiment.

After the medium is released, the gaseous atmosphere within the instrument chamber is cleaned of carbon dioxide by the exposure of a secondary, or "clean-up," getter surface. The rate of uptake of a chemical getter and its capacity is a function of its temperature. The getter surface will be at the air temperature maintained by the heater element, or a higher ambient temperature.

The secondary CO_2 getter used in the detection module is of a special design. It consists of a spool with O-ring seals in both ends. Between the two bulkheads with the O-ring seals, there are two fins spaced so as to present, in the collection position, a maximum area of getter material. This getter material is lithium hydroxide evaporated by vacuum deposition in the exposed areas of the spool. There are six active circular areas to collect CO_2. With a diameter of 0.53 inch (approximately 15 mm.) the secondary getter exposes a minimum of area of 1.2 square inches (7.5 sq. cm.). During tests, for the sake of expediency, instead of coating the fins, filter paper washers were placed in the space between the fins and wetted with a saturated solution of barium hydroxide.

Once the chamber module has been cleaned of nonmetabolic carbon dioxide, the collection of biologically produced gas is effected by exposing the primary getter mounted in front of the radiation detector. In order to close off the secondary getter,the spool is moved one-fourth inch in its housing by means of an explosive piston actuator (Atlas 1 MT 114). This action removes the secondary getter from the

incubation-gas collection chamber. The same action exposes the primary getter on the face of the radiation detector. The end wall of the secondary getter is thick enough to prevent penetration by beta particles emitted by material collected on the secondary getter.

6. Chamber-to-Surface Seal

The experiment is conducted in a nearly closed volume to collect a maximum of metabolically evolved gas and prevent its dilution by the gases in the surrounding atmosphere. However, some leakage is desired to preclude possible exhaustion of an essential gas needed by the organisms. The near seal of the chamber to the surface is effected by means of a polyvinyl chloride bellows that pops out of the module as soon as it leaves the launcher tube. Polyvinyl chloride is not an accepted space material and in a flight model it will have to be replaced by a special bellows made of an elastomer such as Viton which is noted for its good hard vacuum characteristics.

Improvements for a final model can be achieved. The possibility of incorporating heat sensors into the bellows where the seal contacts the soil is a logical one. A special bellows is indicated for a final model, for which a special casting mold will have to be developed. It will provide a large soil area which will make the system more sensitive by exposing a larger sample.

7. Medium Ampul

The medium is carried in a sealed glass ampul, Figure No. IV-5. A 0.25 ml. quantity of medium can be sealed in this ampul. Alternatively,

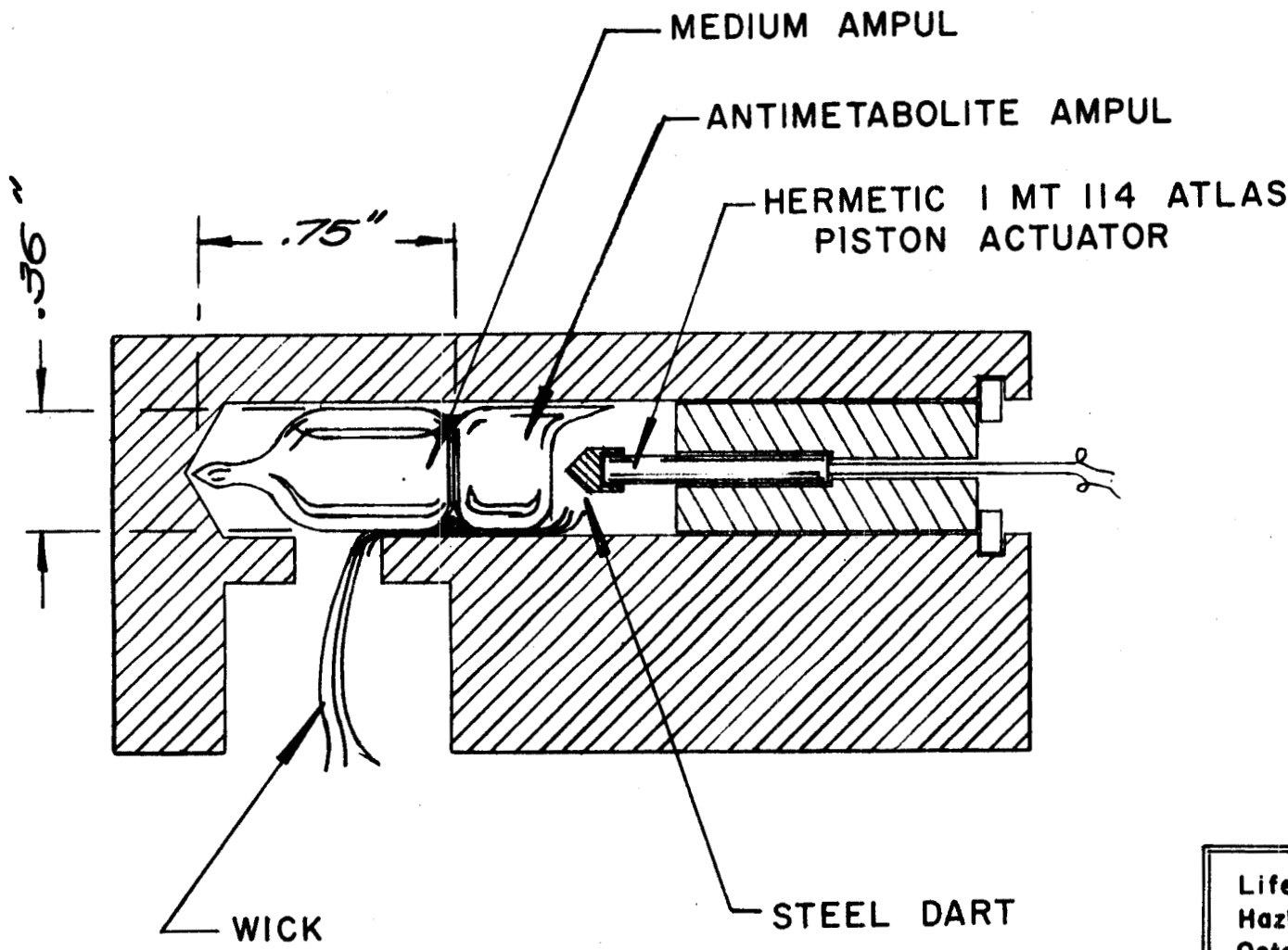

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Figure IV-5 – Medium Ampul and Ampul Breaker

antimetabolite can be incorporated into the medium or placed into a separate section of the ampul.

8. Ampul Breaker

The medium ampul is housed in a cylindrical cavity in the module. The ampul is introduced into the cavity followed by the ampul breaker device (Figure No. IV-5). The ampul breaker consists of a steel dart cemented to the tip of a piston actuator, Atlas 1 MT 114, the same one used for the self-righting legs and secondary getter actuator. Upon firing the piston actuator, the steel dart is propelled 5/16 of an inch to hit the bottom of the ampul. The ampul is shattered, allowing the medium to contact the wick which was in previous contact with the ampul.

9. Medium Dispersal

The liquid medium must be supplied to the sample surface. As stated previously, the volume of the medium is 0.25 ml. If the full quantity were suddenly discharged onto the soil enclosed by the chamber, the surface might be flooded. On the other hand, if the surface is very porous, the liquid might percolate away from the experimental area and curtail the experiment. To solve this problem, the medium is fed to the surface through a wick. This maintains a moist surface through the continuous supply of fresh medium at a rate which precludes flooding.

Two strands of Clark's #10 Mercerized Boilfast seem to give a desirable rate of nutrient flow into the soil.

D. DESIGN AND FABRICATION OF IN SITU INSTRUMENT, ELECTRONIC COMPONENTS

1. High Voltage Power Supply

Figures No. IV-6 and No. IV-7 are block diagrams of the module circuitry and original tentative high voltage power supply circuits, respectively. The tapped Hartley type oscillator utilizing the transformer distributed capacitance was the final selection for this application. The selection was based on the output voltage obtained and the number of required components for equal results from both circuits. Since the output voltage was of sufficient amplitude, a half wave rectifier was used instead of a voltage doubler.

The high voltage power supply (HVPS) is shown in Figure No. IV-8. Components R1, R2, C1, C2, D1, Q1 and T1 comprise the oscillator circuit, and D2, R3 the half wave rectifier. The rest of the components in the figure constitute the preamplifier. Resistor R2 can be adjusted to increase the power output of the oscillator. There is neither line nor load regulation. Since the load is constant, no load regulation is necessary. However, line regulation is essential as the output voltage is dependent on the supply voltage. With a regulated 24-volt supply and a resistor R2 of 330 ohms, the HVPS draws 52 milliamperes dc and delivers 1560 volts dc with a maximum ripple component of four volts (peak to peak). It is possible to obtain the same output voltage with a reduced supply voltage by decreasing the emitter circuit resistor R2, Figure No. IV-8. The oscillator output waveform is sinusoidal and the frequency of oscillation is approximately 100 kc.

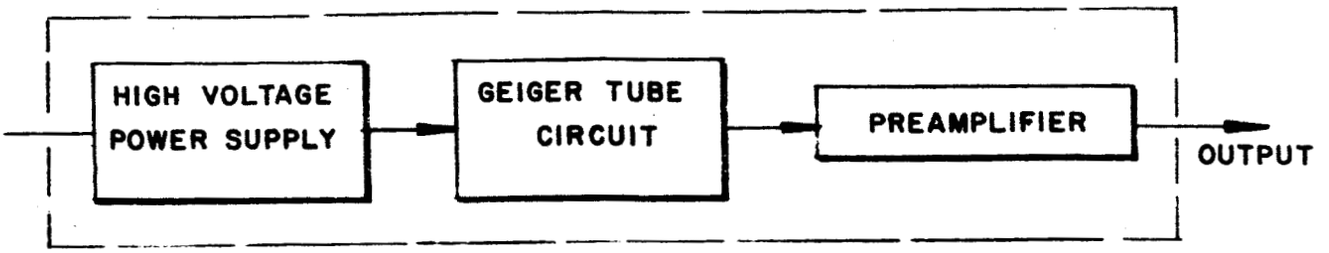
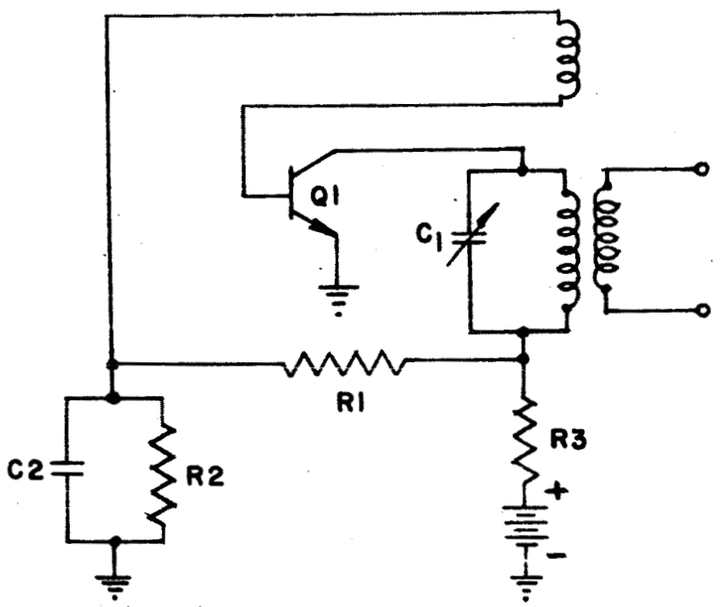
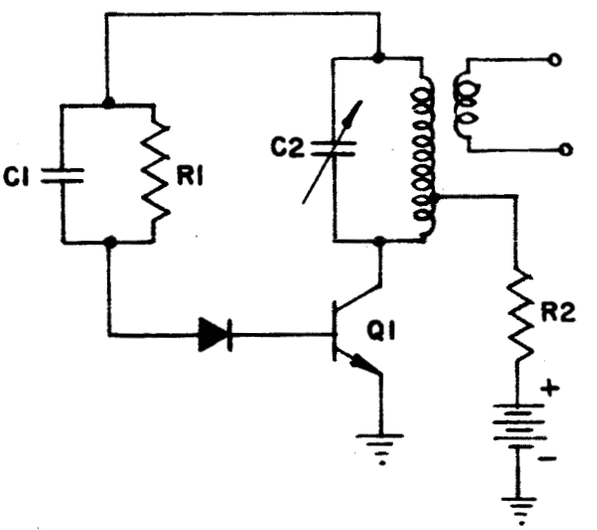


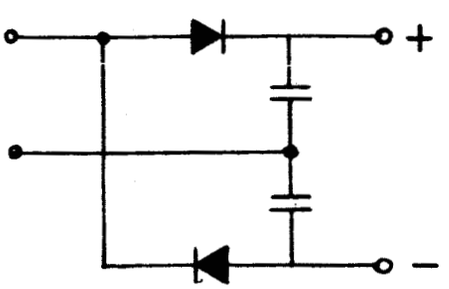
Figure IV-6 — Module Electronic Block Diagram



(a) OSCILLATOR-TUNED COLLECTOR TYPE



(b) OSCILLATOR – HARTLEY TYPE



(c) DIODE VOLTAGE DOUBLER

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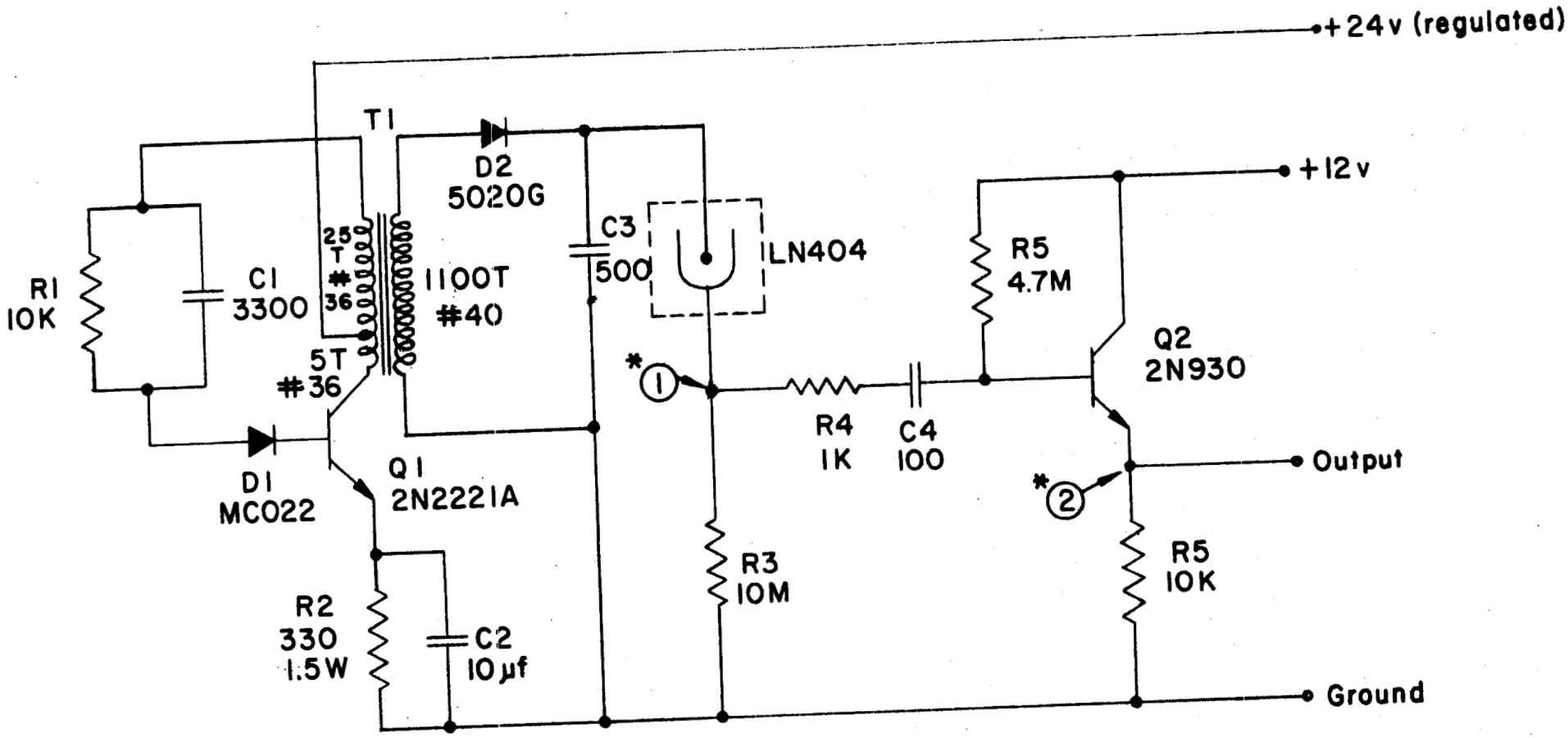
Figure IV-7 — High Voltage Power Supply Circuits



a – Resistors in ohms ± 5%

b – Capacitors in picofarads ± 10%

* Circled numbers denote check points

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Figure IV-8 - High Voltage Power Supply and Pre-amplifier

Lack of space prevented the addition of components to compensate for switching transients. As a result, switching transients reflect back to the supply, a condition that presented difficulties later on in the amplification of pulses.

Components which are not readily recognizable are the diodes D1, D2 and the transformer T1. D1 is a Microsemiconductor Corporation fast recovery, low capacitance micro-diode and D2 is a Simcor high frequency, 2000 peak inverse voltage rectifier diode. Other types of units were tried in this circuit, but the two mentioned gave the best result mainly because of their fast recovery time and low junction capacitance. Resistors are 1/10 watt, 5% tolerance and capacitors 10% tolerance except as noted on the schematics. The transformer was made by winding 1100 turns of #40 AWG Nyclad wire as the secondary, 20 turns #36 AWG Nyclad wire as the feedback, and five turns #36 AWG Nyclad wire as the primary on the bobbin of a pot core. The pot core used was a Ferroxcube P/N 1408C-A160-3B9. This pot core has a tuning slug. The slug can be used to vary the inductance at the pot core and thus the frequency of oscillation. Since the change in frequency was not significant and the output voltage remained constant throughout the tuning range, tuning was not used.

All components were selected to have operating temperatures of at least 100° C. and storage temperatures upward of 150° C. This preselection was made to insure proper operation of these components after having been subjected to sterilization temperatures.

The HVPS was operated continuously for 48 hours at room temperature with periodic checks made on the dc output level. Throughout this test there was no observable variation or change in the dc level. However, since the module case doubles as a heat sink, its temperature rose to approximately 38° from an ambient temperature of 24°, an increase of only 15° C.

2. Detector

The detector was designed and manufactured especially for this use by LND Incorporated, 3230 Lawson Boulevard, Oceanside, Long Island, New York. It was designated LN404 and is a Geiger-Müller (GM) proportional counter, Figure No. IV-9. It measures one-half inch in diameter at its widest point by one inch in length overall. The ionization chamber itself is just one-half inch in diameter by seventeen thirty-seconds inch long. Electrical connections are made to wires which are welded to the tube. The tube has a mica window 1.5 mg/cm^2 thick, operates at 1100 to 1450 volts dc and has a plateau greater than 250 volts with a slope less than 3% per 100 volts. The fill is argon with 10% methane at a pressure of one atmosphere.

The detector circuit consists simply of a resistor in series with the GM tube. This is shown in Figure No. IV-8 as LN404 and R2. Other possible combinations that could be used are shown in Figure No. IV-10. The configurations usually used are those of (a) and (b), but (a) requires capacitor C to have a dc working voltage at least as high as the GM tube supply and (b) requires an extra wire for use as

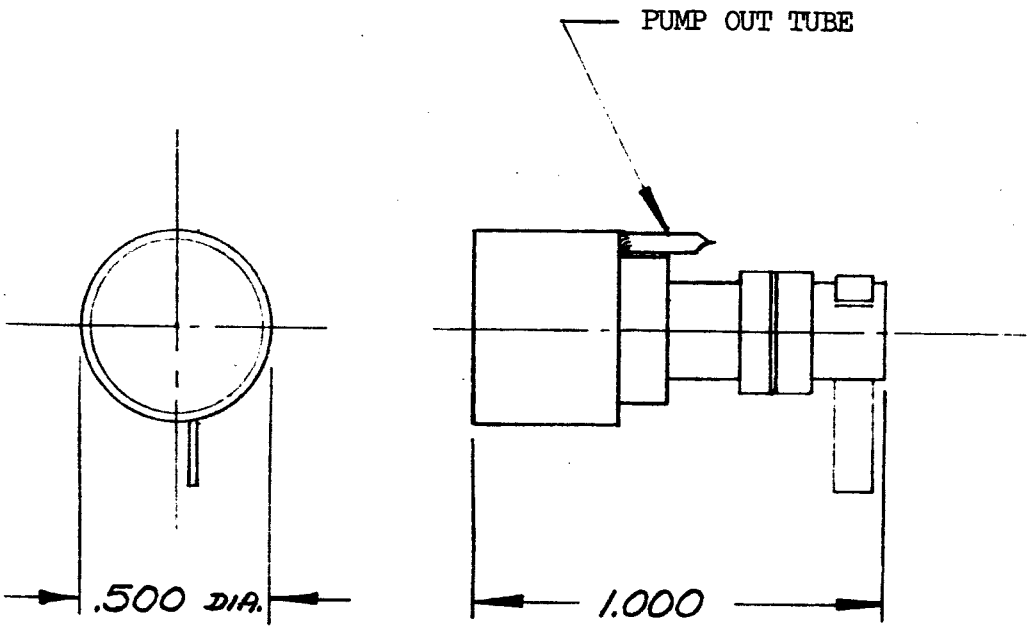

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Figure IV-9 - LND 404 Geiger Tube

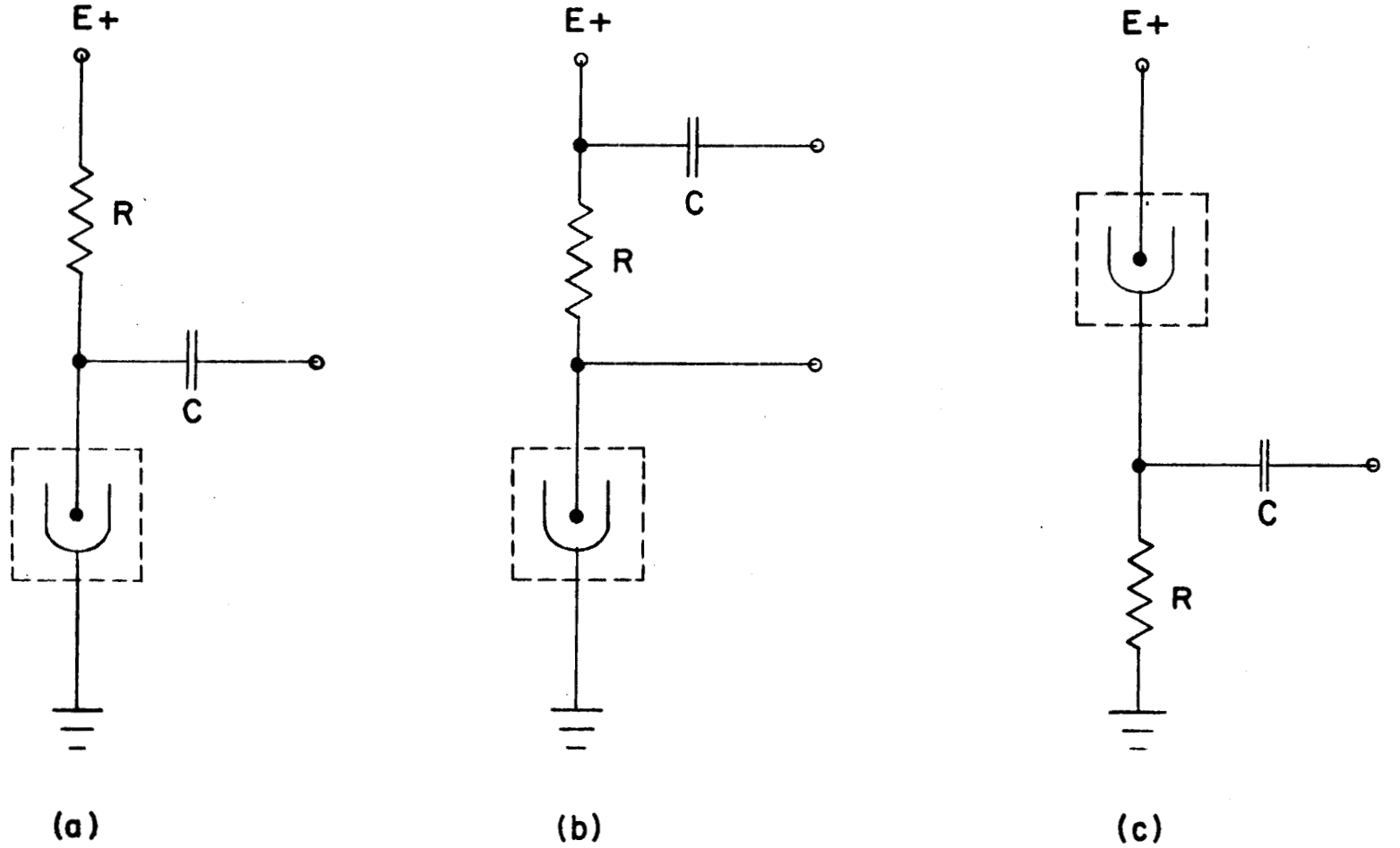

Figure IV – 10 - Detector Circuits

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a common in any following circuits. Unfortunately, capacitors meeting the high working voltage requirement are comparatively large in size. In these two configurations the full ripple component of the GM tube supply would appear as part of the signal pulse. For these reasons the circuit of Figure No. IV-10 (c) was used. It was necessary to isolate the cathode of the tube from ground but since the cathode is, for all practical purposes, at ground potential, this proved to be a relatively simple matter.

The tube came provided with a wire for connections to the anode but none on the cathode. Using a Weldmatic Power Supply Model 1024B, Control Unit Model 1028C, and a copper electrode set at seven pounds pressure and 20 watt-second energy, a wire was successfully welded onto the cathode of the tubes.

Pulses obtained across resistor R3, test point 1, were 0.1 to 3.0 volts in amplitude with the majority being greater than 0.5 volts. The pulse width varied with the pulse amplitude, but was in the order of 0.3 milliseconds. This implies a count rate of 200,000 counts per minute as a maximum. The noise or ripple component was 0.4 volts. Since this is a peak-to-peak value, it was possible to count any pulse greater than 0.2 volts amplitude. The breadboard model gave less ripple but this was attributed to the remoteness of the HVPS from the rest of the circuit. High density packaging presented pick-up problems.

3. Preamplifier

As the pulses obtained from the GM tube were in the order of 0.1 to six volts, it was thought that the attenuation that would be experienced in the umbilical cord could be considered negligible. This turned out to be the case, although the resistance of the wires in the umbilical cord was high, namely 0.14 ohms per foot. An impedance matching device was needed, however, to match the high impedance of the GM tube circuit to the low impedance of the cord and following stages of amplification. The emitter follower circuit of Figure No. IV-8 composed of components R4, R5, R6, C4 and Q2 serves this purpose. This constitutes the preamplifier. A video pulse integrated circuit amplifier was tried for this application but proved unsatisfactory for impedance matching reasons.

The pulses appearing at the output of the preamplifier, check point 2 on Figure No. IV-8, were 0.1 to six volts in amplitude with the majority greater than 0.8 volt. This compared favorably with the input pulses since, at best, the gain of an emitter follower is one. The pulse width, however, decreased to 0.1 millisecond. This was due to the differentiator composed of capacitor C4 and the input impedance of the emitter follower. The ripple or noise increased from 0.4 volt to 1.2 volts. This was unfortunate, but the signal was still usable as the majority of the pulses were greater than 0.8 volt. The signal at the capsule end of the umbilical cord did not change except for a higher noise component, attributable to pick up through induction along the line.

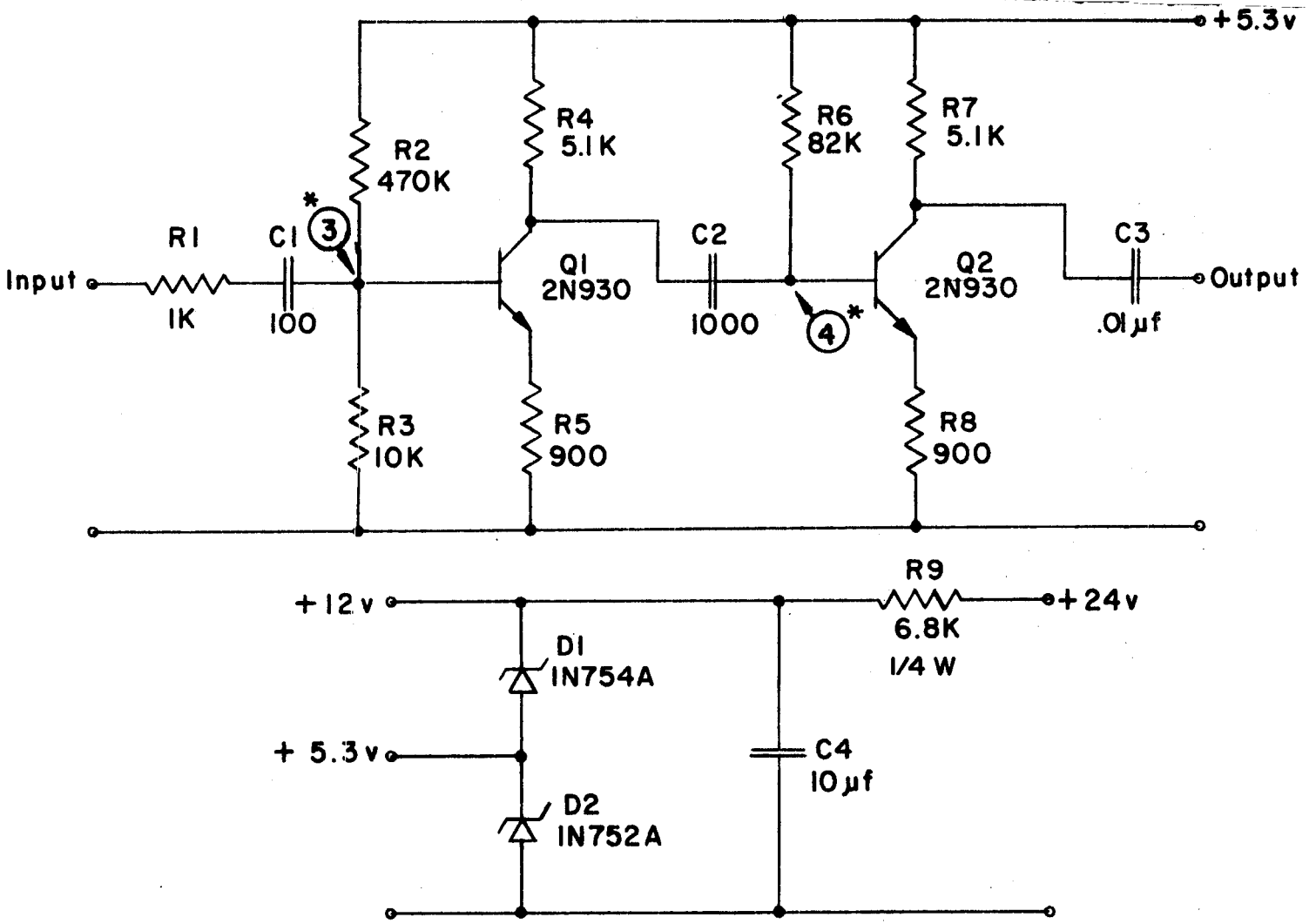
The noise level had risen to 1.7 volts. To offset this undesirable effect, the signal and ground wires were twisted together and a subsequent reduction in noise level was achieved. The use of a shielded signal wire should clear up this difficulty. The signal obtained at the capsule end of the cord was of sufficient amplitude and energy to drive a counter. The counter had a level selector that was used to block out the noise.

In an effort to keep both the noise and current level low, 12 volts dc were used as a supply to the preamplifier. The voltage transients that appeared on the 24-volt supply were thus suppressed. This accounted for the increase in noise level in the preamplifier since any transient in the line would look like a signal to the amplifier.

4. Pulse Amplifier

Originally, the amplifier used on Gulliver III was to be used. As it turned out, operation was erratic. Output pulses were not one to one in relation to the input pulses. At times, there were more, and at other times less, output than input pulses. The Gulliver III amplifier was replaced with the two-stage amplifier of Figure No. IV-11. Figure No. IV-11 also shows the voltage regulator that was used to supply the voltages for the module and capsule amplifiers.

In operation, transistor Q1 is biased in the off state and transistor Q2 is biased on. When a pulse of sufficient amplitude to override the level established by R2 and R3 comes along, Q1 conducts and its collector voltage goes negative, yielding a negative going pulse that is applied to the base of Q2. Since Q2 is biased on, a negative



a - Resistors in ohms

b - Capacitors in picofarads except as noted

*Circled numbers denote check points

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Figure IV-11 – Pulse Amplifier and Voltage Regulator

pulse will tend to turn it off, thereby increasing its collector voltage and producing a positive going pulse at the output. The voltage level established by R2 and R3 prevents most of the noise from passing. The pulses are differentiated twice by the differentiators consisting of C1 and the input impedance of the amplifier and C2 and the input impedance of the second stage.

The output of the pulse amplifier may be amplified further, used to operate a Schmitt trigger or fed directly into a counter as was done by this laboratory. The noise level of the output signal is just 30 millivolts or 15 millivolts zero to peak. Anything to which the output of the amplifier is attached must therefore have a level selector. Output pulses are 0.5 to four volts in amplitude with the majority being greater than 1.0 volt. The pulse duration is two microseconds. The signal was fed directly into a Nuclear-Chicago Counter Model 182 with the level set at 59 mv. Good results were obtained. The amplifier was also used to trigger a Schmitt trigger, but, as there was not much point in that, it was left out of the circuit.

Again a supply of only 5.3 volts was used in an effort to reduce line voltage excursions and to minimize current consumption.

5. Packaging

The space available for packaging the electronics was one inch long by 3/4 inch wide by 3/8 inch deep. The technique used was that known as cordwood packaging and point-to-point soldering.

The first step was to slip the HVPS transformer into a phenolic sleeve, pot it and test it to insure that the ferrite core had not cracked. The rest of the components were placed between two epoxy-glass boards and the proper connections were made by point-to-point soldering. At this point, the module case was modified to simplify insertion and removal of the package and to make it possible to use a mold for potting purposes. Although this modification made it possible to remove the electronics when clean-up time came between experiments, it was not done. It was desired to know what effects sterilization processes would have on the electronics. While the NASA sterilization requirements were not applied, it was found that if the package was baked at 100° C. for an hour after clean-up, it would dry completely. The module has gone through this cycle several times without suffering any deleterious effects. The only component washed and dried separately was the GM tube, as extra care had to be taken to avoid damage to the mica window. The tube was merely air-dried.

To the removable part of the modified case a fuse type clip was mounted. This clip was electrically insulated from the part as its function was to hold the HVPS transistor so that the module case could double as a heat sink. Next, the umbilical cord and transformer were attached, the entire assembly placed in a mold, potted, and cured for one hour at 100° C.

After curing, the package was trimmed, put in place in the module, and made fast by means of four screws. The GM tube was then

insulated, set in place, and connected. The three squibs that operate the legs, break the ampul, and move the baffles were connected last for safety reasons. That completed the packaging. The module was then ready for tests and experimentation.

To insure good heat transfer, a heat sink compound, Dow Corning 340, was used on all metal-to-metal joints and other applicable points. The potting compound used was Sylgard 182 resin because of its resiliency property and also because component changes, if necessary, could have been made. As it turned out, this was not the best choice, as explained in the next section.

6. Problems Encountered

Some problems arose that were particularly annoying because they wasted time. To mention two, it was discovered that the manufacturer's data sheet for a pulse-video amplifier that was tried as a preamplifier was erroneous and, secondly, the Gulliver III amplifier could not be used as a follow-up amplifier. The most important difficulties encountered were with the potting, the GM tube, and noise. These problems are taken up in the following paragraphs.

a. Potting

The electronic circuit was checked at various points in time during the encapsulating procedure. The transformer was checked both before and after potting. When the entire circuit was ready for the mold, it was checked again and again when it came out of the mold. In all instances it was observed that potting resulted in a decrease

in high voltage. The drop was in the order of 300 volts. It was found that spraying with an epoxy insulating varnish before potting reduced this loss considerably. To compensate for this drop, it was necessary to decrease the resistor in the emitter circuit of the HVPS. The solution to this problem would be to use a more suitable potting compound. One such would be a mixture, by weight, of 70 parts Epon B15 Epoxy, 30 parts Thiakol LP-3 as a resiliency agent, and seven parts Deapa as a curing agent. This mixture has been used successfully in the past in similar applications. It was not used here because it sets to a much harder consistency than the Sylgard resin. However, now that the circuit is a proven circuit, it could be used in future models.

b. GM Tube

Of the six tubes that were purchased, only two usable tubes are left. It was found early in the program that a wire was needed on the cathode, as well as on the anode in order to make electrical connections. The tubes came from the factory with a wire on the anode, but none on the cathode, and, prior to the successful welding on of cathode wire, an unsuccessful attempt was made to solder a wire on one of the tubes. That left five tubes. Two of the remaining tubes never functioned. It is assumed that the gas leaked out of one of these since it had a cracked window. The other, although it appeared undamaged, failed to function for some unknown reason. The fourth tube quit after approximately 10 hours of use. Of the remaining two tubes, one has been in use for at least 40 hours and the other for approximately 24 hours. Both, as of this writing, are performing well.

Although the manufacturer's specifications state that the operating voltage of these tubes is 1100-1450 volts dc, it was found that, in order to obtain a usable counting rate from the soft beta of C^{14}, 1560 vdc had to be used. It is a characteristic of GM tubes that, if the voltage is raised to a point beyond its plateau, the counting rate increases, but the counter may produce self-sustained, continuously repeating pulse discharges even in the absence of external ionizing radiation. Operation beyond the tube's plateau is likely to damage the counter. To determine if the upper limit could be reached, the supply voltage to the HVPS was raised to 30 volts dc. This increased the GM tube voltage to 1630 volts dc. The above mentioned characteristic was not observed. The only result was a slight increase in pulse count. Since a usable count was obtained at 1560 volts dc and maximum tube life is obtained when operation of the tube is near the lower end of its plateau, it was decided to set the voltage at 1560 volts dc with a maximum of 1600 volts dc.

c. Noise

The effect of noise was that it set a limit to the size of the smallest pulse that could be detected. The noise was quasi-sinusoidal and had a frequency of approximately 100 kc, the same as the oscillator frequency. The main source of noise was the HVPS. It produced a ripple on the high voltage and switching transients on the line. The ripple appeared directly on the signal while the switching transients were introduced through induction in the umbilical cord and the components in

the high density package. It was also found that dust and fingerprints on high voltage components caused some noise and that transients in the line were amplified by the amplifiers.

Several things were done in an effort to minimize the noise. Some resulted in a decrease and some did not. One that resulted in negligible results was magnetic and electrostatic shielding of the HVPS transformer. The negligible results were attributed to the inherent shielding of the pot core. Since it was not needed, and took up needed space, the added shield was discarded. It would have been better to shield the entire HVPS but lack of space prevented this. Since components could not be added to compensate for transients and ripple, for the same reason, it was decided to shield the lines. The ground and 24-volt supply lines in the umbilical cord were twisted together to simulate some form of shielding. This reduced the noise pick-up significantly.

It was next decided to shift the components around in the module package. The components that make up the preamplifier were placed as far away from the transformer and its associated circuitry as possible. Short leads were used and everything was cleaned with alcohol before potting to remove dust and fingerprints. The results of all this maneuvering were that the noise was reduced to a level lower than the level of the majority of pulses as seen at the input of the pulse amplifier. Some pick-up in the umbilical cord was experienced in the field when in the vicinity of high tension lines, but this should be eliminated by using a shielded signal line. In this instance, a different location

was the easy solution as the module was already packaged and it would not have been a simple matter to change the umbilical cord.

7. Other Possible Design Arrangements

As proposed, this instrument was to have self-contained modules. By this, it was meant that each module was to be completely independent from all the others so that, if any failed to function, none of the others would be affected. Rather than becoming involved in the problem of transporting high voltages long distances, as through the umbilical cord, it was decided to construct a module containing a high voltage power supply, a GM tube and a preamplifier. Any further amplification was to be done in the capsule.

Another configuration that could have been used without losing the individuality of the modules would have been to place the HVPS and pulse amplifier in the capsule and the GM tube and preamplifier in the module. This method was thought to present a high voltage transportation problem and possibly to introduce a higher noise component by induction in the umbilical cord. It was decided to investigate this approach because it had some decided advantages.

A breadboard model was modified to simulate this technique. The results were encouraging. It was found possible to transport the high voltage without any difficulty and the noise level was not prohibitive if the signal and ground wires were twisted together. No shielding was used. The advantages of this configuration would be a lighter module, more space in the module for other circuits such as

thermal control circuit, etc., and a better signal to noise ratio. The noise level could be reduced substantially by adding a few components to the HVPS to compensate for transients and ripple. The entire HVPS circuit could also be packaged separately and completely shielded. This would also make it possible to use 24 volts dc throughout. The main disadvantage would be that a heavier umbilical cord would be required as the best way to transport the high voltage would be through a coaxial cable. The capacitance of the coaxial cable would assist in damping out any residual ripple so that would be an asset. The signal wire would also have to be a coaxial line.

A further extension of the same idea of reducing noise would be to have only the Geiger-Müller tube in the module. This idea was not tried, as there was nothing further to be gained.

With the present HVPS it was found possible to operate at least two GM tubes. With a slight modification, which does not require any additional components, it should be possible to service several tubes with one supply. One HVPS could then serve all modules from a location in the capsule. This could result in an overall weight reduction. Reliability could be assured by including a back-up supply - in other words, having a dual system.

E. PRELIMINARY TESTING OF IN SITU INSTRUMENT

1. Mechanical Components

Before the electronics components were available, routine preliminary electromechanical testing was performed in which simulated drops after capsule ejection were carried out.

a. Self-Righting Mechanism

Operation of the self-righting mechanism was tested. Initially, it was found that the short legs located on the back of the module had a tendency to lift one end of the module instead of rotating it about its longitudinal axis. This was due to the fact that the plane of action of the legs was away from the center of mass of the module. To eliminate the resulting movement between the plane of action of the legs and the center of mass of the module, the legs were extended forward until the ends were in the approximate plane of the center of mass of the unit, Figure No. IV-3. This modification has provided a double benefit. First there is no moment when the legs are released. The module rotates until it comes to rest with its flat surface on the bottom. The extremely fast action of the piston actuator imparts enough kinetic energy to the wire legs so that they overtravel the stroke of the actuator and continue their travel past the elastic limit of the wire material. The legs are, in effect, permanently deformed and, thereby, they acquire a longer effective length which gives better footing for the rotational motion and better stability when the module comes to rest.

b. Ampul Breaker

In the original design, provision was made to break the ampul by means of a steel dart sealed with an O-ring to prevent contamination of the medium by the explosive gases generated by the Hercules Powder OP 152 BO actuator. This actuator, loaded with 8.0 mg. of lead Styphanate, proved to be too powerful. The ampul was shattered into

glass powder. Although this did not affect the nutrient or its flow into the wick, it did, on occasion, cause the failure of the squib barrel retaining ring. A test fixture was made to see if an actuator similar to the ones used for the legs and baffle could be used. Successful operation of this device led to a change in the squib barrel in the module to house the new steel dart and actuator (Figure No. IV-5). This change has been fortuitous in that all the actuators in the unit are now identical. A small saving in weight was also achieved.

c. Medium Dispersal

Various wick materials and configurations were studied by breaking ampuls to release water onto the wick. The first tests were performed using absorbent paper rolled and flattened into a cross section of 1/64 x 1/8 inch. The medium was quickly conducted into the soil. The rate of flow, however, appeared to be too fast for average soil conditions. Later, strands of cotton string were tried out, starting with four strands of Clark's #10 Mercerized Boilfast. Again, the flow of medium into the soil was considered to be too fast. Reducing the number of strands to two seemed to produce the desired flow. During our simulated tests in the laboratory, soils of very loose, small grains have remained wet for periods of up to 16 hours. However, it would seem desirable to increase the amount of medium provided to a minimum of, say, 0.5 ml.

d. Module-to-Surface Seal

Testing of the flexible bellows was done in conjunction with the tests performed to ascertain the effectiveness of medium dispersal. The sealing properties of the bellows were found to be quite satisfactory

in that they would prevent evaporation of the medium for long periods of time. However, disappointingly low responses occurred in the preliminary soil tests with the instrument. Investigation of the various component systems indicated that the bellows diameter restricted gas diffusion severely. The effective bellows opening was only 0.076 square inch. The flexible bellows was replaced by a smooth, hollow cylinder. A short aluminum bushing 3/4" O.D. x 11/16" I.D. with the edge in contact with the soil sharpened to a knife edge served this purpose. The sharp edge digging in the soil effected the desired seal. The increase in cross-sectional area, now 0.37 square inch, gave immediate response improvements of approximately twofold.

e. Carbon Dioxide Getter

In addition to the bellows problem, low responses in the early tests were, in part, attributed to the fact that the getter was applied to 0.4 mil aluminized Mylar film rather than directly to the geiger tube window. The film was fixed to the window. This arrangement was used to prevent contamination of the mica window. In the final tests, the $Ba(OH)_2$ was applied directly to the mica window. This produced a twofold increase in readout efficiency. (The ultimate plan called for sputtering of LiOH onto the window.)

After each test, the geiger tube was decontaminated by cleaning with acetone applied with a very soft camel hair brush. This procedure was repeated not less than six times with no residual contamination or other ill effects on the tubes which are still usable.

The barium hydroxide coating was applied to the mica window by the following method: Clear spray coating Krylon 1301, by Krylon Inc., Norristown, Pa., was sprayed in abundance into a small petri dish. A soft camel hair brush was wetted with the material in the petri dish and, very quickly, the Krylon was brushed onto the mica window. Immediately, another clean brush was dipped into dry barium hydroxide powder and gently tapped over the geiger window to spread the powder over it before the Krylon had a chance to dry. Excess barium hydroxide was removed by careful sweeping with the brush after the Krylon had dried.

The following combinations of getter materials and binders were tried in order to improve the $C^{14}O_2$ collection efficiency for the tests:

$Ba(OH)_2$ + Plaster of Paris (5% in H_2O)
Ascarite powder + Krylon
$Ba(OH)_2$ + Silicone grease
LiOH powder + Krylon

None of these was as effective as the barium hydroxide and Krylon. Unfortunately, time did not permit experimentation with LiOH sputtering to produce getter surfaces.

2. Electronic Components

a. Detector Efficiency

Several radioactive sources were prepared by the radiation laboratory for use as standards in determining the efficiency of the module. The sources were prepared by plating six one-half-inch-diameter filter paper discs with 0.01-0.02 ml. glucose-1-C^{14} solution having a

specific activity of 2.5 μc/ml. One-minute counts of each "pill" were taken on a laboratory counter whose efficiency for soft beta was 30%. From this and the count obtained on the module, it was determined that the efficiency of the module for soft beta radiation was 6%. Table No. IV-1 gives the data from which computations were made. The average of several counts is what is noted under module counts. Source No. 1 gave a low count but that was because it had been used previously and was frayed by the time the data were taken. Source No. 5 gave a high count. Any explanation for this occurrence would be merely speculation. Since the sources were used previously several times, with No. 1 source being used most frequently and the rest randomly, it is possible that No. 5 source was less worn than any of the others. The rest of the sources gave comparable results as evidenced by the percentages obtained from each.

b. Carbon Dioxide Getter

In the preliminary experiments using soil samples, it was decided to add a Mylar window to the GM tube to facilitate cleaning between experiments. The Mylar was 0.4 mil thick. Krylon was sprayed on the Mylar to act as an adhesive for the barium hydroxide getter. The Mylar window Krylon getter combination proved too absorbent. With the Mylar window in place and no getter, the counts obtained with the standard sources were at best one-third of that without the Mylar. It was then decided to put the getter directly on the mica window. Good results were obtained using this method. To insure that no pulses were

Table IV-1 - Module efficiency for soft beta radiation

STANDARD SOURCE NUMBER	ONE MINUTE COUNTS		COMPUTED MODULE EFFICIENCY %
	LABORATORY COUNTER*	MODULE	
1	8013	1413	5.30
2	7290	1447	6.04
3	5642	1173	6.24
4	6977	1415	6.09
5	5141	1206	7.05
6	7567	1542	6.11

*Sharp Lab., Inc., Widebeta proportional gas flow counter (30% efficiency)

being generated or lost, counts were taken at various check points. Circled numbers on Figures No. IV-8 and No. IV-10 denote these check points. There was observed a one-to-one correlation between the output of the tube, check point 1, and the output of the amplifier. The very small pulses appearing at check point 1 were blocked out in the amplifier along with the noise in the level selector circuit consisting of resistors R2 and R3, Figure No. IV-10. Since the number of very small pulses was not large, it was thought that this compromise could be made - a slight count loss in favor of a considerable reduction in noise. It was felt that, in subsequent designs, the placement of the HVPS in the capsule rather than in the module would be advantageous in this respect.

c. Power Requirements

The overall power requirements, exclusive of heating requirements, were 57 milliamperes at 24 volts dc. In addition, there are three squibs that open legs, break the nutrient ampul, and move the baffle. These squibs require 1.0 ampere for 10 milliseconds. These are momentary loads, but it is recommended that the circuit be opened after firing the squibs as they are not guaranteed to be open circuits after firing.

F. FIELD TESTING OF IN SITU INSTRUMENT

Following is a description of a typical test performed in the field in back of the Life Systems Division laboratory at Reston, Virginia.

For the purpose of "landing" the module, an undisturbed area free of grass was chosen. AC power, obtained through an extension cord

100 feet long, was used to operate the Nuclear-Chicago Model 186 counter, a Harrison Labs 6202 A DC power supply, and an oscilloscope, Tektronix 561 A, with dual trace amplifier, Type 3A1, 3B3 time base and high voltage probe P6013 (Figure No. IV-12).

A junction box housed the electronic components which would be in a landing capsule. This box also contained the push botton switches to operate the squibs in the module. The junction box was connected to the module by means of an umbilical cord through which power was transmitted and output signals were returned and directed to the Nuclear-Chicago counter.

In preparing for the test, the medium ampul and the ampul breaker were assembled into the module. The secondary getter was fitted with filter paper discs to receive the saturated solution of barium hydroxide. The geiger tube without getter material was temporarily assembled in the module and electrical connections were made to the electronic module. The geiger circuit response was checked out by applying power, placing a standard source in front of the clean window and observing the count rate in the Nuclear-Chicago counter. If the count rate was normal for that particular source, it was assumed that the unit was working properly. The geiger tube was then carefully removed from its housing without breaking the electrical connections. The $C^{14}O_2$ getter was then applied to the window of the geiger tube as previously described. The geiger tube was reinstalled in its working position in the module and again checked electrically. Immediately

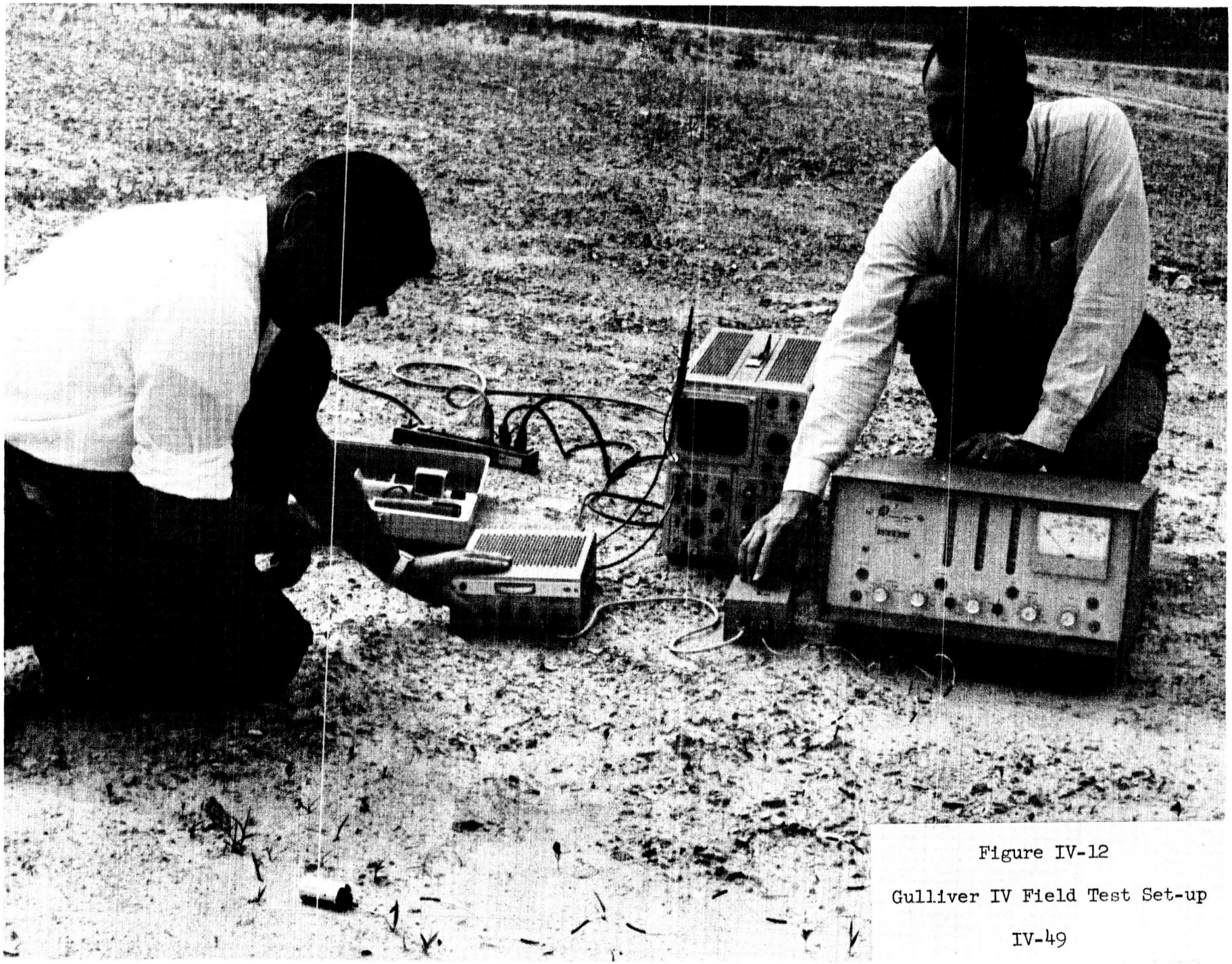


Figure IV-12

Gulliver IV Field Test Set-up

thereafter, the secondary getter filter paper washers were wetted with barium hydroxide solution and the secondary getter installed, protecting the primary getter in the geiger window from the atmosphere.

The preceding operations were usually carried out in the laboratory after the field test site has been chosen and the rest of the experiment was readied on site.

The module was then carried out to the test site, all electrical connections made, and the equipment turned on. Check-out was usually carried out by taking a 15-minute background count. Once it was established that the systems were working, the module was dropped on the ground from a height of several feet and the test begun.

First, the squib that releases the legs was activated. The module then assumed the test position on the ground. The metabolic chamber was formed by this action as the bellows contacted the ground. Another background count was taken, usually five muntes long, after which the medium ampul was broken. The secondary getter began to collect non-metabolic $C^{14}O_2$. The count rate was monitored every minute during the initial phase of the test, the only lost time being that necessary to reset the counter to zero after each minute interval. Five minutes later the medium ampul was broken, and the secondary getter was moved out of collecting position by firing of a squib. The primary getter on the window of the geiger tube was thus uncovered. From then on, the metabolic phase of the experiment continued for the time required. During field testing, readings were taken and recorded nearly every minute.

Figure No. II-3 is the curve produced by the field test performed on May 27, 1966. Biological details of this test can be found in Section II, D.

V. RECOMMENDATIONS CONCERNING RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

A. RATIONALE

Additional knowledge to be gained by impending observations of Mars may affect the design of the radioisotopic biochemical probe in minor or major ways. Advanced technological developments might do the same in the time remaining before the experiments must be "frozen" for an actual space flight. However, at this point in the development program of this experiment and in NASA's planetary exploration program, it seems appropriate to take stock and make some recommendations.

B. INSTRUMENTS

Breadboard models of Mark III Gulliver have been repeatedly tested in the laboratory and in the field with success. It is recommended that this type of instrument be included in the experiment.

Testing of the Mark IV, in situ model, has only begun. It cannot be evaluated on the same basis as the Mark III. Intensive testing of the Mark IV should be conducted. If this model also proves itself, the experiment should be conducted in both the Mark III and Mark IV instrument configurations. The Mark III, Figure No. V-1, retrieves a soil sample and conducts the experiment inside a closed, or almost closed, chamber. The "sticky string" sampler integrates the sample over any linear distance desired. Conducting the experiment in the chamber permits better environmental control over such factors as temperature, pressure and humidity. The Mark IV, or in situ instrument, Figure No. IV-3, has the advantage

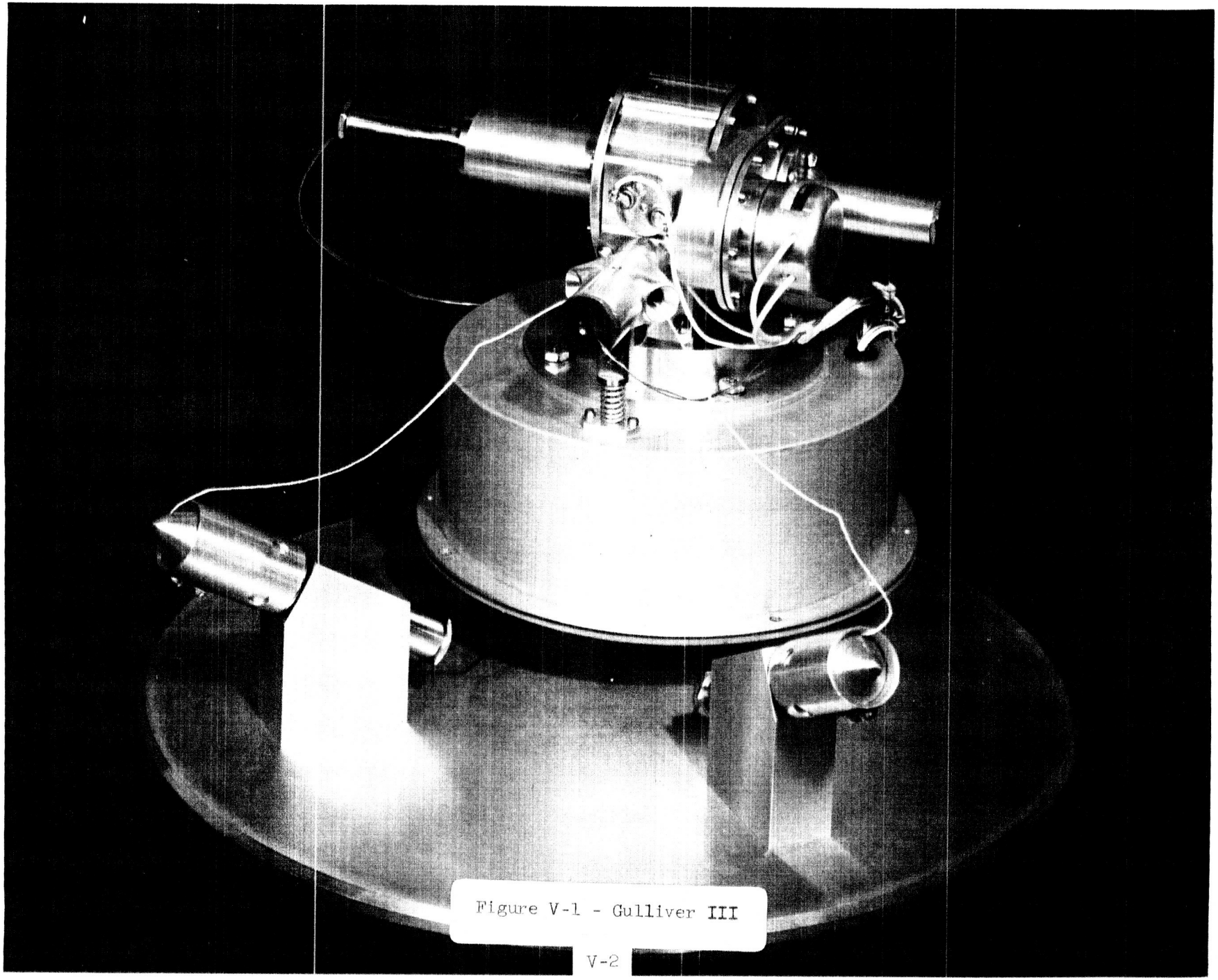


Figure V-1 - Gulliver III

of conducting the experiment in the undisturbed environment. This is especially important if photosynthetic organisms are encountered. In addition, however, this instrument also samples in depth in that the medium is free to percolate through the soil. Should the ultraviolet flux at the planetary surface be very high, it is possible that the majority of the microorganism population would lie beneath the protective surface.

An important problem associated with the in situ instrument needs to be resolved. Under the low atmospheric pressure on Mars, will the liquid medium evaporate too rapidly through the imperfect instrument-ground seal and by percolating through the ground under the instrument wall followed by capillary rise to the atmosphere? If this problem exists, perhaps it can be overcome by increasing the amount of medium carried. The diameter of the bellows might also be increased to decrease the loss of medium through the percolation route. A related problem deserving attention is determining to what degree the CO_2-getting capacity of the getter will be spent on ambient CO_2 on Mars contained in and leaking into the incubation chamber.

Photosynthesis tests have been developed for both types of instruments. All of this work has been confined to the laboratory. No attempts to detect photosynthetic activity in the field have been made. Such tests should now be made. They may adequately justify the instrumental approaches. However, it is possible, or even likely, that the numbers of photosynthetic microorganisms encountered on land may not produce adequate responses. Should field tests find this to be the case, it is recommended that more sensitive photosynthesis detection tests be

developed because the photosynthesis question on Mars is an extremely important one.

The Mark III instrument would include the heterotrophic-photosynthetic experiment through the incorporation of a midget lamp timed to give alternate light and dark periods, each of one hour duration. The Mark III instrument should be supplied in pairs, one test unit and one control unit, with the number of pairs depending on the capacity of the lander. The timing sequence and logic system for the Mark III instrument are described in the Second Annual Progress Report (11).

A temperature and a pH probe should be incorporated into the incubation chamber and readings of temperature and pH made with the same frequency as those on radioactivity of collective gas.

If the in situ field testing program is successful, multiple in situ units should be incorporated into the lander for injection onto the planetary surface. The number of units will again depend upon the capacity of the lander. Two different types of in situ units should be provided if the photosynthetic experiment is included. One type will perform the basic, labeled substrate experiment and also incorporate a light so that the heterotrophic-photosynthetic experiment can also be conducted in this unit. The second type of unit will perform the strict phototroph experiment through the provision of a light source similar to that in the first type of unit and, in addition, a supply of $C^{14}O_2$. A means for expelling the excess $C^{14}O_2$ and replacing it with the planetary atmosphere will be provided. These features have yet to be incorporated

into the in situ model. Both basic types of in situ units should also contain temperature probes.

The timing sequence and logic systems for both types of in situ units remain to be engineered. However, the unit conducting the basic Gulliver experiment and the heterotrophic-photosynthetic experiment should be programmed to achieve the same operations as that of the Mark III instrument.

It is suggested that the unit in which the strict phototroph experiment is conducted provide for a six-hour period of simultaneous exposure of the surface sample to light and the $C^{14}O_2$. Following replacement of the labeled carbon dioxide with planetary atmosphere, the light should be extinguished and the gas collection and counting system activated. The logic system should provide for the institution of a suggested one-hour light-dark cycle once the radioactivity collected from the test unit exceeds twice the level of the control. A temperature probe should also be installed in this unit and readings obtained at the same time as the radioactivity readings.

Either of the two types of in situ instruments can function as test or control units, both being provided with antimetabolite. A logic system to determine which of the units should become tests or controls has yet to be designed. In its minimally sophisticated form, this system should base the decision on the number of ejected units which survive up to the point of initiation of the experiment.

The number of in situ units which can be accommodated in the lander will determine the degree of redundancy desired within each unit. Such redundancy can be achieved by using duplicate squibs, ampul breakers and leg release systems.

As electronics developments occur, it may be possible to substitute a radio link between the capsule and in situ modules for the umbilical cord. If this were done, it would also be necessary to equip each module with a power supply. The state of the art at the time of final design will determine the trade-off.

One electronics improvement which is advisable now is the centralization of the individual high voltage power supplies for the in situ units into the lander capsule. The high voltage can be, as was demonstrated in this program, supplied over the umbilical cord without a penalizing voltage drop. Two such central high voltage power supplies should be used for the sake of redundancy. The net effects will be to decrease the weight of the individual in situ units and to decrease the weight of the overall lander.

Provision for insuring that all squib circuits are open after firing should be made.

C. MEDIA

1. Basal Media

The test organism spectrum has responded to both types of basal media developed in this program: the relatively simple medium, finalized as M9, and the dilute complex one, finalized as M10. While M11 (M10

made with M9 as diluent rather than with water) has not been tested as extensively as M10, because it was developed rather late in the program, the improved ionic strength did show to advantage. It is most doubtful that any of the species tested would have been inhibited by the inorganic salts in concentrations up to those present in M9 since M9, itself, proved to be a "universal" medium for the test species. However, an extensive test program for M11 should be conducted to prove this.

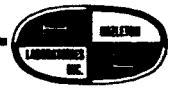
Thus, the two basal media for the experiment are M9 and M11 with the possibility that M10 will be substituted for M11 if the latter tests unsatisfactorily.

How much to infer from terrestrial biology in planning exobiological experiments is a difficult matter to decide. In the area of media development, two principal arguments are possible:

a. Use aqueous solutions containing inorganic salts and labeled substrates as the total medium. This approach adds the fewest ingredients and thus minimizes potential toxicity to the alien organisms.

b. Use the labeled substrates in a dilute complex medium. This offers a wide array of candidate nutrients to the organisms.

The experimenters have not reached agreement on a medium recommendation. One considers that the chances of success would be maximized by the use of simple media only; the other that both simple and complex media should be employed. This question is not urgent at the present time, however.



We suggest that a decision be postponed until the matter can be given fuller consideration.

If the spacecraft has sufficient capacity, additional Gulliver units should contain a duplicate set of media to which 10 g/L and 5 g/L of K_2HPO_4 and KH_2PO_4, respectively, are added for buffering capacity to protect against possible acid soil of the interfering type.

Racemic mixtures of optically active media constituents should be used.

2. Labeled Substrates

Essential to the development of the final media was the selection of appropriate labeled compounds. Several criteria were used to determine the radioactive nutrients: they must be metabolized and incorporated into an evolved, detectable gas; be utilized by a wide range of organisms; be chemically and radioactively stable; and be compatible with other media constituents.

Detailed studies were conducted on the following substrates listed in Table No. V-1.

Each labeled substrate was tested with pure cultures and with soil inocula. Each was tested individually and with those other carbon labeled substrates, generally M5, M9 and M10, that had already been incorporated into the developing "standard" media.

Once a substrate showed promise, various concentrations and radioactivity levels were investigated to adjust toward optimal sensitivity. To preclude undue inhibition of some species, particularly

Table V-1 - Labeled substrates tested in Gulliver program

1. Sodium formate-C^{14}
2. D-glucose-U.L.-C^{14}
3. Sodium acetate-1-C^{14}
4. Sodium pyruvate-1-C^{14}
5. Glycine-1-C^{14}
6. Glycine-2-C^{14}
7. DL-aspartic-4-C^{14} acid
8. DL-glutamic-1-C^{14} acid
9. DL-sodium lactate-1-C^{14}
10. randomly labeled C^{14} yeast extract
11. DL-α-alanine-C^{14}
12. DL-malic acid-3-C^{14}
13. Nitrilotriacetic acid-C^{14}
14. DL-tyrosine-1-C^{14}
15. Urea-C^{14}
16. Acetaldehyde-1-2-C^{14}
17. DL-phenylalanine-1-C^{14}
18. DL-serine-1-C^{14}
19. Succinic-1-C^{14} acid
20. DL-tyrosine-1-C^{14}
21. Cysteine-S^{35}
22. Sodium thiosulfate-S^{35}
23. Sodium sulfate-S^{35}

some in soils, the carbohydrate concentrations were kept lower than those generally incorporated into standard laboratory media. The benefit of these reduced concentrations was demonstrated. Here the radioactive method offers another advantage in that its sensitivity conveniently permits the use of concentrations of carbohydrates too low to produce visible growth by conventional microbiological methods. In the latter cases, exponential growth must be supported for one or two days or more.

Of the labeled substrates listed, experimental data strongly support the use of formate, glucose, lactate and glycine from the standpoint of bacterial responses obtained. However, the formate, while frequently the most active substrate in terms of $C^{14}O_2$ production with a wide variety of microorganisms, was the chief offender in evolving non-metabolic $C^{14}O_2$ from those few acid soils producing this undesirable effect when tested in media not additionally buffered. To a lesser degree, glycine reacted similarly.

Of these four primary substrates, formate, glycine and lactate were identified among the products of the Urey-Miller reaction. Thus, on the theoretical grounds that these three compounds were likely to have been available life precursors on Mars as they were on Earth, their selection as logical substrates for the experiment is reinforced.

While succinic acid did not improve test responses when added to the other carbon labeled substrates, it is selected because of its importance in intermediate metabolism on Earth and hence its potential importance on Mars. Furthermore, it has been shown (12) to enhance substrate phosphorylation of glucose by wild populations of microorganisms.

As in the case with succinic acid, the S^{35} compounds selected did not materially improve test responses when added to the C^{14} substrates. However, when used alone, they did elicit good responses in a number of instances, especially with the sulfur bacteria. They are selected for use - preferably in a separate Gulliver - because it is possible that alien organisms might respond to the sulfur compounds and not to the carbon offerings.

The specific activities recommended were determined from experiments with compounds labeled in one position only. These values were used to calculate values for the corresponding uniformly labeled compounds. The precise specific activities were sometimes dependent on the particular batch produced by the suppliers. The recommended labeled substrates are shown in Table No. V-2.

Except for the strict phototroph detection experiment, this full complement should be incorporated into the media. If sufficient capacity exists in the capsule, additional portions of basal media, again in the priority originally recommended, containing all of the labeled substrates except the formate and glycine, should be added to additional instrument units.

The substrates listed in Table No. V-2 should be uniformly labeled where more than one isotope position is possible. Since there is no basis for anticipating stereoisomer preference by alien organisms, racemic mixtures of the labeled substrates - where applicable - should be supplied.

Table V-2 - Recommended labeled substrates

SUBSTRATE	SP. ACT. (mc/mM)	mM	uc/ml	% (w/v)
C^{14} sodium formate	20.0	0.25	5.0	0.002
C^{14} glucose	21.6	0.36	7.8	0.006
C^{14} sodium lactate	16.5	0.24	3.9	0.002
glycine	10.2	0.22	2.6	0.002
succinic acid	13.2	1.26	16.4	0.015
S^{35} cysteine	4.3	0.58	2.5	0.007
S^{35} sodium thiosulfate	14.0	0.70	10.0	0.110

D. ANTIMETABOLITES

1. Bard-Parker

On the basis of the experimental work performed, recommendation is made for the use of Bard-Parker germicide as the antimetabolite. It demonstrated clear superiority over any other of the chemicals and preparations listed in Table No. V-3.

The composition of the Bard-Parker is given in Table No. V-4.

2. Heat

If spacecraft power and capacity permit, heat applied to the sample should also be used as a control. The sample or ground surface tested should be heated to 90° C for two minutes and permitted to cool to the ambient or experimental temperature (whichever governs) prior to application of the medium. In this manner, the heat will not degrade the substrates and thereby produce radioactive gas from the medium.

Table V-3 - Antimetabolites tested in Gulliver program

Bard-Parker Germicide (isopropanol, methanol, formaldehyde hexachlorophene)

Acetic acid

Barium hydroxide

Calcium hypochlorite

Airken A-3 (n-alkyl dimethyl benzyl ammonium chloride, sodium carbonate, tetrasodium ethylene diamine tetracetate, nonphenoxyethylene, ethanol, essential oils)

Hysol (soap, orthohydroxydiphenyl, alcohol, pine oil, propylene glycol, glycerol)

Amphyl (potassium ricinoleate, o-phenylphenol, p-tertiary amphylphenol, alcohol, propylene glycol, glycerol)

Acrolein (CH_2 = CHCHO)

Argyrol (commercial preparation of 10% silver protein)

Mercuric chloride

Bard-Parker in conjunction with dimethyl sulfoxide (DMSO)

Brook Aleotex Concentrate (a commercial iodine-detergent complex)

Formaldehyde

Beta propiolactone

Ethylene oxide

Table V-4 - Bard-Parker disinfectant

CONSTITUENT	PERCENT
Isoproponal	65.26
Methanol	2.75
Formaldehyde	8.00
Sodium salt of 2,2' methylenebis (2,4,5-trichlorophenol)	0.50
Inactive ingredients	23.49

VI. MANAGEMENT, PERSONNEL, CONFERENCES, PUBLICATIONS

A. MANAGEMENT

On August 1, 1965, Hazleton Laboratories, Inc., established the Life Systems Division with Dr. Gilbert V. Levin as Director. The Gulliver personnel and program were immediately transferred to the new division which opened a fully equipped, 12,000 square foot laboratory at Reston, Virginia.

The Life Systems Division contains its own bioengineering staff in which the engineering design for the program was executed. Fabrication of the parts so designed was performed in the adjacent Instrument Development Company, a sister division of Hazleton Laboratories, Inc.

B. PERSONNEL

During the year, George R. Perez, the original design engineer on the Gulliver instrumentation subcontract, joined Hazleton Laboratories, Inc., as Senior Design Engineer in charge of the bioengineering section of the Life Systems Division. He assumed responsibility for the engineering aspects of the program.

Other technical personnel who worked on the reported areas were:

Dr. Richard Pledger, microbiology

Paul Wilkins, instrumentation

Luis Baez, instrumentation

Mrs. Rose Pock, microbiology

Mrs. Judy Onopchenko, microbiology

Lesley Hulse, microbiology

Mrs. Mary Frances Thompson, no longer with Hazleton Laboratories, Inc., kindly assisted in the preparation of this report.

Co-experimenters on the project continued to be Dr. Gilbert V. Levin, Hazleton Laboratories, Inc., and Dr. Norman H. Horowitz, Biology Division, California Institute of Technology.

C. CONFERENCES AND PUBLICATIONS

The following papers were presented in connection with this program:

Life Detection by Means of Metabolic Experiments, Gilbert V. Levin and George R. Perez. Presented at the 12th Annual Meeting of the American Astronautical Society, May 24, 1966.

Extraterrestrial Life Detection with Isotopes and Some Aerospace Applications, Gilbert V. Levin. Presented at 1st Symposium on Radioisotope Applications February 15-17, 1966, Dayton, Ohio. Radioisotopes for Aerospace, Vol. II, Systems and Applications. In Press, Plenum Press, Plenum Publ. Co., New York, N. Y., 1966.

Dr. Horowitz presented space biology lectures before the Biophysical Society in San Francisco in February, 1965 and at Cornell, Chicago, and Seton Hall Colleges in October, 1965.

Drs. Levin and Horowitz attended the JPL-Caltech Lunar and Planetary Colloquium at the California Institute of Technology, September 13-17, 1965.

On April 22, 1965, Dr. Levin appeared on the Tonight Show with Dave Garroway and discussed Gulliver.

The CBS Special Reports team photographed and taped scenes with Gulliver for use on a CBS Special Report on Mars presented in July, 1965.

VII. REFERENCES

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Contract No. NASr-10

Respectfully submitted,

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