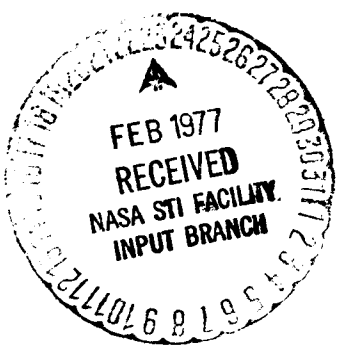
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

PRELIMINARY APOLLO 12 BIOLOGICAL SCIENCES AND CONTAINMENT REPORT



MANNED SPACECRAFT CENTER
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APOLLO 12 QUARANTINE AND BIOLOGICAL SCIENCES REPORT

I. INTRODUCTION

Man has now made his second successful trip to the moon and has safely returned. On this trip, referred to as the most complex manned space flight ever attempted, man was able to perfect and demonstrate the feasibility of pinpoint landing on an extraterrestrial body, establish a functional long-term scientific base on the surface of the moon, examine and reclaim parts from an early unmanned vehicle sent to the moon, and collect some seventy-five pounds of lunar material for return to earth and detailed scientific investigation. This lunar mission, as was the case for Apollo 11, necessitated the implementation of a quarantine program to prohibit the introduction of possible lunar originated contamination into the terrestrial biosphere. The Apollo 12 flight crew have successfully completed their quarantine stay within the Lunar Receiving Laboratory Crew Reception Area without exhibiting any deleterious affects that can be attributed to their flight to the moon. The lunar samples collected by the Apollo 12 crew have been biomedically evaluated by the Lunar Receiving Laboratory staff and it has been determined that they do not represent a hazard to terrestrial life forms. The samples have been released to the scientific community without additional biological containment restrictions. All items contaminated by the Apollo 12 lunar samples have also been released without decontamination or biological containment restrictions.

II. QUARANTINE OPERATIONS

A. Inflight

Quarantine operations for the Apollo 12 mission were initiated on the lunar surface with the crews collecting the lunar samples. The Apollo 12 flight crew was able to obtain all planned sample types; that is the selected sample, the documented sample, a contingency sample, and selected rocks in a tote bag. The total amount of lunar sample collected by the Apollo 12 crew was approximately seventy-five pounds. This sample ranged from surface fines and rocks, to material collected in the bottom of shallow trenches, to material collected to a depth of approximately 18 inches below the surface using a double core tube arrangement. A combination of house-cleaning procedures, leaving certain equipment items on the lunar surface, and the bagging of materials in the LM were again utilized to reduce the contamination level within the LM. The environmental control system and its associated vacuum cleaning capabilities were also utilized to minimize the amount of residual, loose lunar material that would be available for possible movement from the LM to the CM during crew transfer in lunar orbit. Apparently these systems were not as effective in this mission as they were in Apollo 11. The flight crew and lunar samples were successfully transferred from the LM to the CM in lunar orbit and housekeeping procedures similar to those utilized in Apollo 11 were performed in the CM to minimize the amount of lunar material that was present at reentry.

B. Landing and Recovery

The Apollo 12 reentry procedures were normal and the recovery procedures utilized were those described in mission operations documentation. See attached Apollo XII Recovery Surgeon's Report for details. Briefly, these procedures consisted of the crew remaining inside the CM until the primary recovery forces transported clean flight garments and biological respirators to the astronauts. After donning the clean flight suits and the biological respirators, the flight crew exited the CM and were flown to the recovery carrier, the USS Hornet, via helicopter. The hatch of the CM was sealed at sea and the hatch area, the area around the post-landing ventilation valve, and the recovery raft and associated equipment were decontaminated. Upon reaching the carrier the helicopter was wheeled adjacent to the Mobile Quarantine Facility (MQF) and the crew exited the helicopter and entered the MQF. The MQF containing the flight crew, the flight surgeon, and an MQF engineer was then sealed for the return trip to Houston. The helicopter was sealed and decontaminated with formaldehyde by a Navy team from the U.S. Army Biological Laboratories at Fort Detrick.

The CM was retrieved by helicopter, returned to the USS Hornet, and placed adjacent to the MQF. A plastic panel was afixed between the MQF and the CM hatch and the CM was then downloaded into the MQF. After the downloading was completed, the CM hatch was sealed, the tunnel and hatch area decontaminated, and the tunnel retrieved back into the MQF. The preliminary physical examinations were completed and the MQF engineer was successful in packaging and transporting of items out of the MQF for flight back to the Lunar Receiving Laboratory. The items to be returned to the LRL were flown off of the USS Hornet by COD aircraft to Johnson Island and transferred by jet aircraft for the flight back to Ellington Air Force Base. The MQF remained on the carrier deck until reaching Hawaii where it was off-loaded, placed aboard a jet aircraft, and flown back to the Lunar Receiving Laboratory. All operations involving landing and recovery and return of items to the Lunar Receiving Laboratory occurred according to approved documentation without potential breaks in biological containment.

C. Lunar Receiving Laboratory

The secondary biological barrier of the LRL Sample Laboratory was activated on November 19, 1969. At that time all entrances and exits to the Sample Laboratory area were controlled in the same manner utilized after lunar samples have been returned. The positions of Quarantine Control Officer and Laboratory Test Director were merged for support of the Apollo 12 mission into a single functional position referred to as Operations and Containment Officer. This position was filled by the Apollo 11 Quarantine Control Officers. From the time the secondary biological barrier was activated until the samples were released, the operations of the Lunar Receiving Laboratory were managed by the Operations and Containment Officer from the LRL Central Status Station on a 24 hour a day, seven day a week basis.

The support personnel entered the Crew Reception Area on November 24, 1969, to make final preparations for receipt of the lunar samples and crew. The first Apollo 12 Apollo Lunar Sample Return Containers (ALSRC) arrived at the LRL at 1520 hours on November 25, 1969. It was passed into the Crew Reception Area and removed from the shipping container. Visual inspection revealed that the outside plastic biological barrier had been torn during shipment. As a result, the Crew Reception Area was then considered as lunar contaminated. Additional plastic barriers were placed around the rock box, heat sealed, and then the exterior sterilized with sodium hypochlorite as it was transferred from the Crew Reception Area to the Sample Laboratory. Since the Crew Reception Area was then considered lunar contaminated, all transport containers exiting the CRA were decontaminated. The first ALSRC was transported

into the Sample Laboratory and up to the Vacuum Laboratory R-cabinets for pressure measurement and passage into the F-201 vacuum complex. The second Apollo 12 ALSRC arrived at the Lunar Receiving Laboratory at 2340 hours on November 25, 1969. This box was passed into the Crew Reception Area, downloaded from the shipping container, and the exterior biological barrier sterilized for passage of the ALSRC into the Sample Laboratory. The second ALSRC was also transported into the Vacuum Laboratory R-cabinets for pressure measurements. Pressure measurements in the R-cabinets revealed that the ALSRC containing the bulk sample had the lowest pressure and would therefore be opened and processed in the vacuum complex while the ALSRC containing the selected sample was transported to Room 1-126 for opening and processing under a nitrogen atmosphere. Flight film and biomedical samples were transported out of the Crew Reception Area according to approved Apollo 12 documentation.

At 0730 hours on November 29, 1969, the MQF containing the Apollo 12 flight crew arrived at the Lunar Receiving Laboratory. The MQF was successfully docked to the facility; the flight crew, the flight surgeon, and the MQF engineer entered the CRA; and the MQF was demated and placed in quarantine storage to await sample release. Contingency sample and the tote bag samples were placed in heat sealed plastic biological barriers within the Crew Reception Area, sterilized through the CRA to Sample Laboratory airlock using sodium hypochlorite, and transported to the cabinetry of the Physical-Chemical Test area. The surveyor parts and flight items returned in the MQF were placed in bonded storage in the Crew Reception Area. At 1330 hours on December 2, 1969, the Apollo 12 CM entered quarantine in the LRL Crew Reception Area. The preliminary analysis of flight results indicated that there were no major anomalies occurring during flight that would require the CM to undergo extensive testing prior to release of the Apollo 12 samples. Therefore, the CM was downloaded and serviced for prolonged storage by the MQF engineer. Certain equipment items that had experienced anomalies such as the lunar surface camera, a CM color camera, and a urine filter were required outside of the Crew Reception Area prior to release of the lunar samples. These items were sterilized with formaldehyde or dry heat transported out of the Crew Reception Area.

Because of the detailed operational procedures that are conducted during downloading of each of the ALSRC's, there is a considerable time delay between the time the rock boxes first enter the LRL and the time the samples are made available for biological testing. Therefore, an early biological sample was obtained to permit several

days of incubation of in vitro test systems prior to crew release. This early biological sample consisted of material from two of the core tubes. This material was inoculated into bacteriological and mycological culture media and tissue cultures on December 2 and 3, 1969. At the time of release of Apollo 12 crew on December 10, 1969, there were no signs of bacterial or fungal growth in the culture media and no deleterious effects or signs of replicating vial particles in the tissue cultures.

For conduct of the complete biological test protocol a representative sample of all material in the two ALSRC's is utilized. These samples were compounded and made available for biopreparation on December 6, 1969. The biological preparation and direct observation procedures were conducted on December 7 and 8, 1969. The unsterilized lunar sample for biological testing was available for each laboratory on December 8, 1969, with the sterilized lunar sample control available on December 9, 1969.

During operations within the Sample Laboratory one incident occurred which required the quarantining of personnel in the Crew Reception Area. The incident involved the Physical-Chemical Test Laboratory and occurred on December 1, 1969. An operator in the gloves attached to the cabinet unit noticed a hole in one of the gloves. He proceeded to draw the gloves back outside of the cabinetry to examine the hole. After verifying the presence of a hole, the operator then tied the glove between the hole and the cabinet. This operation resulted in loss of the negative pressure within the glove and thereby the escape of the air from the glove into Room 1-124. At the time of this occurrence there were eleven personnel in the room. It was determined that it was necessary to quarantine all the occupants of the room in the Crew Reception Area. The room exits were sealed and the entire room sterilized with formaldehyde gas before personnel reentered the room and operations resumed.

The Lunar Receiving Laboratory facility systems performed well during Apollo 12 operations. The two minor problems experienced on Apollo 11, that is sewage system and autoclave door gaskets, were repaired between missions and the problems did not exist during Apollo 12 operations. One normal power supply failure occurred during mission operations but all containment system equipment is connected to the emergency power generator and this generator performed flawlessly with no delay or loss of containment capabilities. Due to the excellent manner in which all systems performed in support of Apollo 12, there are no plans for any modifications to facility systems prior to the next mission.

On January 7, 1970, the 30 day quarantine test period for Apollo 12 lunar samples was completed. At that time, data from the extensive biological testing program indicated that there were no deleterious

effects to terrestrial living systems as a result of exposure to lunar samples returned by the Apollo 12 crew. In addition, there were no indications of replicating microbial life forms by any of the cultural procedures utilized. Therefore on that date the quarantine restrictions on the Apollo 12 lunar samples were lifted.

APOLLO XII RECOVERY SURGEON'S REPORT

I. Recovery Procedures

Recovery quarantine procedures were exactly as planned. All swimmers remained in full SCUBA gear, and on closed SCUBA breathing system, while in the vicinity of the spacecraft. The astronauts donned their coveralls and respirators before exiting the spacecraft, and nothing was brought from the spacecraft. There was some difficulty in closing the hatch, with the hatch being opened and partially closed three times.

The Recovery Surgeon maintained isolation technique by wearing a respirator in the helicopter until the crew had donned their respiratory protection. Before pickup of the astronauts, the helicopter pilots had donned oxygen masks and were on 100% oxygen. The crew chief and hoist operator had donned BIG's without hoods, and oxygen masks attached to their regular flight helmets. The masks hose terminated in a biological filter.

After uprighting of the spacecraft, recovery operations proceeded normally and without incident, with one exception. On the second astronaut pickup the swimmers failed to place the net sea-anchor into the net. This resulted in a hazardous situation with the sea-anchor line coiled in the life raft as the net was jerked clear by the helicopter. The crew later pointed out that the net with which they trained in the Gulf did not have a sea-anchor, and feel that a sea-anchor does create a hazardous situation.

As the astronauts were brough aboard the helicopter they were helped from the net by the Recovery Surgeon with no contact between astronauts and helicopter crewmen occurring. After all three astronauts were aboard, the two helicopter crewmen in the aft compartment were helped from their BIG's by the Recovery Surgeon while they maintained their helmets and masks. At no time did they come in contact by the exterior of their BIG's. The two crewmen then went to the forward compartment of the helicopter for landing. All helicopter crewmen maintained respiratory protection until they exited the helicopter.

Capt. Bean, first crewmen brought aboard, was noted to have an adhesive bandage over high right eyebrow. When questioned about it, he reported that a 16mm camera mounted in the right-hand window had come loose at impact and struck him in the head. He denied any loss of consciousness; however, later reports by his crewmates indicate that he was stunned for approximately 5 seconds, with resultant late release of the parachute risers. All three crewmen appeared to be in excellent condition in the helicopter, with no evidence of orthostatic intolerance, nor undue fatigue. The Recovery Surgeon communicated the astronaut's condition to the Hornet Surgeon on the way back to the ship, primarily to explain the bandage on Capt. Bean's head.

The helicopter, with the aft door closed, became quite hot and humid, but the astronauts suffered no ill effects. Handling time by the deck crew was very short, and therefore the exposure of the crew to high temperatures were not sufficiently long to produce demonstrable heat stress. The Recovery Surgeon subjected to the heat stress for an hour and one-half in the helicopter prior to recovery, was completely sweat-drench upon entering the MQF.

II. MQF Activities

A. MQF time-line. (All times are local ship time).

1050-- Astronauts and recovery surgeon enter MQF.
1055 - Commence microbiology specimen collection, weights, showers, and clothes change
1130 - Presidential phone call
1145 - Begin hematology specimen collection
1230 - Lunch
1320 - Repair of Capt. Bean's laceration
1340 - Spacecraft hatch opened
1400 -
1430 - Spacecraft microbiology (except for suits)
1430 - Rock boxes out of spacecraft
1500 - First rock box out of spacecraft
1500 - Hematology specimens ready for bagging
1515 - All flight film bagged and spacecraft microbiology completed
1545 - First COD load left MQF area
1600 -
1830 - Physicals and ECG's
1830 - Supper
2100 - Completion of ECG's and physical exams
2130 - Completion of chest films
2140 - Last crewmember in bed
2200 -
0100 - WBC's, differentials, hematocrits, and uranlyses

B. The crew requested that the Presidential phone call be delayed for approximately 5 minues, in order that they could complete showering and changing clothes and open the curtains for television coverage. This was done.

Microbiology swab and gargle specimens were collected without difficulty, and each crewmember weighed himself and recorded his weight on his way to the shower.

Hematology specimen collection went well, with the MQF engineer assisting. The Commander experienced an hypotensive episode, manifested by palor bradycardia and mild naeusea. After lying down for approximately 3 minutes, he was completely recovered. (Due to space limitations, all hematology specimens were drawn with the crewmbmer sitting beside the table).

Both Mr. Stone and Capt. Gordon helped at various times during the laceration repair.

The COD schedule could not have been met without the help of the crew. Capt. Conrad assisted Mr. Stone in collection of the initial spacecraft microbiology samples. All three of the crewmembers participated in unstowing and sorting of the film into two shipments. While Mr. Stone was bagging the rock boxes, Capt.'s Conrad and Gordon assisted Dr. Jernigan in completing the spacecraft and pressure suit microbiology specimen collection.

All medical requirements objectives were fully met, with the two following exceptions:

1. Toxicology - A preliminary ear, nose, and throat examination was performed at the time of the microbiology specimens were taken. Each crewmember had been blowing his nose and wipping his eyes after entering the MQF and removal of the respirators. No particulate matter was seen in nasal passages or conjunctival sacs. Therefore, swabbing of these areas was not performed. However, three specimens for toxicology investigation were returned. These consist of:

   a) dust and lint from the spacecraft ECS screens collected in flight on a piece of tape.

   b) swab samples of dust and lint which had accumulated on the left hand circuit breaker panel from an ECS hose.

   c) grey colored lint removed from the MQF air conditioning filters on R+3, when the filters became so clogged that it was necessary to clean them in order to improve air conditioning efficiency.

2. Orthostatic tolerance test - Blood pressures and pulse rates were recorded on the standard MEDATA form used for annual physical examinations. Through oversight and force of habit, the test performed was the standard two minute standup following recumbancy, rather than a five minute test.

III. Physical Findings

		ORAL TEMP	WEIGHT	RECUMBENT PULSE	RECUMBENT B.P.	STANDING PULSE	STANDING B.P.
CDR							
	R+0	99.0°F	145 lbs.	86	124/78	98	118/80
	R+1	98.3	147	78	114/69	76	108/70
	R+2	98.2	147				
	R+3	98.2	148				
	R+4	97.5	147				
CMP							
	R+0	98.4	148	90	126/78	100	100/32
	R+1	97.1	152	76	134/88	100	130/92
	R+2	98.4	152				
	R+3	98.8	152				
	R+4	98.8	152				
LMP							
	R+0	98.4	140	68	105/72	112	100/99
	R+1	97.6	143	66	102/70	92	110/82
	R+2	98.0	142				
	R+3	98.9	147				
	R+4	98.9	146				

A. Commander. The nasal mucosa was red and glistening when first examined, but had returned to fairly normal pink appearance within 8 hours. The mucosa of the posterior pharynx was slightly erthematous with some lymphoid prominence when first examined. There were lesions present at all biosensor sites, which appeared to be healing pustules. There were some which occurred in the center of the areas as well as the margins. There were three crusted lesions, just posterior to the auditory meatus of the left ear, and chapping and cracking of the distal crease of the right pinna. There was also a fine papular erythematous rash on the right forearm volar surface. Many of the lesions had a prominent central point. This rash had almost entirely dissappeared by 24 hours after recovery. On R+1 Capt. Conrad experienced progressive left sided nasal congestion throughout the day with left maxillary pressure symptoms and a small amount of moderately thick yellow tinge discharge from the left side. A nasal culture was taken from the left side and he was started on actifed and tetracycline. Some 24 hours later the symptoms had all abated except for a moderate amount of mucoid discharge.

B. CMP. The nasal mucosa was red and glistening upon initial examination, but was a normal pink color some 8 hours later. The mucosa of the posterior pharynx was moderately erythematous in patches, with a pebbling effect of the lymphoid tissue. There were multiple pustules both at the margins and center of the sensor sites, as well as excoriations corresponding to the sensor margins. There was a healing apthous ulcer in the floor of the mouth on the left side which the CMP states was present before flight.

C. LMP. There was a 2cm jagged laceration with downward beveled edges over the lateral right eyebrow. The laceration did not extend to the bone and there was no underlying boney defect. No foreign material was seen. The wound was cleansed and investigated by probing. It was repaired under 1% lidocaine anesthesia, using one subcutaneous 3-0 plain gut suture, and six 5-0 permalon skin sutures. Twenty-four hours later there was a small area of redness and two small vesciles at the inferior lateral corner of the wound. On R+3 when Capt. Bean arose in the morning there was edema around the superior portion of the right orbit, and the right eyelid was discolored, being reddish-purple in color. The vesicles had developed into three pustular lesions, and one was pointing. There was no tenderness in the immediate vicinity of the wound and no induration nor heat. The pus was streaked on blood agar, but there was no growth. Ampicillin 250mg QID was started about 1000X on 27 November and continued for 5 days. Twenty-four hours after initiation of the antibiotic the swelling of the eyelid and supraorbital region was completely gone and the pustules were subsiding. The skin sutures were removed on 29 November after arrival at the LRL.

Immediately after recovery Capt. Bean was noted to have clear fluid and bubbles behind the right tympanic membrane. Valsalva maneuver was effective. There was moderate injection over the handle of the malleolus on the left. After 24 hours of Actifed therapy both conditions had returned to normal. The nasal mucosa was bright red and glistening at the time of the recovery, but some 8 hours later was pink and normal appearing. No foreign material was noted.

There were red areas and small pustules noted about all sensor sites. There was one small pustule on the left elbow with a hair in the center. There was moderate scaling and cracking of the skin between the left little toe and the fourth toe.

IV. Laboratory Findings

Laboratory data with the exception of the MQF bacteriology will be reported with the rest of the crew lab data in Apollo Flight Data Astronaut Resumes.

Bacteriology Data: Three separate organisms were isolated from the commander's nasal secretions. The predominant organisms as judged by colony characteristics and gram staining characteristics appeared to be a staphalbus. Each colony type was picked and inoculated into tryptocase soy broth and veal infusion broth. These tubes were then incubated at approximately 85° until return to the LRL. The blood agar plates were taken from the incubator (37°C) at 24 hours and placed in the refrigerator, and also passed to the microbiology laboratory upon return to the LRL.

V. Medical Kit Usage History.

A. CDR: Actifed tablets, approximately 5, one after ascent from the lunar surface and then one each night thereafter taken for nasal congestion.

B. CMP: Actifed, three tablets. One the day before re-entry, one the morning of re-entry and one just before re-entry. Asprin, two tablets, approximately day 8 "as a precaution against a developing sore throat."

C. LMP: Capt. Bean took two asprins the first night for a generalized headache which accompanied the full headed sensation which all three crewmen experienced for the first 24 hours of flight. He also noted nasal congestion on the second day of the mission and took approximately two Actifed tablets per day for the duration of the mission. Additionally, six seconal capsules were used. Capt. Bean states that he would sleep for approximately five hours, wake up, take a seconal and sleep for another five hours. This occurred each night of the mission except for the lunar surface stay and the night immediately preceeding lunar descent.

Medical kit inspection - The outer container had two rips on the top. The following items were missing: 6 asprin tablets, 6 seconal capsules, 18 Actifed tablets, 2 adhesive bandages, 4 UCD roll-on cuffs, 1 sternal electrode harness, and 1 axillary harness, 5 electrode assemblies, and 1 bottle of Afrin nasal spray.

The crew reported unsatisfactory operation of the Afrin spray bottle Og. That is, they were unable to obtain sufficient medication from the bottle, by squeezing the bottle. When checked in the MQF all three Afrin bottles delivered a fine spray when sharply squeezed.

Clarence A. Jernigan, M.D.
Recovery Surgeon

III. CREW OPERATIONS AND THE OPERATION OF THE CREW RECEPTION AREA; APOLLO XII

The Crew Reception Area and the Clinical Laboratories located in the Lunar Receiving Laboratory supported the Apollo XII crew quarantine activities during November and December, 1969. A professional staff consisting of eight laboratory analysts, two physicians, three stewards and one recovery engineer, was quartered in the quarantine space of the laboratory. All logistical and clinical duties were performed by the attending staff during the quarantine period. Additionally, the Apollo XII Command Module was housed within the Crew Reception Area and subjected to a controlled down-loading exercise during the quarantine period by individuals cross-trained to effect this program according to specified protocols. The Command Module was not subjected to decontamination procedures and will remain behind the biological barrier until sample release.

During the normal course of quarantine within the Crew Reception Area, eleven individuals from the Sample Operations Laboratory were involved in a "spill" condition which resulted in the transfer of these eleven personnel into the Crew Reception Area for quarantine. These individuals, principally members of the Preliminary Examination Team and several support personnel, were quartered within the Crew Reception Area until crew release. A laboratory which normally functions within the Clinical Laboratories was made available for the use of the PET members, and with the addition of sterilized Apollo XI and Apollo XII fine and thin section material, the individuals were able to continue their preliminary examination work while quartered within the CRA.

A. Crew Operations Time Line

The attached table describes the time profile characteristic of the Apollo XII mission. Pre- and postmission activities are noted in temporal sequence with the Clinical Laboratory protocols exercised during the mission sequence. Crew exposure profiles are portrayed in this figure from F-30 (9 October) through quarantine termination (10 December). Postmission medical surveillance was initiated at crew release and will continue in effect for a period of one calendar year.

The Apollo XII crew spent approximately 21 days (504 hours) in quarantine following final closure of the LM hatch on the lunar surface (135 hours GET; 20 November). The entirety of this period was spent in the confines of the Lunar Module, the Command Module, the Mobile Quarantine Facility and the Crew Reception Area. Crew release was effected approximately eleven hours early since improved data management techniques allowed completion of release clinical examinations ahead of schedule.

The crew, in general, worked a nominal 5 1/2 - 6 day-week while in quarantine. The work cycle was divided between scientific and technical debriefings and compilation of the pilot's report. Exercise periods, relaxation and free-time occupied the remainder of their time spent in the CRA. The Apollo XII crew accepted the mission philosphy of quarantine and other than separation from their family and the outside world, voiced no significant complaints. It is worthy of note that the Apollo XII

crew, much the same as the Apollo XI crew, commented on the desirability and efficiency of quarantine in terms of compilation and completion of pilot reporting and the orderly progression and completion of scientific and technical debriefings.

B. Clinical Laboratory Operations

The Clinical Laboratory supported the Apollo XII misson from F-30 through the release of the crew at the termination of quarantine. Specific protocols in the areas of Hematology, Immunology and Biochemistry were employed as specified in the MSC document entitled, "Medical Requirement; F Type Mission Lunar Landing." The temporal sequence of specific protocols is depicted in the attached table.

The Data Management Program of the Clinical Laboratory provides for continuous update of all laboratory data pertinent to individual crew members as well as categorizes all medical data on individuals. Laboratory analytical controls pertaining to specific analyses are also continuously updated to insure the reliability of generated data. Through the operation of this laboratory data management and quality control system, data collected previously on the three crewmen were correlated with the data generated during the entirety of mission activities for Apollo XII. The results of the Apollo XII Clinical Laboratory determinations indicate that no findings directly attributable to extraterrestrial exposure are apparent. In the specific areas of Hematology, Immunology and Biochemistry all values were within established normal limits and are unremarkable. However, it should

be noted that postmission protocols are incomplete and that continued surveillance will be effected.

C. Quarantine Aspects of Sample Operations Laboratory Personnel

The eleven individuals transferred into the Crew Reception Area following the "spill" condition in the Sample Operation Laboratory precipitated a minimum of logistical requirements on the resident staff and support personnel of the CRA. The requirement for housing and accommodating these eleven individuals was met and was reported in a postmission debriefing of the individuals involved to have been extremely adequate and caused no significant problems concerning the effectivity of the PET Program.

With the exception of one individual transferred from the Sample Lab into the Crew Reception Area following the "spill", the entirety of the group was healthy and remained so throughout the quarantine period. One individual developed, during the course of his stay in the CRA, an infectious disease which required this particular individual to be confined to the Mobile Quarantine Facility for a period of approximately 72 hours which was at that time a part of the CRA. Other than confinement and lonliness and the inability to visit under comfortable conditions with his family, this individual voiced no complaints regarding his stay in the Mobile Quarantine Facility. The MQF was operated as an infectious disease ward with the individual's food being passed in under control conditions and access limited only to the Crew Surgeon in charge of the area. The

individual involved subsequently returned to the Crew Reception Area following the 72 hour stay and was released with the remainder of the individuals in quarantine at crew release time.

D. Conclusion

The Crew Reception Area operated in an efficient manner throughout the quarantine period, although the increased contingent of personnel posed logistical constraints uncommon to normal operations. The Clinical Laboratory processed in excess of 2500 samples in direct support of the quarantine mission. It is singificant to note that MSC Dispensary samples were processed in a routine manner on a daily basis throughout the quarantine period. This was accomplished by daily transfer of samples from the Dispensary into the Crew Reception Area for analysis. Results were reported via the Clinical Laboratories data link.

NASA-S-69-5076-V

CREW OPERATIONS/CLINICAL LABORATORY TIMELINE

APOLLO 12

MONTH DAY	10 12	10 31	11 7	11 9	11 13	11 14	11 18-20	11 24	11 25	11 26	11 27	11 28	11 29	11 30	12 1	12 2	12 3	12 4	12 5	12 6	12 7	12 8	12 9	12 10	12 11
QUAR DAY							1	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
CREW ENVIRON	CRA	KSC	KSC	KSC	KSC CRA	CM	LM	CM MQF	MQF	MQF	MQF	MQF	MQF CRA	CRA	CRA	CRA	CRA	CRA	CRA	CRA	CRA	CRA	CRA	CRA	
CLINICAL LAB	V_{CM}	V_{CM}	V_S	V_{CM}	V_S			V_{CM}						V_{CM}	V_S		V_S		V_S				V_{SCM}		
H AND P	V_{CM}	V_{CM}	V_S	V_{CM}	V_S			V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	V_{SCM}	
EVENT	F-30	F-15	F-8	F-5	F-1	L	EVA	R	R+1	R+2	R+3	R+4	R+5	R+6	R+7	R+8	R+9	R+10	R+11	R+12	R+13	R+14	R+15	R+16	R+17
COMMENT	A	A		A				A,B,C	C					A	D		SC		D*				E	F	S

COMMENT LEGEND

- **A** CONTROL SAMPLES DRAWN ON MISSION SCHEDULE
- **B** BARRIER ERECTED; SUPPORT PERSONNEL ENTER CRA 24 NOV 69 0800 HOURS
- **C** ASAP SAMPLE ON CREW AND CONTROLS DRAWN; RETURNED TO CRA FOR ANALYSIS ON R+1
- **D** SAMPLE OPERATIONS LAB PERSONNEL ENTER CRA; LABORATORY EXAMINATION PERFORMED
- **D*** FOLLOW-ON LABORATORY EXAMINATION FOR SOL PERSONNEL
- **E** LABORATORY EXAM ON CREW; CRP ON SUPPORT PERSONNEL
- **F** RELEASE OF CREW, SUPPORT PERSONNEL, AND SOL PERSONNEL
- **SC** CM 108 QUARANTINED IN CRA: REMAINS UNTIL SAMPLE RELEASE
- **S** CREW/FMO SURVEILLANCE INITIATED

CLINICAL LAB/H AND P LEGEND

V_C - CREW (3)

V_M - MQF (2)

V_S - SUPPORT (13)

- SUPPORT (SOL - 11)

IV. BIOPREPARATION - DIRECT OBSERVATION AND APOLLO BACTERIOLOGY/MYCOLOGY

A. General

The lunar material obtained during the Apollo 12 mission and used for biological investigations was divided into two different groups based upon the method of collection. The two samples were tested independently and never mixed.

1. The early bio-sample was composed of one-half (47.239) of core tube #12026 and ten grams from the bottom of core tube #12028 (deep core). The combined portion, used in the early biological protocols, consisted of 57.23 grams of finely powdered lunar material (most particles less than 100 microns in diameter).

2. The conventional bio-sample pool contained a combination of rock chips, which were the result of preliminary geological examinations, and fine material scooped from the other ALSRC. This sample weighed approximately 500 grams.

B. Preparation of Conventional Lunar Bio-Sample Pool Material for Use in Biological Systems

Lunar sample was sieved through a normal laboratory sieve with a pore size of 104 microns. Particles larger than 104 microns were ground with a motor-driven sample crusher (Model 150, LeMaire Instruments, Reno, Nevada) and added to the 104 sample.

The resulting composite was then ground with an electric mortar grinder (Torsion Balance Company, Model MG-2). A riffle sampler was used to homogenize and distribute the resulting sample.

A microscopic examination was performed on dried, n-butyl alcohol suspensions of homogenized lunar material. The volume mean diameter (VMD) was calculated for each sample. The volume mean diameter was found to be 20 microns for both samples.

Sterile control samples (when required) were heated in a 160°C drying oven for sixteen hours before passage out of the Class III cabinetry.

C. Direct Observation of Lunar Material

Samples of lunar material were observed for traces of recognizable life forms using the following methods:

1. Ground and unground dry samples were observed at 7X and 25X magnification, using white light and ultraviolet light (= 2537Å) illumination.

2. Aqueous concentrates were observed at 430X and 1,000X magnification by:

a. Phase contrast microscopy
b. Typical gram stain procedure
c. "cold" acid fast stain procedure

The above observations were recorded on high-speed Ektachrome film.

3. Aqueous diluents were observed and photographed at 20,000X employing the electron microscope. These diluents were fixed in three per cent gluteraldehyde for twenty-four hours before being passed out of the Class III cabinetry for electronmicroscopic examination.

4. No objects of biogenic origin were observed by any of the test methods.

D. Methods for Detection of Viable Microorganisms in Lunar Material

Portions of each category of lunar material were cultured with the addition only of melted 1.5 per cent agar. This culture method was used to promote the growth of replicating agents using lunar material as the only nutrient and energy source. Each Petri plate containing 669 mg of lunar material in ten ml of agar was incubated, along with an uninoculated control, at one of two temperature/atmosphere combinations. No growth was observed on any of these plates throughout the twenty-one day incubation period.

For all other test systems, the lunar material was suspended in sterile phosphate buffer and filtered through Whatmann No. 3 filter paper. Both the filtrate and the residue were divided into aliquots, each representing wash or residue from 233 mg of lunar material.

Aliquots from the early bio-sample were inoculated onto Blood Agar (BA), Trypticase Glucose Yeast Extract Agar (TGY), and Thioglycollate Broth (Thio.).

Aliquots from the conventional bio-sample pool were inoculated onto all of the above, in addition to Czapek Dox Agar (CD), Sabouraud dextrose agar (SDA), terrestrial soil extracts in agar, and six different aquatic/mud media. Each of these media, except the terrestrial soil and water types, were used in two concentrations: (1) the normal concentration recommended by the vendor, and (2) a dilution of 1/100 the normal concentration.

All solid media, both inoculated and uninoculated controls, were incubated under one of three different atmospheric conditions. These conditions were: (1) ten per cent carbon dioxide, (2) one hundred per cent dry sterile nitrogen, or, (3) ambient to room (and cabinet) air.

Likewise, all control and test systems were incubated at one of the following four temperatures: 4°C, 24°C, 35°C, and 55°C.

To accomplish these varied environmental requirements, the plates were placed in groups of three into a specially designed controlled environment enclosure. All plates were incubated for twenty-one days with periodic observations for growth.

Results

No growth was observed on these plates during the 21-day incubation period. If growth had occurred, the microorganisms would have been isolated and identified using appropriate morphological-biochemical procedures.

APOLLO CREW BACTERIOLOGY/MYCOLOGY

A. General

Eleven microbiological samples were taken from each crewmember immediately after recovery. These included seven external body swabs, nasal passage swabs, throat-mouth gargle, urine, and feces. In addition, the spacecraft was sampled at four sites, and each set of clothing was sampled at three sites.

These specimens were transported to the LRL Sample Lab where the contents were evaluated by standard microbiological methods. The resulting isolants were categorized on the basis of their morphological and biochemical integrity. Any microorganisms which were not readily identified were compared with preflight baseline date (T-30, T-14, and T-0) to assist in establishing their origin in relation to the crew. The various microorganisms isolated from each sample were also enumerated.

B. Results

A greater number of microbial isolations were made on Apollo 12 than on any previous Apollo mission. A total of 861 bacterial isolations and identifications were made in addition to approximately 200 fungi. Of these 220 isolations were made from the postflight samples.

Staphylococcus aureus was found on only one crewman at the immediate preflight sampling period. The microorganism was found at a number of sites on all three crewmen at the postflight period. A number of S. aureus isolations were also made from the clothing and spacecraft samples.

As on previous Apollo flights high counts of gram negative rods were obtained from samples of the UCD's. Proteus mirabilis was the predominate microorganism found at this site.

No microorganism with unfamiliar morphological structure, or unusual response to biochemical tests, was found.

V. LUNAR SAMPLE AND CREW VIROLOGY

The virology program conducted in support of the Apollo 12 mission consisted of (a) an analysis of lunar material for replicating agents, (b) a characterization of the viral and mycoplasma flora of the Apollo crewmembers, and (c) an analysis of specimens obtained as a result of the mission personnel surveillance program designed to ascertain the nature of illnesses in personnel who were either contacts of the Apollo 12 crew or worked behind the biological barrier. In the analysis of the lunar material for replicating agents, nine types of cell culture, embryonated eggs, and mycoplasma media were inoculated with supernatant fluids from 50% suspensions of lunar material. Specimens obtained from the Apollo 12 crewmembers 30 days and immediate preflight and immediate postflight were inoculated into embryonated eggs, suckling mice, and three types of cell cultures. This report describes the virology procedures employed and the results obtained from studies conducted in support of the Apollo 12 mission.

A. Materials and Methods

Primary African green monkey kidney (GMK), primary human embryonic kidney (HEK), and human embryonic lung (WI-38) cultures were obtained from HEM Research Incorporated, Rockville, Maryland. The cultures were ampouled in 100 culture tube equivalents and were maintained frozen in liquid nitrogen. They were determined to be free of contaminating agents prior to use. Hetereploid bovine kidney (MDBK) hetereploid porcine kidney (PK_{15}), and primary duck embryonic fibroblast (DEF) cultures were obtained from Microbiological Associates, Bethesda, Maryland. These cultures were also ampouled in 100 culture tube equivalents and were maintained frozen in liquid nitrogen.

Primary rhesus monkey kidney cells, suspended in monkey kidney medium A with 10% fetal bovine serum, were received weekly from National Communicable Disease Center, Atlanta, Georgia.

Rainbow trout gonad, Salmo gairdneri (RTG-2); fathead minnow, Pimephales promelas (FHM); and grunt fin, Haemulon sciuras (GF) tissue cultures were obtained from the American Type Culture Collection, Rockville, Maryland. These tissue cultures were maintained by continuous passage.

For culturing, Eagle's basal medium and 10% fetal bovine serum in Hank's balanced salt solution (BSS) was used for the HEK, WI-38, RTG-2, and FHM cultures; monkey medium A with 10% fetal bovine serum was used for the GMK cultures; Eagle's minimal essential medium and 10% fetal bovine serum in Hank's BSS was used for BEK and PEK cultures; Eagle's minimal essential medium and 10% fetal bovine serum in Eagle's BSS was used for DEF cultures; and Eagle's basal medium, 10% fetal bovine serum, and 10% human serum in Hank's modified BSS containing 0.206 molar sodium chloride was used for the GF cultures. The mammalian and avian cultures were incubated at 35°C, the GF and FHM cells were incubated at 27°C, and the RTG-2 cells were incubated at 20°C. For

challenge, the growth media from the mammalian and avian cells was replaced with a maintenance medium consisting of 49.5% Eagle's minimal essential medium, 49.5% medium and 199 and 1% fetal bovine serum. The cultures from the poikilothermic animals were maintained on the growth media. All of the tissue culture media contained 100 units of penicillin and 100 µg of streptomycin per ml.

Embryonated eggs from White Leghorn chickens, bred resistance inducing-factor-free, were used. (Kimber Farms, Fremont, California).

Pregnant albino mice (Webster strain) were obtained from Texas Inbred Mice Company, Houston, Texas.

1. Lunar Soil Virology

All of these procedures were conducted in Class III cabinets. Two samples of lunar material were studied - an early sample composed of material from one of the cores and a conventional pooled sample.

The early sample was used for toxicity determination and preliminary analysis of the lunar material. Six screwcap tube cultures of GMK, HEK and WI-38 cells, containing maintenance medium, were challenged with 0.2 ml of the supernatant from a 65% suspension of the early sample and incubated at 35°C. The cultures were examined daily for toxicity and evidence of viral replication until crew release. By this time, the complete virology protocol on the conventional pool sample had been initiated and the study on the early sample was terminated.

Six screwcap tube cultures of GMK, HEK, WI-38, MDBK, PK_{15}, and DEF cells were challenged with 0.2 ml of the supernatant from a 50% suspension of the conventional pooled sample in maintenance medium. Controls were unchallenged cultures and cultures challenged with the supernatant from a suspension of sterilized lunar material. The cultures were incubated in stationary racks - the DEF cultures at ambient temperature and the remaining cultures at 35°C. The cultures were observed daily for evidence of viral replication and were subpassaged after 10 days incubation. Two subpassages were made.

In the final passage, detection of possible inapparent infection was investigated by (a) presence of interferon by challenge with vesicular stomatitis virus, (b) use of the plaque technique, (c) hemadsorption with guinea pig, sheep, goose, monkey, rat and human O red blood cells, (d) histological examination of cultures stained with hematoxylin and eosin stain and safranin stain, and (e) ultramicroscopic examination of the cultures.

Falcon flasks containing RTG-2, FHM, and GF cells were inoculated with supernatants from 50% suspensions of the conventional pooled sample. The cells were incubated at 15°C and were observed daily for

cytopathogenicity. After 10 days incubation, the RTG-2 cultures were challenged with infectious pancreatic necrosis virus for the determination of possible plaque reduction. After 20 days incubation, all of the cultures were subjected to ultramicroscopic examination.

Embryonated eggs were inoculated with 0.2 ml of the supernatant from a suspension of pooled lunar material in phosphate buffered saline. Controls were eggs inoculated with supernatant from a sterile lunar material suspension and eggs inoculated with phosphate buffered saline. Each specimen was inoculated into 5 eggs by 3 routes of inoculation - yolk sac, amniotic-allantoic sac, and charioallantoic membrane (CAM). Six-day-old eggs were used for the yolk sac inoculation and 10-day-old eggs were used for the amniotic-allantoic sac and CAM inoculations. The yolk sac inoculated eggs were incubated for 6 days, and the amniotic-allantoic sac and CAM inoculated eggs were incubated for 4 days at 36°C. Three subpassages were carried out. The allantoic and amniotic fluids were tested for hemagglutinating particles with guinea pig and chick red blood cells, and the yolk sac and CAM from the final passage were examined histologically and ultramicroscopically.

For mycoplasma isolation, the supernatant from the pooled lunar material suspension was inoculated into media developed by Chanock, Hayflick, and Barile (Ref. 1 and 2). Broth cultures and agar plates were inoculated and incubated at 35°C. The plates were incubated in a "Gaspak". The broth cultures were subpassaged after 3 days, and three blind passages were made. The plates were incubated for 14 days, and the cultures were examined daily for the appearance of microcolonies.

2. Crew Virology

The isolation of viruses and mycoplasma was attempted from specimens obtained from the Apollo 12 crewmembers 30 days, 14 days, immediate preflight, and immediate postflight. The virology on the immediate postflight specimens was carried out in Class III cabinets. The specimens were whole blood, urine, feces, and a pharynegal swab. The whole blood was inoculated into six-day-old embryonated eggs via the yolk sac and into a litter of 24-48 hour old suckling mice (at least 6 mice) intracerebrally and intraperitoneally. The urine was adjusted to neutrality with Tris (hydroxymethyl) aminomethane buffer; treated with penicillin, streptomycin, and Fungizone; and inoculated into HEK and WI-38 cells. A 10% suspension of the stool was made in Hank's basal salt solution with 0.5% gelatin. The supernatant was treated with penicillin, streptomycin, Fungizone and trypsin inhibitor and inoculated intracerebrally and intraperitoneally into suckling mice and into HEK and GMK cells. The pharyngeal swab was immersed and rinsed in TPB containing 0.5% gelatin. The TPB was treated with penicillin, streptomycin, and Fungizone and inoculated into HEK, GMK, and WI-38 cells. A similar specimen with the Fungizone omitted was used to inoculate six 10-day-old eggs via the amniotic-allantoic sac.

All of the specimens were treated with antibiotics and inoculated into mycoplasma media. The mycoplasma, tissue culture, and embryonated egg procedures described in the lunar soil virology section were followed. The suckling mice were observed daily for 14 days for signs of infection. One blind subpassage was made.

3. Mission Personnel Surveillance Program

Two weeks before lift-off and until the end of lunar sample quarantine, specimens were obtained from personnel, exhibiting illness, who were possible contacts for the Apollo 12 crew or who worked behind the biological barrier. The specimens for virology were usually throat and fecal swabs. Acute and convalescent sera, 14-21 days apart, were also obtained.

The specimens were treated with antibiotics and were routinely inoculated into HEK, WI-38, and rhesus monkey kidney cells. Embryonated eggs and suckling mice were used when indicated by the symptomatology. One passage, after 7 days, was carried out in the tissue cultures. The agents isolated were identified.

B. Results and Discussion

1. Lunar Soil Virology

There was no evidence of viral or mycoplasma replication. These results are similar to the results obtained from the analysis of Apollo 11 lunar material.

2. Crew Virology

No viruses were isolated from the specimens obtained from the Apollo 12 crew 30 days, 14 days, and immediate preflight and immediate postflight. Since isolation rates of 2-4% would be extreme in healthy adults the results were as expected. Mycoplasma were isolated from the throat and urine of all of the astronauts (Table 1). Although these organisms are considered to be of no medical importance the data indicates a high carrier state for these organisms. The possible pathogenicity of these organisms during extended spaceflights should not be ignored.

3. Mission Personnel Surveillance Program

There was a good amount of gastrointestinal and respiratory illness in the personnel supporting the Apollo 12 mission. Nine viruses have been isolated from the specimens obtained.

Adenovirus 5 and 2 rhinoviruses have been identified. The remaining 6 viruses have been tentatively identified as rhinoviruses and confirmatory procedures are in progress.

C. Summary

No evidence of viral replication was obtained by an analysis of the Apollo 12 lunar material in 9 tissue culture systems and embryonated eggs. Attempts to isolate mycoplasma were negative.

Mycoplasma were isolated from the urine and throat specimens obtained preflight and postflight from all of the astronauts. Virus isolation attempts from blood, urine, feces, and pharyngeal swabs were negative.

Nine viruses have been isolated from personnel supporting the Apollo 12 mission. Two rhinoviruses and an adenovirus 5 have been identified, and the remaining 6 viruses have been tentatively identified as rhinoviruses.

D. References

1. Chanock, R. M.; Hayflick, L.; and Barile, M.F., Proc. Nat. Arad. Sci., 48, 41-49, 1962.

2. Barile, M. F.; Bodey, G. P.; Synder, J.; Riggs, D. B.; and Grabowski, M. W., J. National Cancer Institute, 36, 155-161, 1966.

TABLE 1

MYCOPLASMA ISOLATED FROM APOLLO 12 CREW

Specimens	Time of Sample	Species
A. Commander		
Throat Swab	30 day preflight	M. salivarium
	14 day preflight	M. salivarium
	0 day preflight	M. salivarium
	0 day postflight	M. salivarium
Urine	30 day preflight	M. hominis I
	0 day preflight	M. hominis I
B. Command Module Pilot		
Throat Swab	30 day preflight	M. salivarium
	14 day preflight	M. laidlawii A
	0 day preflight	M. salivarium
	0 day postflight	M. salivarium
Urine	30 day preflight	M. hominis I
	0 day preflight	M. laidlawii A
	0 day postflight	Unidentified
C. Lunar Module Pilot		
Throat Swab	30 day preflight	M. orale I
	14 day preflight	M. orale I
	0 day preflight	M. laidlawii A
	0 day postflight	M. salivarium
Urine	30 day preflight	M. hominis I
	0 day preflight	M. hominis I
	0 day postflight	M. laidlawii A

VI. BIO-TEST OPERATIONS - MAMMALIAN AND AVIAN

The objectives of this study were: (1) to determine whether lunar material returned to Earth as a part of the Apollo 12 mission contained extraterrestrial, biologically active agent(s) capable of producing acute disease in specific homoiothermic test species; (2) to determine whether the lunar material contained extraterrestrial agent(s) capable of replicating in the test species, and (3) to conduct all experiments so as to contain any hazardous infectious materials.

In order to accomplish the above objectives, one mammalian species and one avian species were chosen. The major considerations for these choices were that the test systems be (1) small and (2) sufficiently tractable to be housed and experimented upon in Class III quarantine isolation cabinets. These cabinets were maintained under negative pressure and test systems were manipulated by technicians wearing flexible gloves and sleeves attached to ports in the face of the cabinets. The animal species and strains chosen were the same as employed during the Apollo 11 mission bio-test protocol, i.e. -- germ-free mice and Japanese quail.

The test plan for Apollo 12 studies was basically that employed for the Apollo 11 protocol with the following changes: The test mice were housed separate from the control mice in positively pressurized isolators. Pre-exposure body weights were not recorded. All the quail were housed within a Class III quarantine cabinet. The exposure of both species to diluent alone was eliminated. The number of quail per test group was reduced from 30 to 20. Spleen weights were measured on all mice killed. No toxicity testing was done on the animals prior to the initiation of the biological activity study. An individually measured aliquot of inoculum was prepared for each animal subject.

A. Materials and Methods

The mammalian test subjects were commercially obtained, random-bred, germ-free, CD-1 male and female mice 4-5 weeks old. They were maintained under positive pressure in the plastic flexible isolators which were in a negatively pressurized Class III quarantine cabinet. The mice were attended in these living quarters through two sets of flexible gloves and sleeves. The mice were housed six to a 7 x 11 x 5 in. "shoe box", polycarbonate cage with a wire top, were fed a sterile moist diet, and were given free access to sterile water. The bedding was ground corn cobs, which were replaced as needed. The acclimation time in the housing quarters prior to the start of the study was three days during which time the individual animals were identified by ear-punch coding.

The avian subjects were commercially obtained, young adult (6-8 weeks old) Japanese quail, Coturnix coturnix japonica (Pharaoh strain). The ratio of females to males was 9:1. All of the birds were housed in a single negatively pressurized Class III cabinet. They were housed one

bird per wire cage in two rows of ten cages each. They had some contact with their neighbor through the wire side common to each cage. The quail were fed a commercial game bird feed and watered ad libitum. The cages had wire floors through which droppings fell to absorbent paper that was replaced daily.

The lunar material used to make up the inoculum was a pool of rock chips and "fines". This material was ground to a size less than 100 microns. The unsterile sample was used without further processing. The sterile sample used for one of the controls was dry heated to 160°F for 16 hours.

The following experimental procedures were undertaken: Direct observation of each animal was done every hour for the first eight days post-exposure to detect illness or death as rapidly as possible. After the eighth day, direct observations were made once every four hours during a worked shift. The egg production of each female quail was noted daily from 16 days prior to exposure.

The following procedure was used to examine mice killed at specific time periods following inoculation of lunar material.

1. The rectal temperature was measured by probe and thermister.

2. The animal was anesthetized with Metofane

3. The total body weight was taken with a top-loading balance.

4. After being restrained in a supine position, the mouse was exsanguinated by incising the axillary vessels. The blood was collected on glass slides, in micropipettes (capillary tubes), and in Pasteur pipettes.

5. A peritoneal swab was obtained by sterile technique and was placed in thioglycolate broth for microbiological culturing.

6. The ventral body mass was dissected away and the spleen removed, weighed, and bisected longitudinally. Half was fixed in Bouin's solution and half in 3% glutaraldehyde. The mesenteric lymph node was dissected out and fixed in Bouin's fixative. The remaining organs from tongue to anus were removed in the manner of Rokitansky and placed in Bouin's solution. Any abnormalities were noted.

7. The blood smear was stained automatically with modified Wright's stain. A differential leukocyte count was done.

8. Using the micropipette technique, the hematocrit was calculated.

9. A total leukocyte count was determined using Unopettes, either manually or with an automatic particle counter.

10. The total serum protein was determined by the biuret spectrophotometric method.

11. Cellulose acetate serum electrophoreses was used to calculate percentages of the serum fractions.

12. Following routine histology procedures for hematoxlyn and eosin staining, thin sections of spleen and mesenteric lymph node were scanned for germinal center development. Sections of the heart, lungs, liver, pancreas, gastrointestinal tract, kidneys, adrenals, and thymus were observed for abnormalities.

13. Selected spleens were prepared for electron microscopy to observe germinal centers in more detail.

B. Specific Protocols and Results

1. Mice

On December 13, 1969, each of the 60 mice in the test (green or T) group was inoculated intraperitoneally (ip) with a suspension of unsterilized lunar material, volumetrically measured to 220 mg quantum sufficit (q.s.) to 1.5 to 2.0 ml with sterile isotonic phosphate buffered (0.04M) saline (IPBS) (pH 7.2 - 7.4). Measurement of the ground lunar material was accomplished with a small scoop calibrated with 220 mg of ground lunar rock from the Apollo 11 mission.

Each of the 60 mice in the control-1 (red or C-1) group was inoculated ip with a suspension of 220 mg of sterilized lunar material q. s. to 1.5 to 2.0 ml IPBS.

Each of the 60 mice in the control-3 (yellow or C-3) group was left uninoculated.

One cage (6 subjects) of each group was killed and processed on post-exposure days 2, 4, 6, 8, 10, 14, 20, 35, and 50. One cage per group has been retained for long term observation. All animals of the same sex were processed on a given day. Males were used on D+2, +6, +10, +20, and +50; females on D+4, +8, +14, +35, and long term. As of this report the protocol has only proceeded through Day+20.

Results

As of this time no mice have died or become visibly ill in either the test or the two control groups, with the exception of those intentionally killed as part of the protocol.

Microbiological data: Routine screening of the housing isolators prior to the exposure of the mice to the lunar material was all negative. The preparation and inoculation of the mice was on December 13. Four days later, December 17, the routine screening of the two housing isolators and fecal samples turned up positive cultures from both. The single organism found in both isolators was a gamma-hemolytic Streptococcus sp. All subsequent tests have been positive. Routine screening on December 23 revealed a gram negative rod in the isolator housing the two control groups. On December 30, a gram negative rod was isolated from the test group isolator.

The peritoneal swabs of the processed mice have had positive results also. In all but two positive cases the bacteria is a gamma-hemolytic Streptococcus sp. like the one contaminating the mice. On D+6 only Pseudomonas aeruginosa, a gram negative rod, was isolated from two animals in the sterile lunar sample group. A mold, Rhizopus sp., has been found as a contaminant of the "sterile" swabs used in the peritoneal sampling.

Clinical data: Body temperatures ranged from 95.0° to 100.4°F. The averages of the uninoculated controls range from 95.6° to 98.4°F while those mice inoculated with lunar material, both test and control groups, overlap greatly and generally between 96.8° and 99.9°F.

The average body weight and average spleen weight per test group per day is charted by sex (Figs. 1 and 2).

The hematology data were analyzed using the analysis of variance technique. Significance was determined at the 0.01 level using a number of different comparisons. The following chart shows this comparison per item per day on non-cumulative data.

	Day								
	2	4	6	8	10	14	20	35	50
hematocrit	-	-	+	+	-	-	-		
TWBC	-	+	-	-	-	-	-		
neutrophil %	+	-	-	-	-	-	-		
neutrophil count	-	-	-	-	-	-	-		
lymphocyte %	+	-	-	-	-	-	-		
lymphocyte count	-	+	-	-	-	-	-		
monocyte %	-	-	-	-	-	0	-		
monocyte count	-	-	-	-	-	0	-		
eosinophil %	0	0	0	0	0	0	0		
eosinophil count	0	0	0	0	0	0	0		

The cumulative average hematocrit values for each of the three test groups over the 20 days were: T = 46.92%, C-1 = 46.54%, C-3 = 46.27%. The values for D+6 were: T = 45.75%, C-1 = 50.33%, C-3 = 54.33% with the significance between T and C-3. The values for D+8 were: T = 52.42%, C-1 = 46.00% and C-3 = 46.50% with significance between both T and C-1 and T and C-3.

On D+2 the neutrophil and lymphocyte percentages show significance in the analysis of variance. The C-1 group average was 35.17% and was higher than either the 14.17% of T or the 18.00% of C-3. Since the lymphocytes are essentially the reciprocal of the neutrocytes in percentage, the C-1 lymphocyte percent was statistically lower than the other two groups.

On D+4 the total WBC's and the actual lymphocyte count of the uninoculated controls (C-3) are statistically higher than the other two groups.

The serum protein values were analyzed using the analysis of variance technique. Significance was determined at the 0.01 level using a number of different comparisons. The following chart shows this comparison per item per day on non-cumulative data.

	Day								
	2	4	6	8	10	14	20	35	50
total serum protein	-	-	-	-	-	-	+		
albumin %	+	-	-	-	+	-	-		
albumin amount	-	-	-	-	-	-	+		
alpha 1 globulin %	+	+	-	-	-	-	-		
alpha 1 globulin amount	-	+	-	-	-	-	-		
alpha 2 globulin %	+	-	-	-	-	-	+		
alpha 2 globulin amount	+	-	-	-	-	-	-		
beta globulin %	+	-	-	-	+	-	-		
beta globulin amt.	+	-	-	-	+	-	-		
gamma globulin %	-	-	-	-	-	-	-		
gamma globulin amt	-	-	-	-	-	-	-		

The total serum protein cumulative mean per test group through D+20 is: T = 5.64 g%, C-1 = 5.65 g%, and C-3 = 5.44 g%. The D+20 values showed that the C-1 average (4.97 g%) was statistically lower than the C-3 average (5.80 g%) yet not different from the T group average (5.50 g%). The range of the means of all groups was 4.85 to 6.63 g% .

The analysis of the serum albumin percentage on D+2 and D+10 shows that the C-3 group was higher than either of the stone inoculated groups.

	Day	
	2	10
Test	55.40%	57.20%
C-1	50.00%	56.17%
C-3	61.67%	62.50%

On D+2 the C-3 group was statistically higher than T or C-1. On D+10 the C-3 group was statistically higher than C-1.

The albumin amount (in grams-percent) average per group through D+20 was: T = 3.14 g%, C-1 = 3.14 g%, C-3 = 3.14 g%. On D+20 the data was: T = 3.13 g%, C-1 = 2.71 g% and C-3 = 2.95 g%. Only C-1 was statistically lower than T.

The alpha 1 globulin percentage on D+2 showed the T group appreciably lower than C-1 or C-3 but only statistically lower than C-3. On D+4 both T and C-1 were statistically lower than C-3 but only the T group was statistically lower than C-3 in actual alpha 1 globulin amount.

The C-1 group was statistically higher than either T or C-3 with the percentage and amount of alpha 2 globulin on D+2 while on D+20 the C-1 percentage only was statistically higher than the T group.

The cumulative averages of each of the test groups are: T = 24.55%, C-1 = 24.35%, and C-3 = 21.47%. Statistically the T and C-1 groups are and have always been higher than the C-3 group. However, using only non-cumulative data these two groups are only statistically higher on D+2 and D+10.

	Day	
	2	10
Test	26.30%	22.58%
C-1	25.55%	23.72%
C-3	16.75%	17.78%

The beta globulin amounts are also statistically different on D+2 and D+10 with T and C-1 being higher than C-3.

	Day	
	2	10
Test	1.43 g%	1.22 g%
C-1	1.35 g%	1.21 g%
C-3	0.82 g%	0.92 g%

Graphs of the non-cumulative daily group averages for the hematology and the serum biochemistry data are included (Figs. 3-~~5~~). ~~There are three graphs per item; one composite of all data through all days~~, one each of the same data per sex.

Histologic examination of the spleen and mesenteric lymph nodes through D+20 is complete. No other tissues have yet been evaluated. Granules of lunar material has been observed in most of the sections of the spleens or lymph nodes in the test and Control-1 groups. The amount of material present is not as concentrated in foci as was seen in the tissues from mice used in Apollo 11. The germinal center production in the spleen is negligible. It appears in both the test and Control-1 not in Control-3. It is virtually absent from any of the lymph nodes.

Ultramicroscopic examination of the splenic white pulp is just underway and is unremarkable to date.

2. Japanese quail

On December 9, 1969, each of the 20 Japanese quail in the test (green or T) group was inoculated ip with a suspension of unsterilized lunar material, volumetrically measured to 220 mg q.s. to 1.5 to 2.0 ml with IPBS.

Each of the 20 quail in the Control-1 (red or C-1) group was inoculated ip with a suspension of 220 mg of sterilized lunar material q.s. to 1.5 to 2.0 ml IPBS.

Each of the 20 quail in the Control-3 (yellow or C-3) group was left uninoculated.

The birds had been housed in their cages in the quarantine cabinet line for 16 days prior to exposure. Individual egg production records were kept from that date on. There were two males and 18 females in each group. This protocol was observational only and included no scheduled laboratory work.

Results

Three quail (one T and two C-1) were killed immediately by the inoculation. Replacement birds were secured and each was appropriately exposed to the lunar material. Two birds in the C-1 group died within 48 hours of exposure. Necropsy revealed that one had its intestine perforated by the inoculating needle and died with a Escherichii coli peritonitis. An abdominal blood vessel in the other bird had been nicked by the inoculating needle and internal hemorrhage was the cause of death. All the other birds in all three groups have been and are well.

The graph of egg production (Fig. 10) shows an increasing volume of egg production in all three groups that has stabilized at about 75 - 80%.

C. Discussion

1. Mice

This report on the results of the murine bioprotocol for the Apollo 12 mission encompasses only the first seven processing days out of a total of nine. It is of the first 20 of 50 days for the complete protocol. It is apparent even now, however, that there is no acutely infectious disease producing agent in this lunar material used to expose mice. It is also apparent that there is no indication of any deleterious toxic effect following intraperitoneal injection of the material. No mice to date have sickened or have died which have received either the unsterilized or the sterilized pulvarized lunar rock. A comparable fact between the mice of Apollo 11 and those of Apollo 12 was their lack of any apparent initial response to the ground lunar rock. They did not react visibly to the injection of this volume of foreign material. The necropsy results of those mice which were killed for data collection have revealed no indication of gross pathology. This inspite of the fact that the mice in all three test groups have become contaminated with at least one bacterial microorganism (gamma hemolytic Streptococcus sp.) on or about the time of exposure to the lunar material. There is at present indication that the two housing isolators have become contaminated with a second species of bacterium (a gram negative rod).

It is also interesting to note that with the known contamination of the mice with one or two bacterial species, the development of splenic or lymph node germinal centers has been negligible and when present the centers have been scattered in intensity and numbers through only the mice which have received lunar material. Histologic evaluation of other organs has not been done yet. Electron microscopic studies of the germinal centers of the spleen have not progressed far enough for comment.

The Apollo 11 mice remained in a germ-free state through out that protocol. Comparison of some of the data from both missions might be interesting. One item worthy of note is that the germinal center production in the spleens of these mice seem comparable to those of Apollo 12. The following figures from both missions are the D+20 cumulative means. The analysis of variance on the cumulative data for Apollo 12 has produced more statistical differences than was apparent in the Apollo 11 data. In spite of the apparent validity of these figures at the 0.01 level of significance, it is difficult to determine a pattern and thus the real value of the information.

	Apollo 11	Apollo 12
Hematocrit		
test	49.85%	46.92%
control-1	51.23%	46.54%
control-3	50.26%	46.27%
Total White Blood Cell Count		
test	4863	4605
control-1	5288	3859
control-3	4739	5011
Neutrophil percentage		
test	15.71%	23.40%
control-1	19.95%	25.83%
control-3	12.44%	19.74%
Neutrophil Count		
test	748	1231
control-1	1108	1044
control-3	588	929
Lymphocyte Percentage		
test	83.67%	75.98%
control-1	79.50%	73.52%
control-3	87.00%	79.82%
Lymphocyte Count		
test	4090	3317
control-1	4154	2787
control-3	4129	4128

The serum gamma globulin fraction in the Apollo 12 mice compared to the Apollo 11 mice is interesting. In neither protocol was there a dramatic change over the first 20 days nor were there dramatic differences between the three test groups. The Apollo 11 mice were germ-free and their gamma globulin percentages were: T = 1.82%, C-1 = 1.86% and C-3 = 1.97%. Like data for the Apollo 12 mice are: T = .86%, C-1 = .93% and C-3 = .89%.

2. Japanese quail

The Japanese quail have been under observation for a period of 28 days following their exposure to the lunar material. In this protocol, in counter distinction from the Apollo 11 protocol, all the birds were housed inside quarantine cabinets. The only untoward effects seen in the subjects occurred as a direct effect of the inoculation, not the inoculum. Five birds total were killed, three immediately and two

within the first 48 hours post exposure. From that point on the birds have appeared extremely healthy and their egg production has risen to its present level (Fig. 54). The rather dramatic bouncing around of this egg production over D+17, +18, +19, and +20 was because of a staffing error. The eggs were not counted at the usual 24 hour interval. The upward spikes were more than 24 hour production and the downward ones, less than 24 hour production.

D. Conclusion

This sample of lunar material returned by the Apollo 12 crew and inoculated into germ-free mice and Japanese quail contained no biological hazard which was in any way deleterious to either of these two signal homoiothermic animal species.

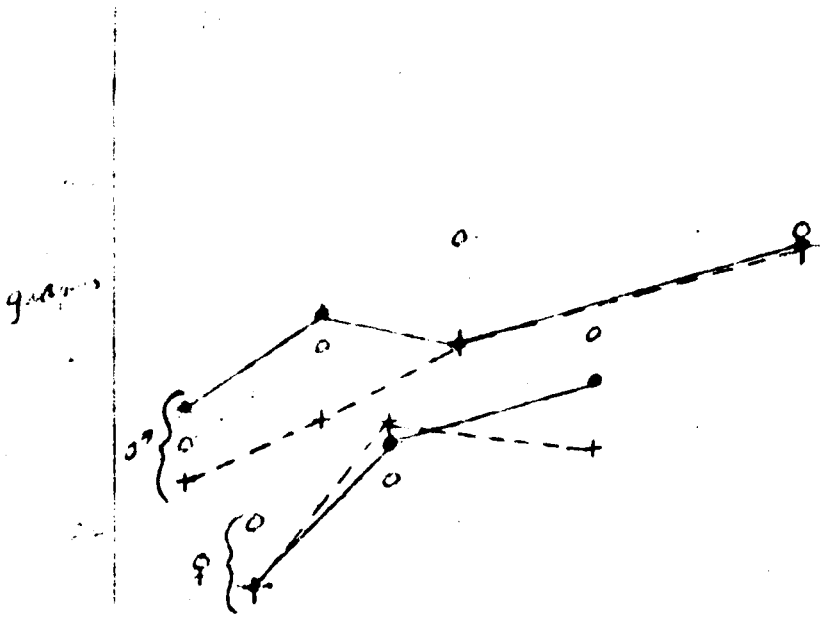
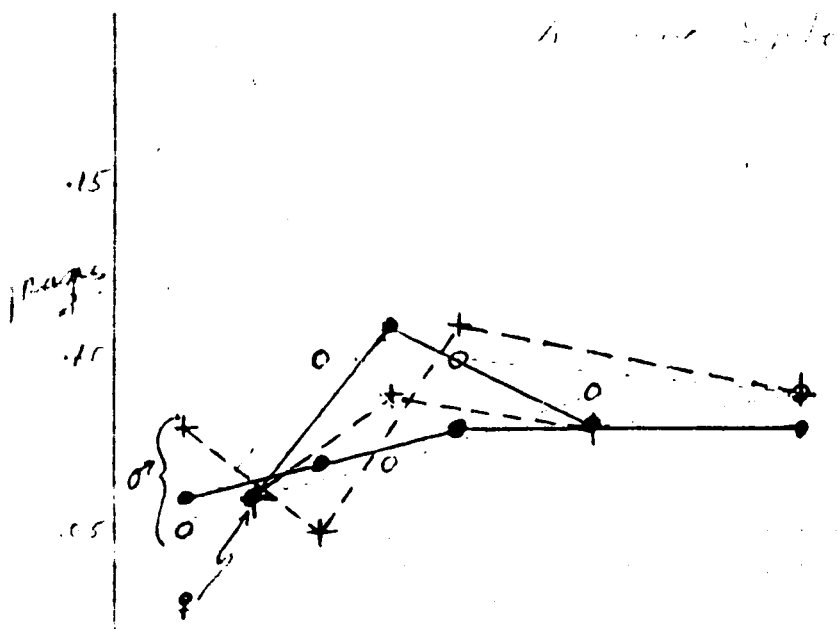


FIG 1



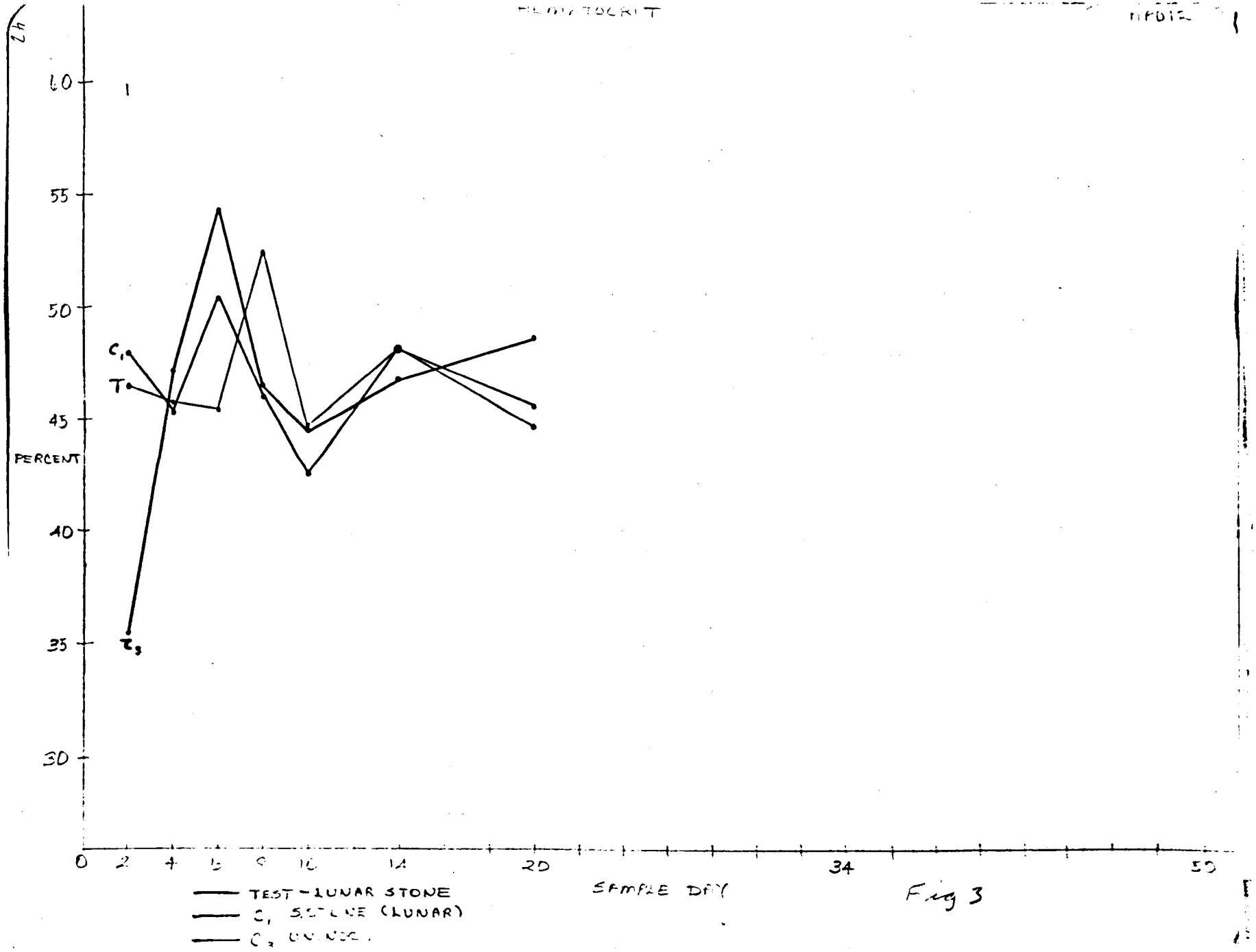

60
55
50
45
PERCENT
40
35
30
C,
T
τ,
0
2
4
6
8
10
14
20
34
55
SAMPLE DAY
TEST - LUNAR STONE
C, STONE (LUNAR)
C, UNKNOWN

Fig 3

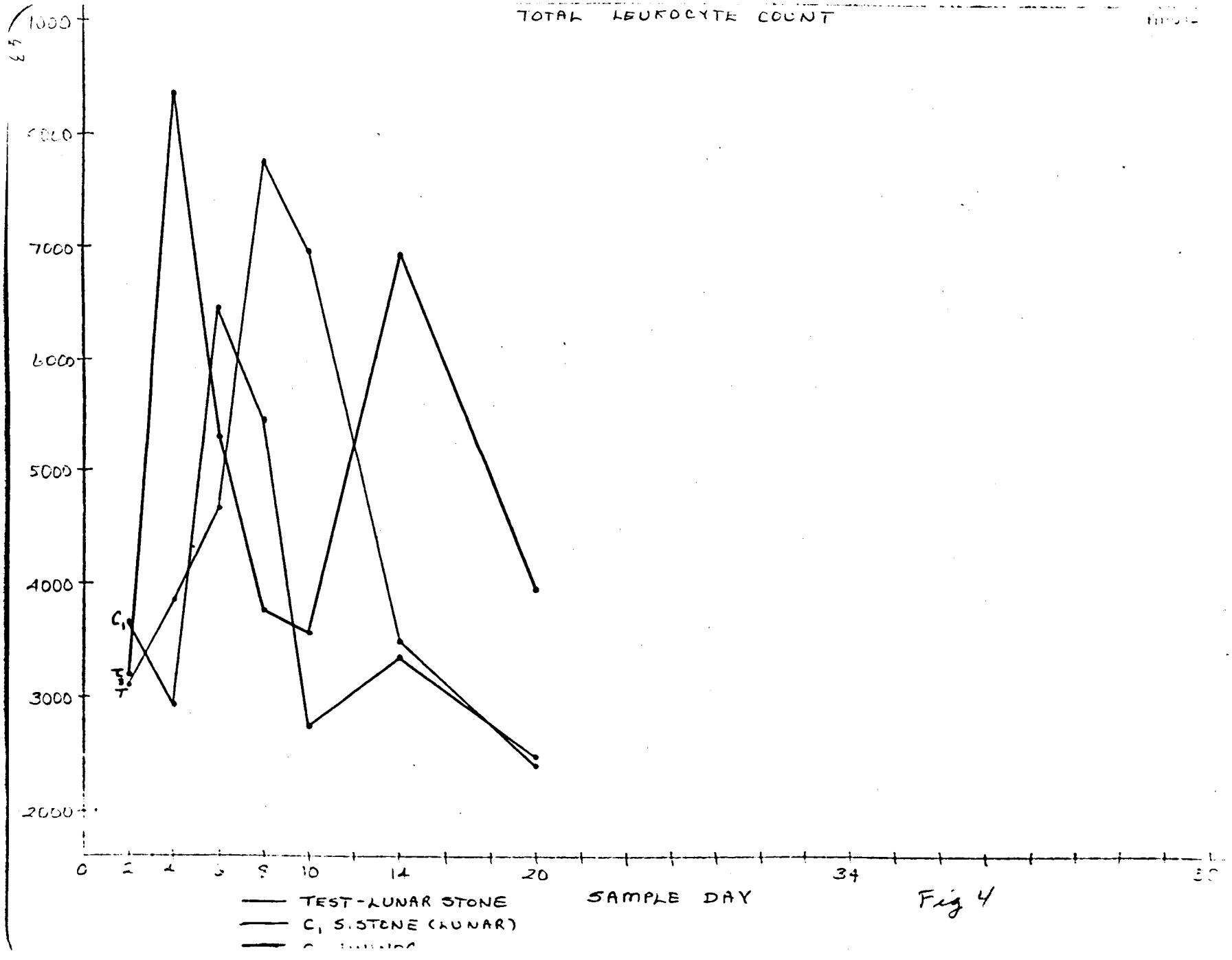

TOTAL LEUKOCYTE COUNT
8000
7000
6000
5000
4000
3000
2000
C1
T
0
SAMPLE DAY
TEST-LUNAR STONE
C1 S.STONE (LUNAR)

Fig 4

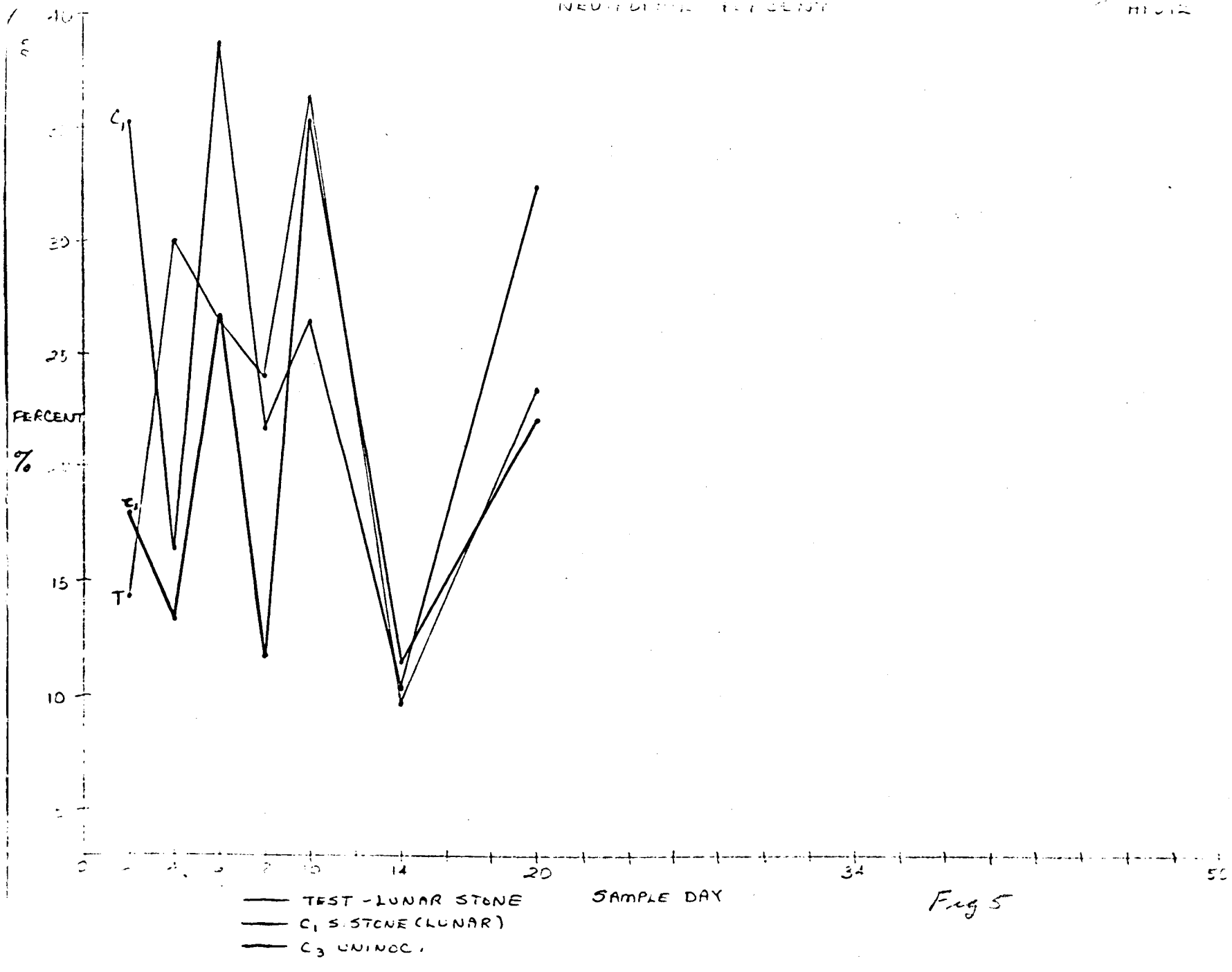

PERCENT
%
30
25
15
10
C_1
E_1
T
0
14
20
34
50
SAMPLE DAY
TEST - LUNAR STONE
C_1 S. STONE (LUNAR)
C_3 UNINOC.

Fig 5

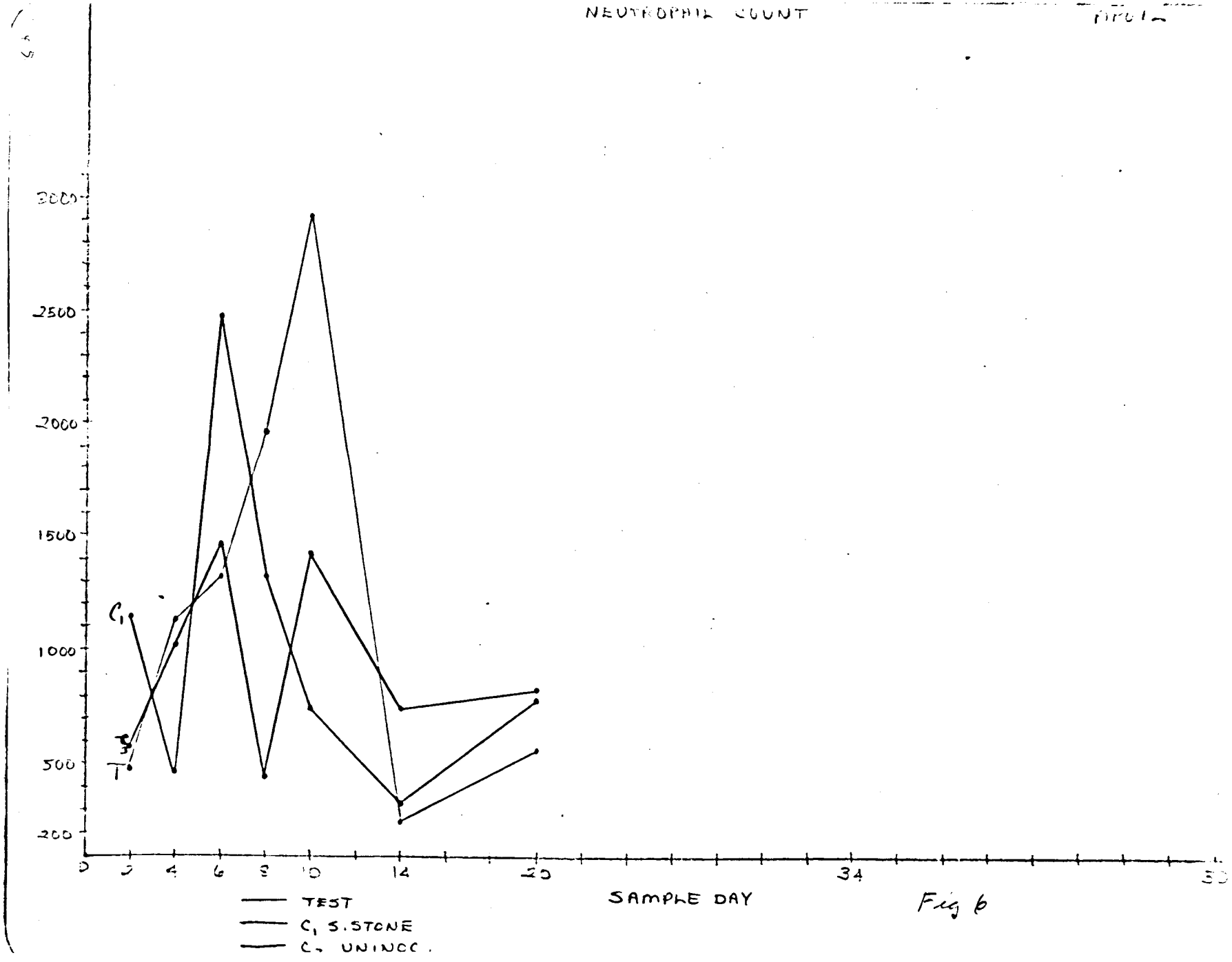

NEUTROPHIL COUNT
3000
2500
2000
1500
1000
500
200
0
2
4
6
8
10
14
20
34
C1
T
TEST
C1 S.STONE
C2 UNINOC.
SAMPLE DAY

Fig 6

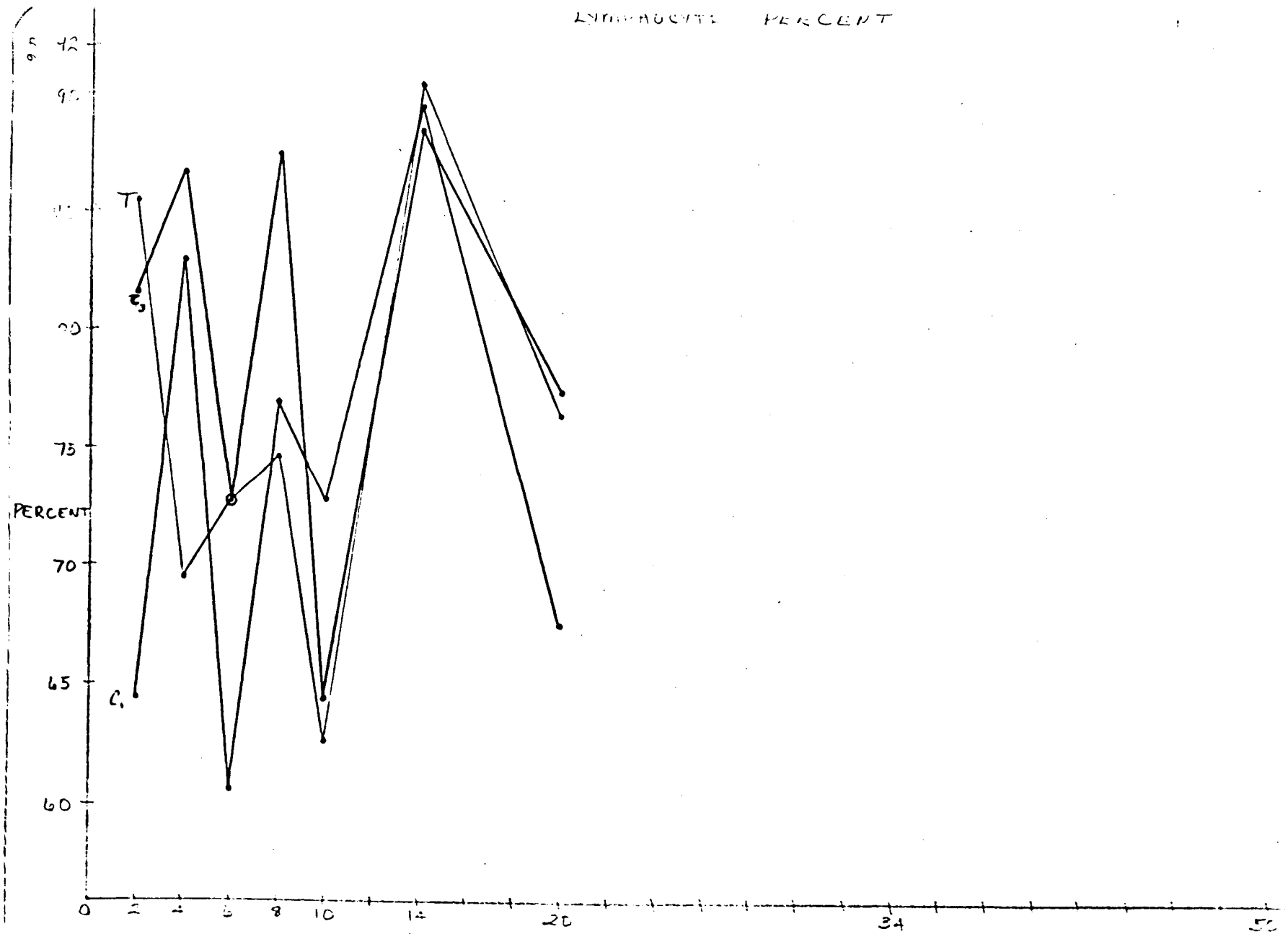

PERCENT
75
70
65
60
0
2
4
6
8
10
12
20
34
T
C1
C2
TEST-LUNAR STONE
C1-S. STONE LUNAR
C2-UNINOC.
SAMPLE DAY

Fig 7

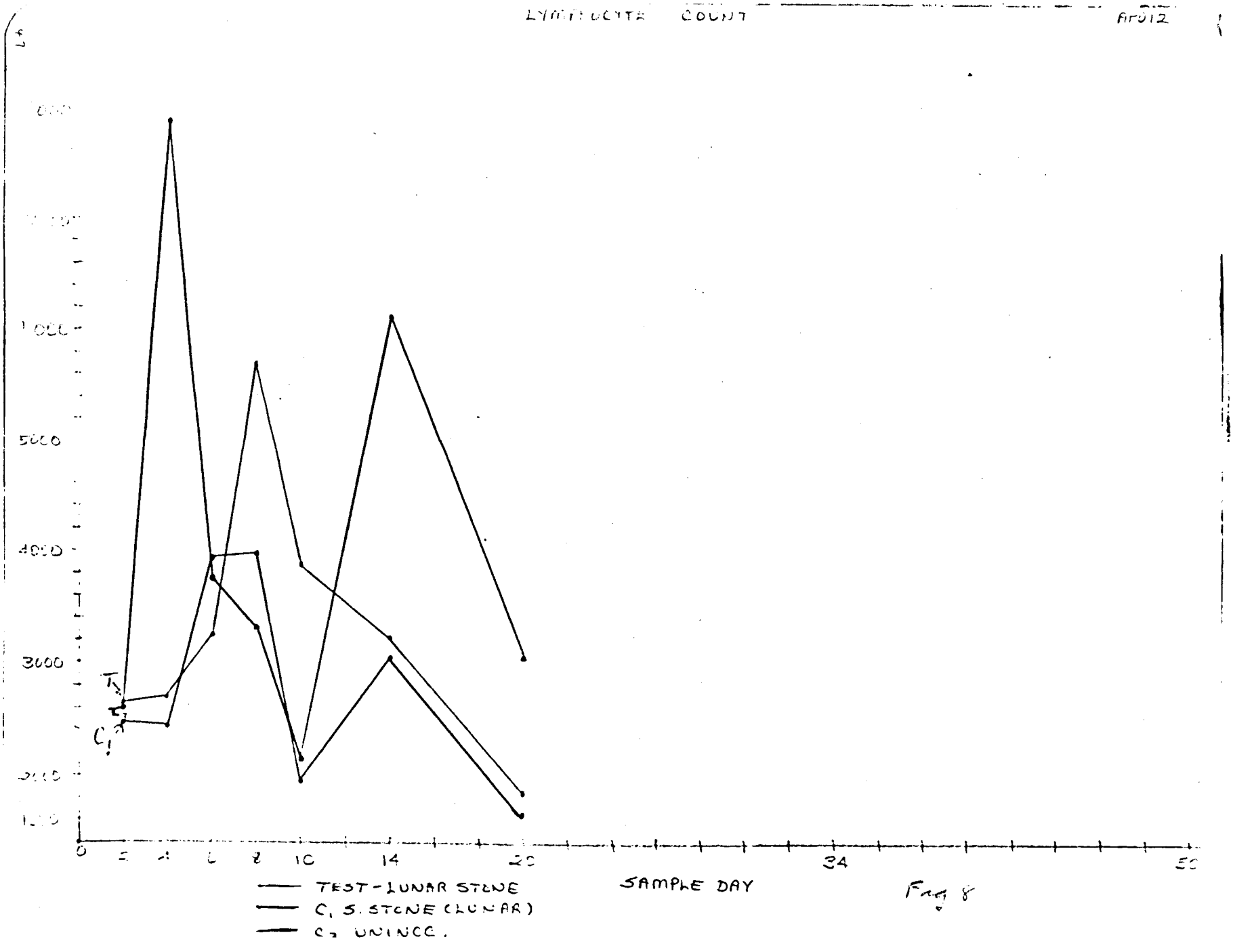

LYMPHOCYTE COUNT
APOI2
5000
4000
3000
0
2
4
6
8
10
14
20
34
50
SAMPLE DAY
TEST - LUNAR STONE
C1 S. STONE (LUNAR)
C2 UNINCC.

Fig 8

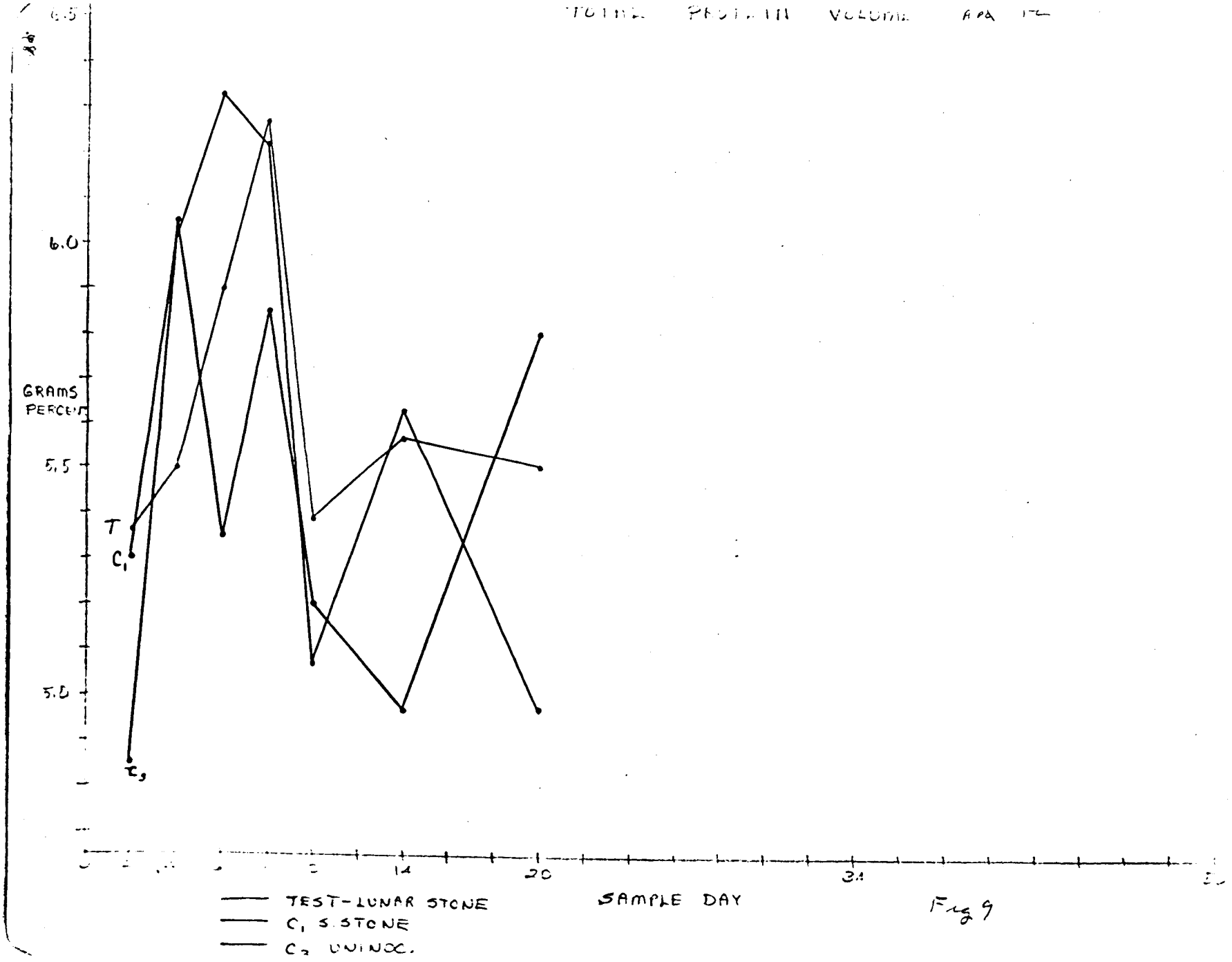

6.5
6.0
GRAMS PERCENT
5.5
5.0
T
C1
C2
14
20
SAMPLE DAY
TEST-LUNAR STONE
C1 S.STONE
C2 UNINOC.

Fig 9

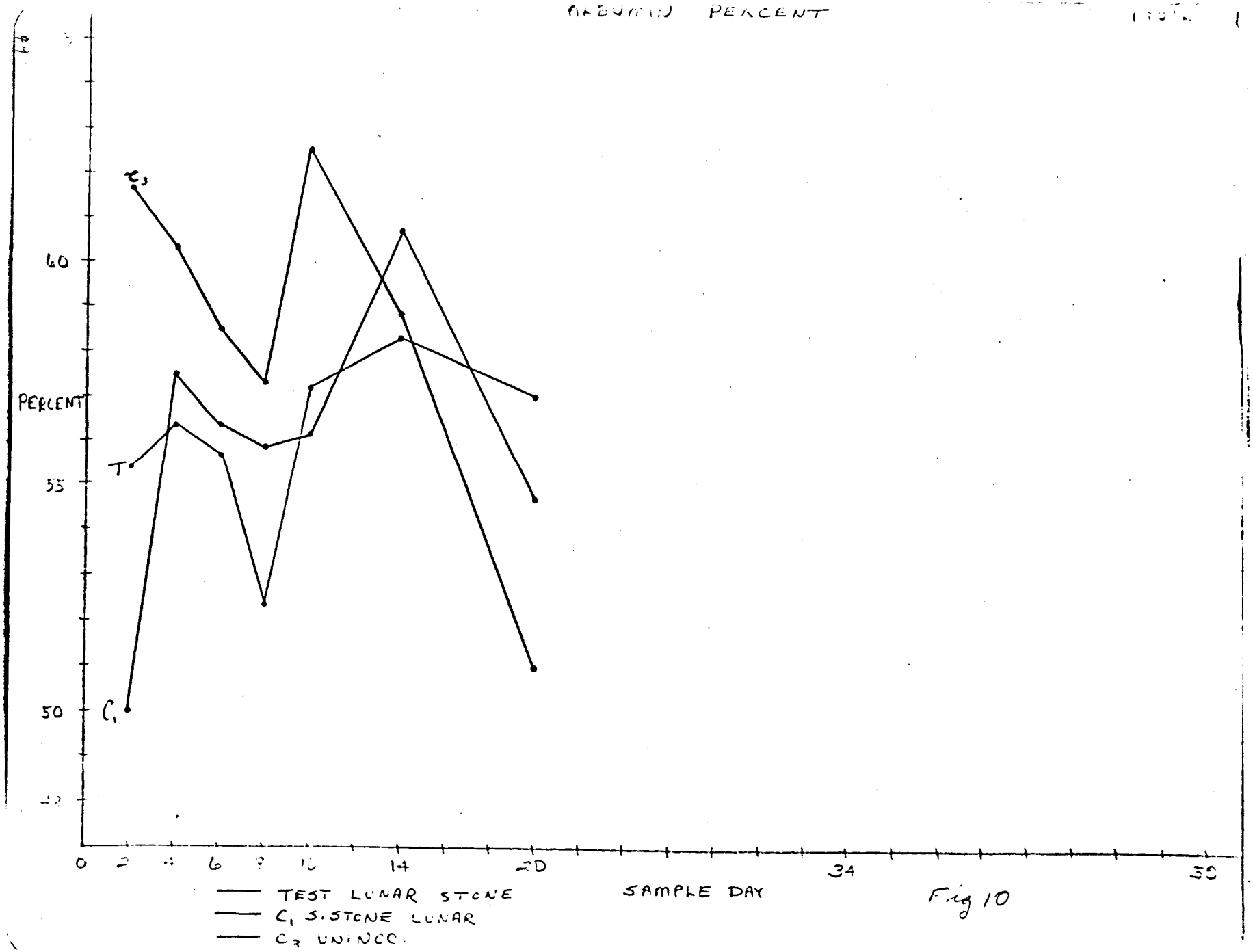

PERCENT
60
55
50
T
C_1
C_3
0
2
4
6
8
10
14
20
34
38
SAMPLE DAY
TEST LUNAR STONE
C_1 S. STONE LUNAR
C_3 UNINCC.

Fig 10

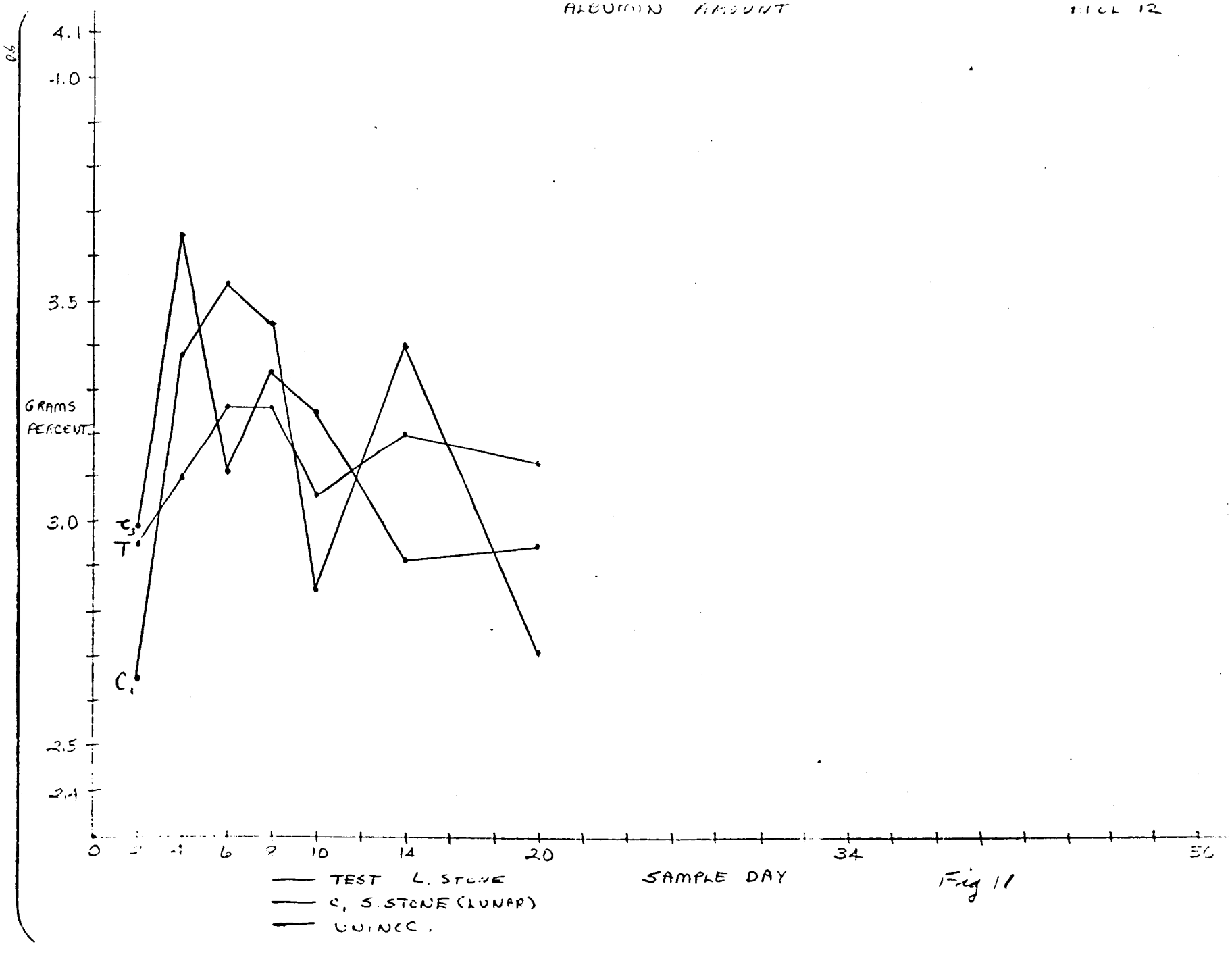

ALBUMIN AMOUNT
4.1
4.0
3.5
GRAMS PERCENT
3.0
2.5
2.1
C_3
T
C_1
0
2
4
6
8
10
14
20
34
50
SAMPLE DAY
TEST L. STONE
C_1 S. STONE (LUNAR)
GUINEA

Fig 11

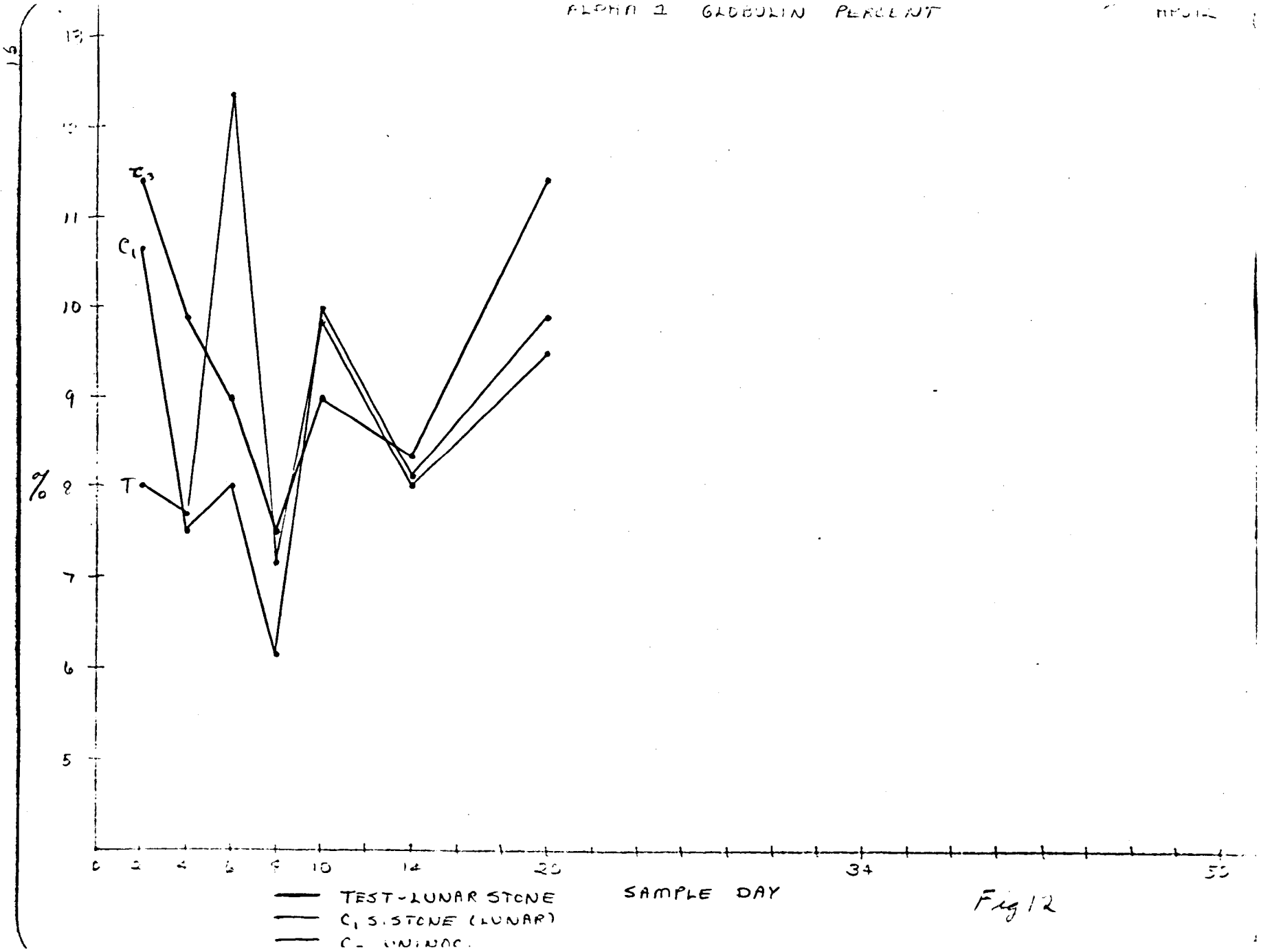

ALPHA 2 GLOBULIN PERCENT
%
13
12
11
10
9
8
7
6
5
T
C1
C2
0
2
4
6
8
10
14
20
34
55
SAMPLE DAY
TEST-LUNAR STONE
C1 S.STONE (LUNAR)

Fig 12

ALPHA I GLOBULIN AMOUNT

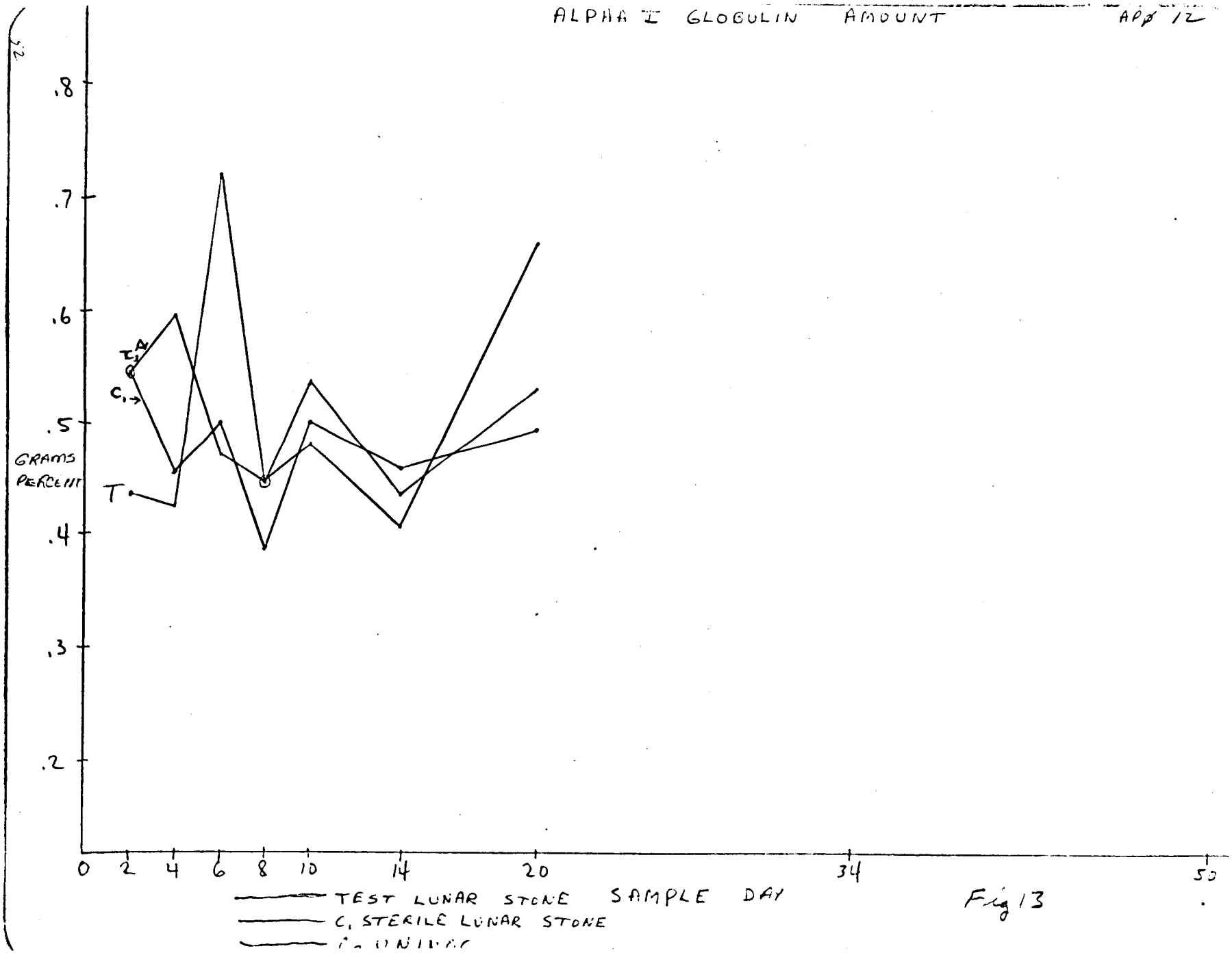


Fig 13

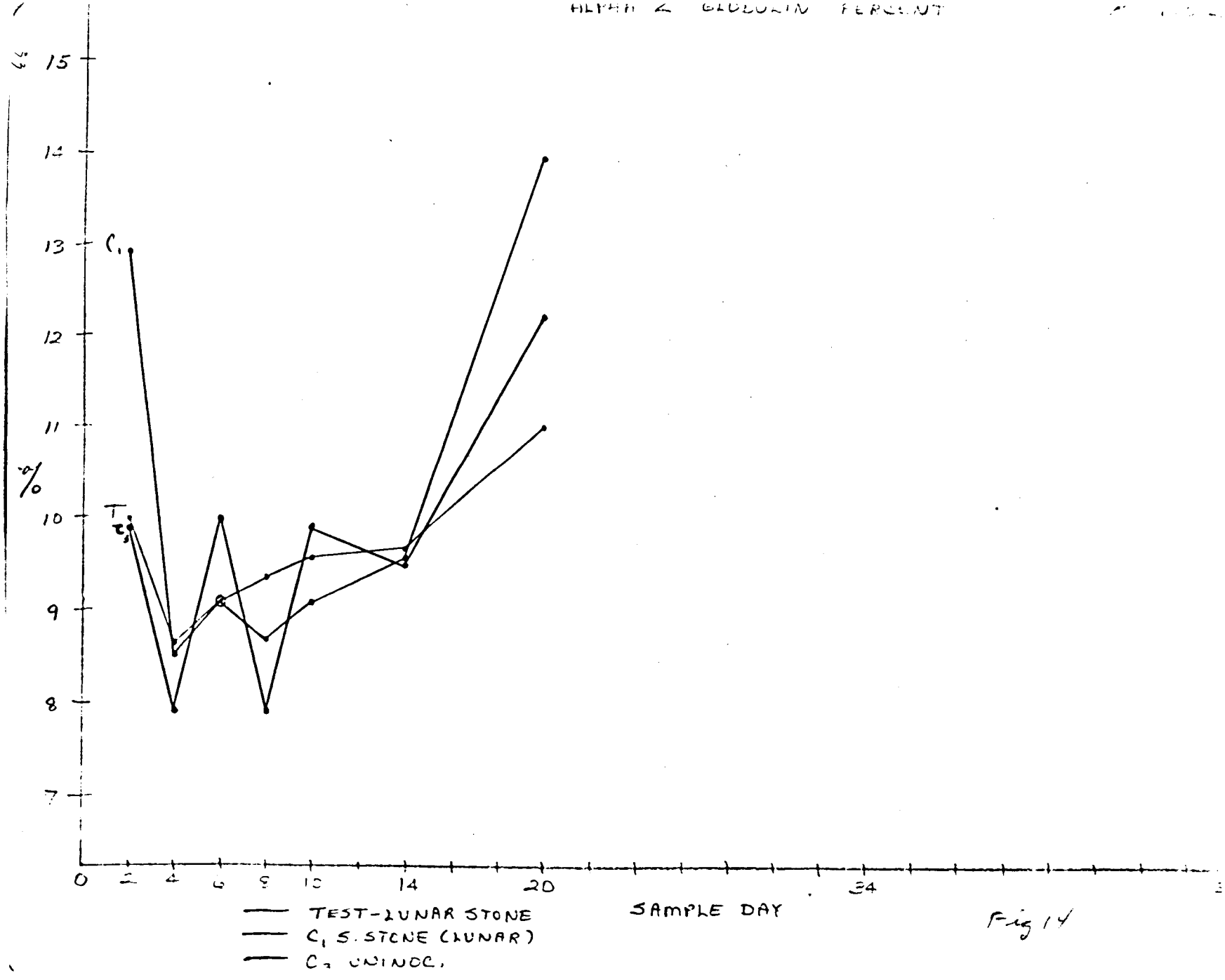

ALPHA 2 GLOBULIN PERCENT
15
14
13
12
11
10
9
8
7
%
C1
T
0
2
4
6
8
10
14
20
34
SAMPLE DAY
TEST-LUNAR STONE
C1 S. STONE (LUNAR)
C2 UNINOC.

Fig 14

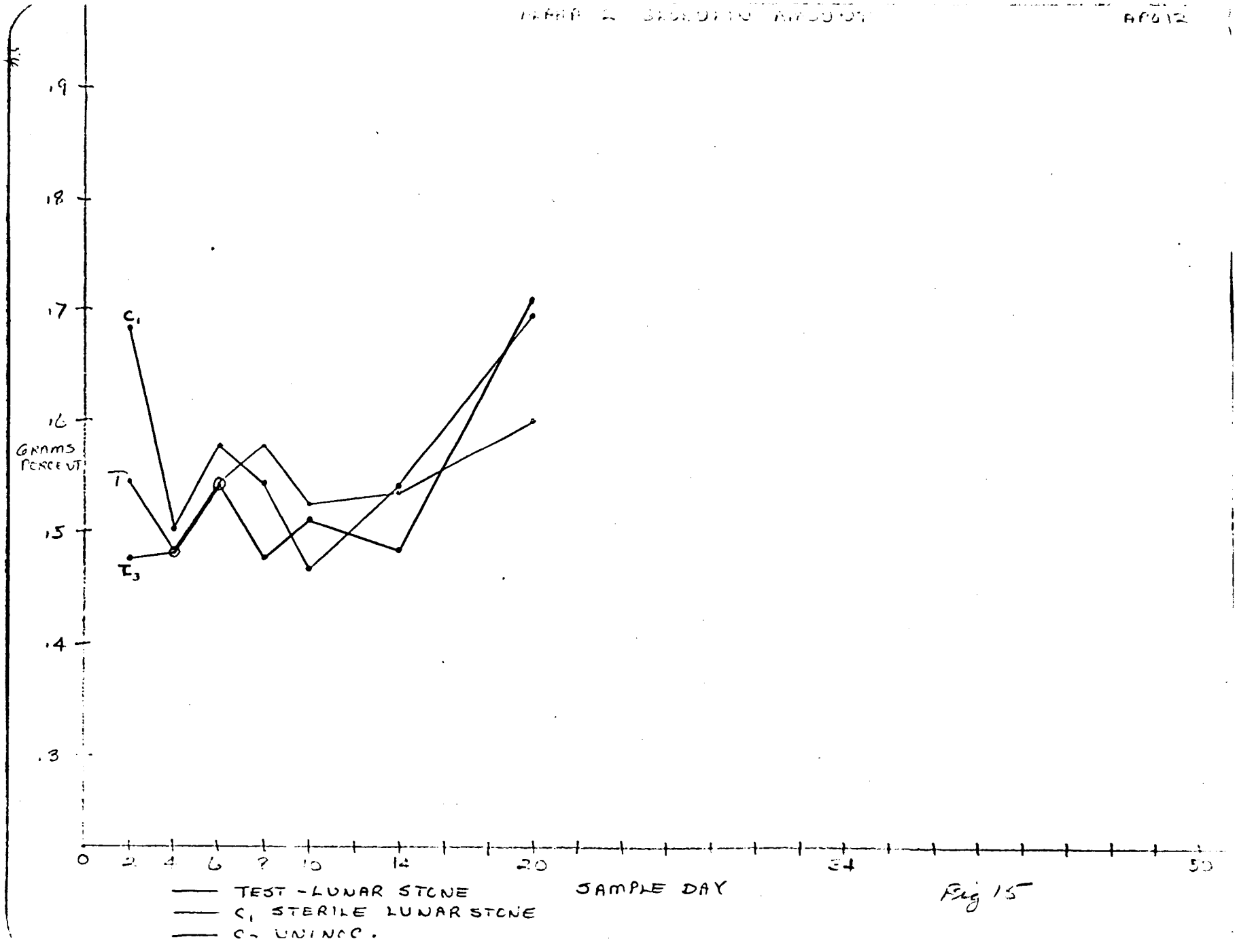

.9
.8
.7
.6
.5
.4
.3
GRAMS PERCENT
C1
T
T3
0
2
4
6
8
10
14
20
34
50
SAMPLE DAY
TEST - LUNAR STONE
C1 STERILE LUNAR STONE
C2 UNINOC.

Fig 15

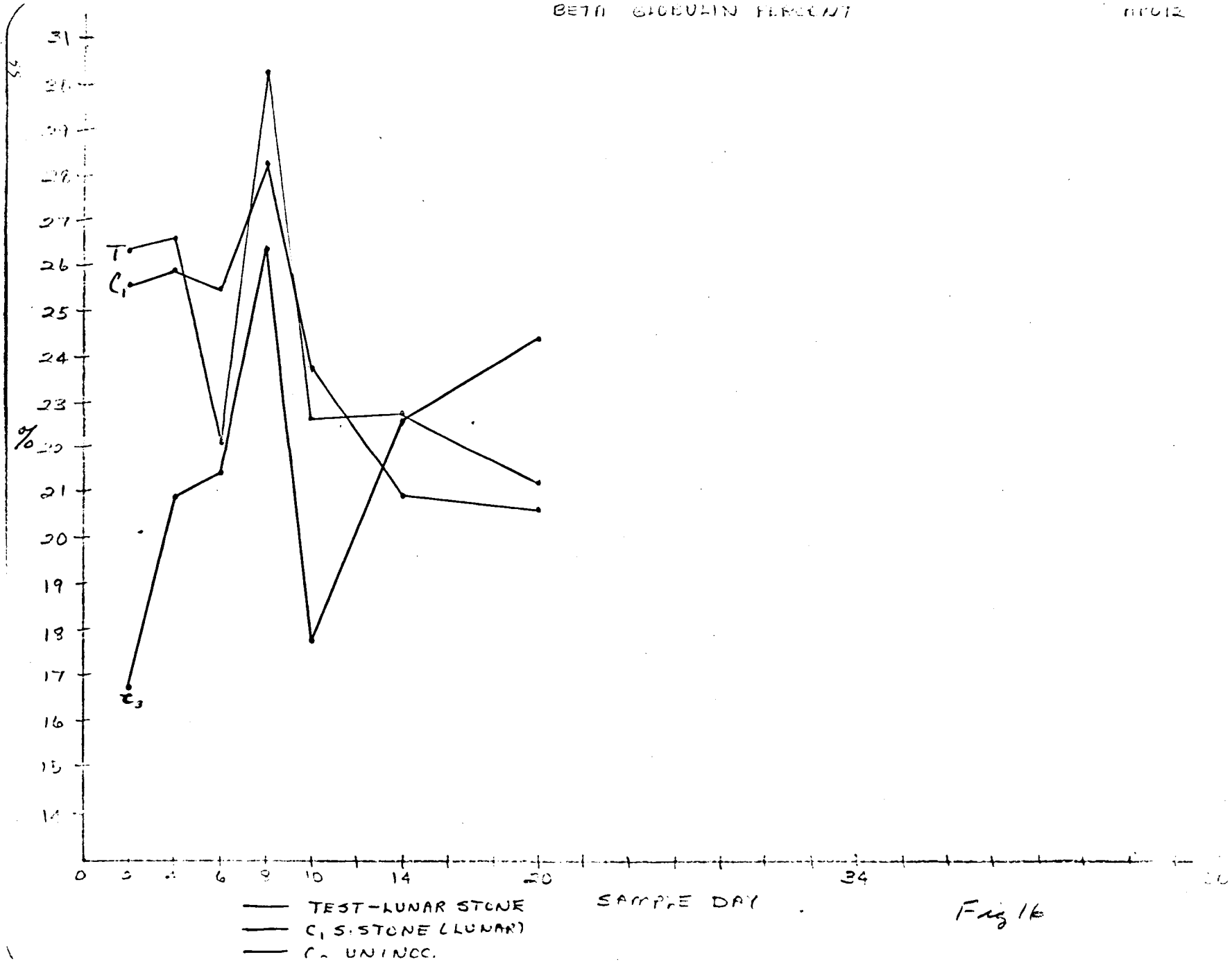

BETA GLOBULIN PERCENT
%
T
C1
t3
0
2
4
6
8
10
14
20
34
SAMPLE DAY
TEST-LUNAR STONE
C1 S. STONE (LUNAR)
C2 UNINOC.

Fig 16

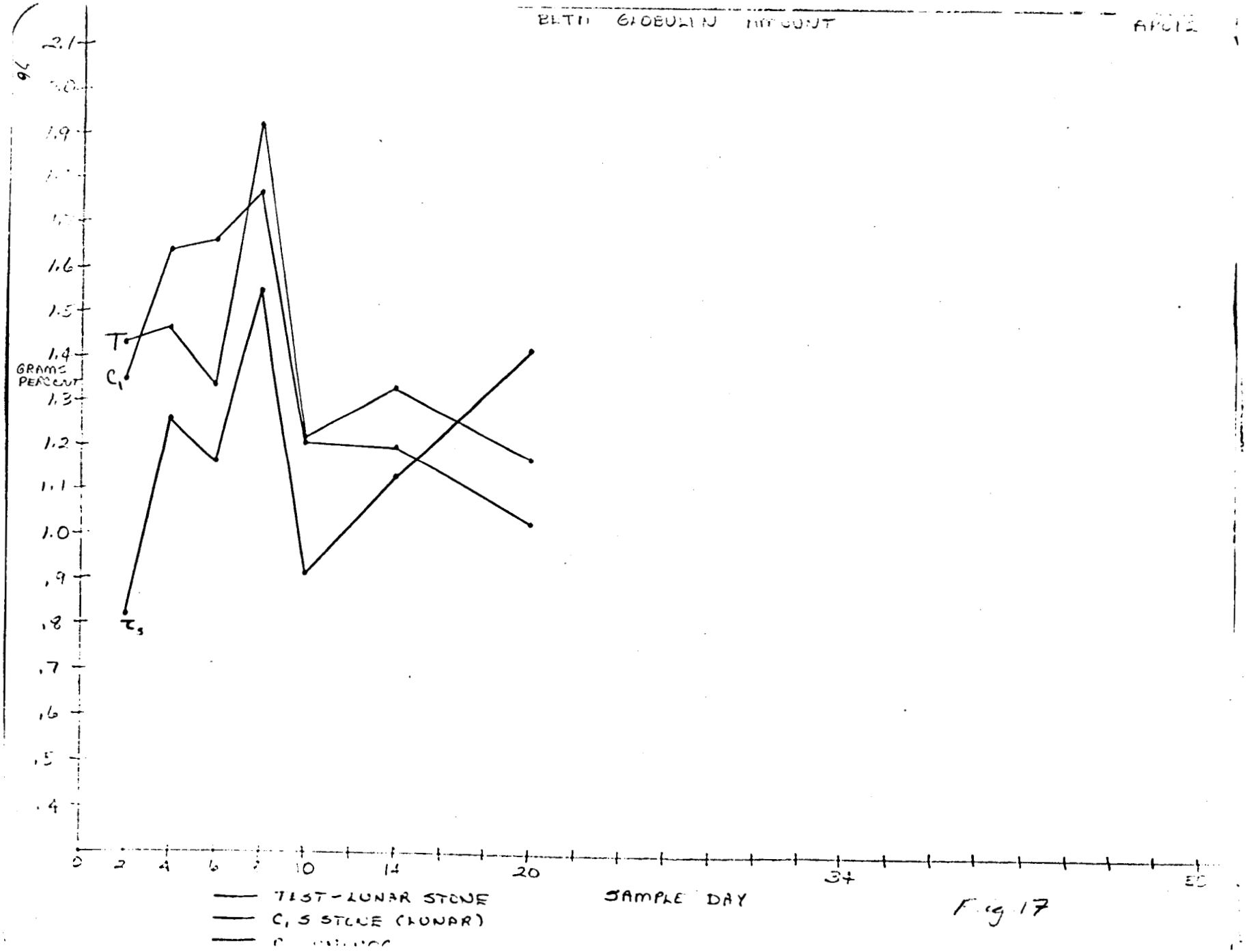

BETA GLOBULIN
APOLLO
2.1
1.9
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
GRAMS PER 100
T
C1
Ts
0
2
4
6
10
14
20
37
TEST-LUNAR STONE
C, S STONE (LUNAR)
SAMPLE DAY

Fig 17

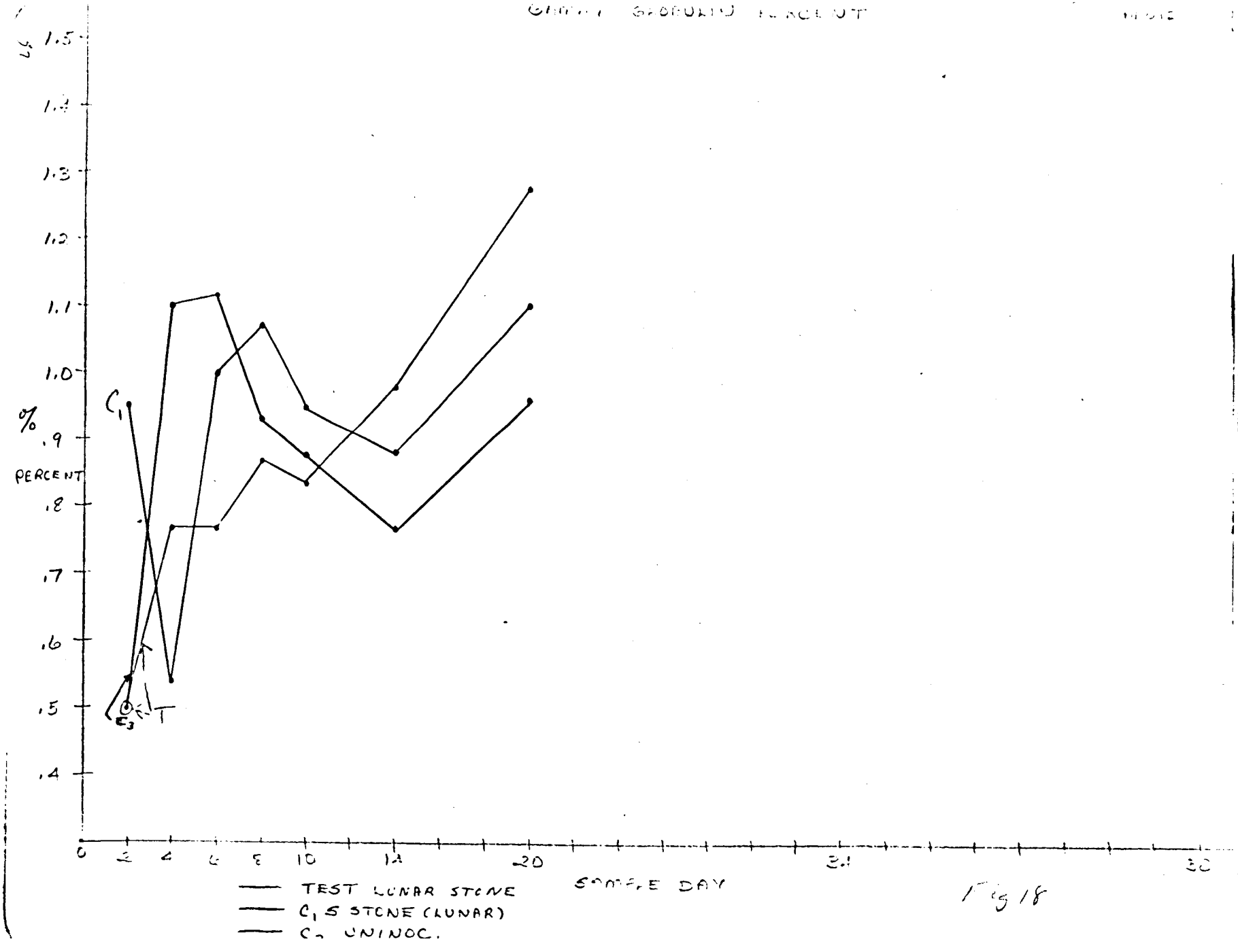

1.5
1.4
1.3
1.2
1.1
1.0
%
.9
PERCENT
.8
.7
.6
.5
.4
C_1
E_3
T
0
2
4
6
8
10
12
20
30
SAMPLE DAY
TEST LUNAR STONE
C_1 S STONE (LUNAR)
C_2 UNINOC.

Fig 18

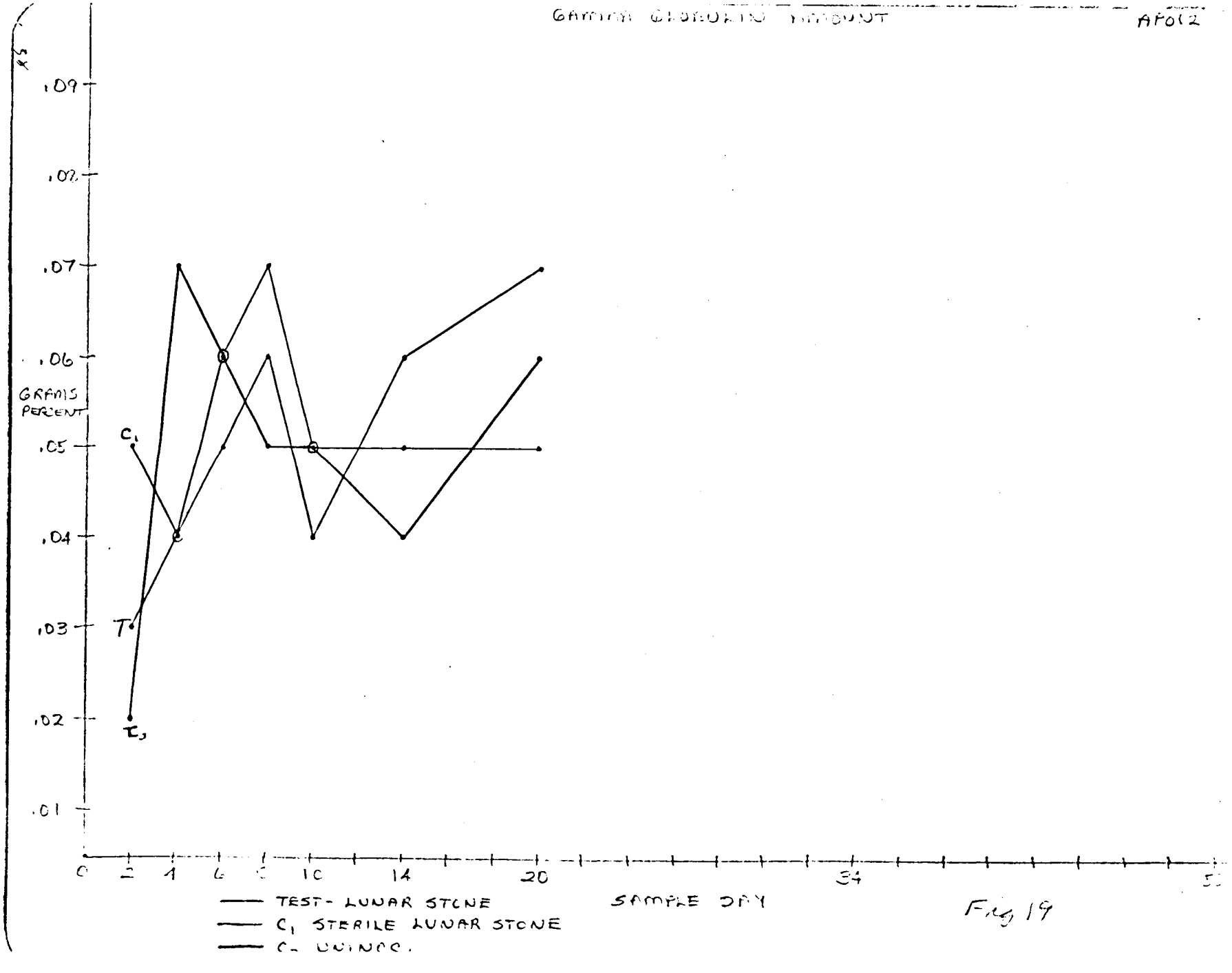

GAMMA COUNTING AMOUNT
AF012
.09
.08
.07
.06
GRAMS PERCENT
.05
.04
.03
.02
.01
C1
T
T2
0
2
4
6
8
10
14
20
34
SAMPLE DAY
TEST- LUNAR STONE
C1 STERILE LUNAR STONE
C2 UNINOC.

Fig 19

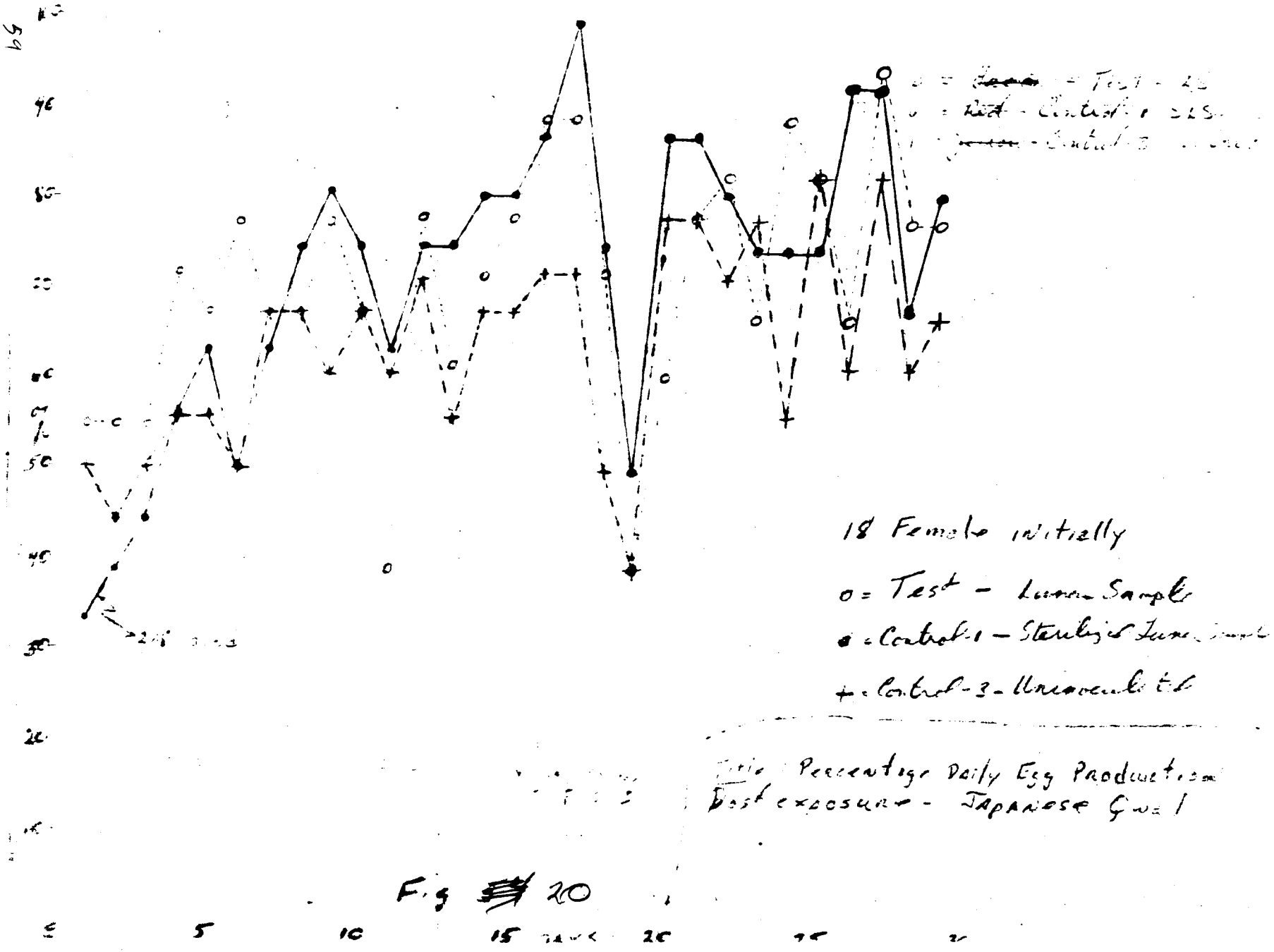

18 Female initially
o = Test - Luna Sample
• = Control-1 - Sterilized Luna
+ = Control-3 - Untreated
Percentage Daily Egg Production
Post exposure - Japanese Quail

Fig 20

VII BIO-TEST OPERATIONS: LOWER VERTEBRATES AND INVERTEBRATES

Apollo 12 lunar samples were quarantined and tested against nine selected lower animal species to determine whether or not infectious agents were present. The biological testing program was carried out in the same facilities and under the same procedures as was the Apollo 11 quarantine test program. The fish and invertebrate species were exposed to lunar material for a period of 29 days beginning on 9 December 1969 in the Lunar Receiving Laboratory (LRL) at the Manned Spacecraft Center, Houston, Texas.

A. Materials and Methods. The animals exposed to lunar materials were those that could be successfully maintained within the confines of the Class III glove-cabinetry essential to the maintenance of the biological barrier system within the LRL. The following animal species were exposed to Apollo 12 lunar samples:

Paramecium	Paramecium aurelia
Planaria	Dugesia dorotocephala
Commercial Oyster	Crassostrea virginica
Pink Shrimp	Penaeus duorarum
German Cockroach	Blattella germanica
House Fly	Musca domestica
Greater Wax Moth	Galleria mellonella
Guppy	Lebistes reticulatus
Mummichog	Fundulus heteroclitus

Optimum temperatures, photoperiods, feed, containers, media or substrates, and stock colony conditions were provided for each species to the extent possible (Table 1).

Only minor modifications in procedures were made with respect to the protocol. The protozoan Euglena was deleted from the Apollo 12 test because of its similarity to several aquatic algal forms tested by the Botany Group. The pink shrimp was used in place of the brown shrimp due to the scarcity of small brown shrimp at the time of year this test was conducted. The guppy, long established in the protocol as a substitute, replaced the fathead minnow because of a mechanical failure of the building air conditioning system in the stock colony area that killed the entire population of fathead minnows. Concern was also felt that the fathead minnow might be too sensitive to toxic agents commonly used to disinfect rooms and hallways within the biological barrier area.

The lunar material used to challenge the test animals was a portion of the pooled conventional sample that had been prepared for all biological tests. This material was ground to a mean particle size of 2 microns. One half of the sample was then sterilized by dry heat, at 160° C., for 16 hours under ambient atmospheric pressure.

Animals were divided into four groups for the test. No fewer than two replicates were established within each test group of each species. At the time of inoculation, all test animals had been acclimated to the cabinet environment for up to two weeks if appropriate. The treatment of each of the four test groups was as follows: one group was inoculated with unsterilized material, a second group was inoculated with sterilized material, a third group was maintained within the glove-cabinetry as an uninoculated control, while the fourth group was maintained in the animal support colony as a cabinetry environment control group.

Due to the differences in cultural methods for the aquatic and terrestrial species, the methods of inoculation providing exposure to the lunar samples differed. In brief, the seven aquatic species were exposed by adding lunar material to the medium in which the animals were living. The three insect species were exposed by mixing the samples with their food. Table 1 shows the amount of lunar material used per animal or culture.

The results of the exposure tests were based primarily upon qualitative observations on the general health and appearance of the test animals supported by periodic histopathological examination of specimens removed from each container. Daily records were maintained and processed into a computer system for analysis and retrieval. In addition to the examination of histological preparations from sampled animals, any mortalities that occurred were immediately examined for cause of death and also subjected to histopathological study. Photographic documentation, consisting of both still and moving pictures, successfully recorded the exact nature of behavioral changes. Supplementary data such as the fission rate of the paramecium and the developmental rates of the insects was also recorded and evaluated.

B. Results. No pathological effects resulted from the exposure of any of the test animals to lunar material. Neither daily observations of general health nor periodic histopathological examinations revealed harmful effects attributable to contact with, or ingestion of, lunar material. Specific findings from each animal system were as follows:

Paramecium (Paramecium aurelia)- Rates of fission of clonal cultures of paramecium did not differ between exposed and unexposed groups. Daily fission counts were made from depression plate cultures that were prepared with lunar-exposed and control media. This system provided exposure of the animals for the entire test period.

Table 1. TEST FACILITIES, ENVIRONMENTAL CONDITIONS, AND LUNAR SAMPLE INOCULATION DATA

SPECIES	CONTAINER	REPLICATES	NUMBER OF ANIMALS PER REPLICATE	TOTAL REPLICATE	DIET	MEDIUM	PHOTOPERIOD LIGHT/DARK	TEMP °C	0.220 gm LUNAR MATERIAL PER
Paramecium	125 ml Flask	25	1.0×10^3 to 1.6×10^4	75	Aerobacter aerogenes	Lettuce Leaf infusion	16/8	22	Flask
Planaria	300 ml Finger-bowl	3	10	9	None	Aged tapwater	16/8	22	Bowl[1]
Pink Shrimp	20 Liter Aquarium	2	20	6	Live brine shrimp	Seawater $25^o/_{oo}$	16/8	27	Shrimp
Oyster	One Liter Jar	10	1	30	None	Instant Ocean $21^o/_{oo}$	16/8	22	Oyster
German Cockroach	500 ml Jar	3/3[2]	20/15	9/9	Mouse[3] diet		16/8	25	Jar
House Fly	500 ml Jar	6/3[4]	25/25	18/9	Compounded[5] diet		16/8	25	Jar
Greater Wax Moth	500 ml Jar	3	20	9	Compounded[6] diet		Total darkness	25	Jar
Guppy	20 liter Aquarium	2	20	6	Live brine shrimp	Aged tapwater	16/8	22	Fish
Mummichog	20 liter Aquarium	2	20	6	Live brine shrimp	Instant Ocean $21^o/_{oo}$	16/8	22	Fish

[1] 1.10gm per bowl

[2] Nymphs/Adults

[3] Charles River prefortified rat-mouse diet

[4] Larvae/Adults

[5] Sugar, albumen, sodium oleate, Wesson salts, B-vitamins

[6] Pablum, sugar, glycerol, water, B-vitamins

Planaria (Dugesia dorotocephala) - No histopathological changes nor changes in morphology were detected in any of the test groups involved in the Apollo 12 tests. All cultures went without food for the test in order to to compare pre- and post-test animal weights. Control and sterilized test group populations remained static throughout the test while those planarians exposed to unsterile lunar increased by 3 (10%).

Oyster (Crassostrea virginica) - No unusual microorganisms or histopathological conditions were noted in the sections prepared from control or lunar sample-exposed oysters. All oysters were in good condition. Moreover, most of the gonadal material had been absorbed in all oysters. This is a normal condition to be expected at this season of the year. Based on the condition of the oysters sampled after 14 days of exposure to lunar material, one may expect good survival for another 30 to 60 days under good conditions.

Pink Shrimp (Penaeus duorarum) - Test shrimp, exposed to lunar samples, showed no abnormal behavior or deleterious effects attributable to exposure to lunar material. Some gross changes in the carapace and appendages were observed in animals in all test and control groups during the first two weeks of the test. Histopathological examination indicated some pathological changes but these changes could not be attributed to lunar exposure. All animals were in generally improved condition near the end of the test as indicated by the regeneration of appendages and the condition of the exoskeleton.

German Cockroach (Blattella germanica) - Daily observation together with histopathological examination of samples collected at one and three weeks showed these animals to be in excellent condition throughout the exposure period. The symbiotic bacteria in the fat bodies appeared normal. The mid-gut epithelium, central nervous system tissue and renal tissues showed no pathology.

House Fly (Musca domestica) and Greater Wax Moth (Galleria mellonella) - Sections were prepared from house fly and greater wax moth larvae which had been exposed to lunar material. Histopathological studies showed no differences between the control and test groups. Gut, nervous, hypodermal, vascular, and adipose tissues were examined and found normal in all respects. No pathogenic microorganisms were detected and no evidence of disease was found.

Guppy (Lebistes reticulatus) - Although most guppy aquaria remained cloudy to turbid during the test, the fish remained in good condition in all test groups. The birth of several young was observed and behavioral characteristics were normal. Both 14-day and 28-day histopathological samples were collected. The results from the first sampling indicated some gill pathology in all groups which could be attributed to the aquarium conditions. No evidence of replicating organisms was observed in the animals exposed to lunar material.

Mummichog (Fundulus heteroclitus) - All groups of mummichogs remained in excellent condition until about day 20 of the exposure period. At this time several fish in each aquarium were noted to be swimming at the surface as if starved for oxygen. Mortality soon followed and fish were lost from each group. Aereation of the water alleviated the condition but bacterial growth leading to gill irritation and anoxia was found to be the cause. Treatment could not be administered without affecting the sensitivity of the test system. Histopathological results from samples collected at 14 days substantiated these findings. These histological preparations gave no evidence of any pathologic changes attributable to exposure to lunar material.

C. Discussion. The results of the Apollo 12 lunar sample testing program involving nine lower animal species closely paralleled the findings of the Apollo 11 experiments. The uniformly negative results coupled with a hint of beneficial effects are the most striking biological similarities. These results support the geo-chemical analyses performed by other workers in the LRL who also reported basic similarity between the two samples. Some minor differences were noted, however.

As reported following the Apollo 11 mission, the sample that had been heat sterilized was hydrophobic, cohesive and required considerable stirring and agitation to suspend it in water. The unsterilized Apollo 11 bio-sample was easily wetted and promptly sank. Both the sterilized and unsterilized Apollo 12 samples were hydrophobic and cohesive making the differences between the two samples was much less marked. These characteristics affected the tests in no way once suspension of the material was achieved.

The results of these preliminary biological tests involving nine species of lower animals have provided no information that would indicate that the lunar material returned by the Apollo 12 mission should not be released from quarantine for further investigation.

VIII. BIO-TEST OPERATIONS - BOTANY

A portion of the lunar material carried to earth by the Apollo 12 astronauts was held in quarantine in the Botanical Sample Laboratory for 30 days. During this period, a variety of plant species was challenged with a mixture of ground rock samples and fine powder collected on the lunar surface. The objective of these tests was to determine if lunar material can cause disease in plants grown under aseptic conditions within the Class III biological enclosures.

The botanical program at the Lunar Receiving Laboratory is one of a number of biological endeavors aimed at insuring that the lunar material is not a threat to the terrestrial biosphere. Maximum diversification in testing was achieved by assessing the effects of lunar materials on a variety of plant systems, including cells in culture, germinating spores and seeds, and young seedlings. Detailed observations were made on both fresh and fixed materials. The appearance of microbes was confirmed by culturing techniques and identified as terrestrial by the Microbiology Group.

The purpose of the present communication is to document the results of the plant testing protocol on Apollo 12 in summary form. Details of the testing procedures are available in unpublished form (Walkinshaw, MSC 00059, Volume III, November 5, 1969). An elaboration of the beneficial effects of lunar material on plants will be given later.

A. Materials and Methods

All plant systems challenged with lunar material were contained in presterilized, gas tight enclosures designated as Class III cabinets. All items passed into the enclosures were sterilized by steam autoclaving or decontaminated by soaking in 5000 ppm sodium hypochlorite for 30 minutes. The only plant materials removed from the cabinets during the 30-day protocol were small pieces of tissue fixed in Bouin's or 6% glutaraldehyde solution for a period of 24 hours. Manipulations of plants could be accomplished only by using rubber gloves. Incoming air was filtered through activated charcoal and a 99.97% biological filter. There were 4 gloves ~~and no filter failures~~ during the 4 months of preparation and testing.

The air temperature for the algae was 25° and for all other species, 21 to 27°C. The turnover rate of air was adjusted for 1 to 4 changes per hour. Air within the cabinets was recirculated with fans. Carbon dioxide was enriched to 1% for the algae. Tissue cultures and algae received from 300 to 500 fc of continuous, full spectrum light while seedlings received 1000 to 2000 fc for a 16-hour photoperiod. The humidity of all systems was above 80%.

A total of 1320 challenge units was employed in an experimental design of four treatments and ten replicates per treatment per species. The challenge unit was an algal culture, a group of 10 seeds or 1000 spores, an individual plant, or a jar of tissue culture. The species tested are listed in Table 1. All species were germ free when introduced in cabinets. Phytopathogenic microorganisms were not detected during the period of testing.

Challenge of the 10 replicate units for each species was made by exposure to lunar material, dry-heat-sterilized lunar material, and dry-heat-sterilized earth material. The challenge dose was 0.22 g which was added as a dry powder or as a slurry in 5.0 ml of 0.05M phosphate buffer, pH 7.0. One set of replicates served as the control and was not challenged.

Observations were made at regular intervals, and all changes were documented on 35 mm film. Health of individual challenge units was defined as the summation of numerical scores (1 = poor, 2 = questionable, and 3 = good) for a set of descriptive characters. Four to thirteen characters were graded three times per week alternately by two observers. Tabulations were accumulated on magnetic tape, and analyses of variance were made to determine treatment and species significance. Histological examinations and analyses of cellular morphology were performed on 200 of 1320 challenge units.

B. Results and Discussion

Cultures of algae challenged with lunar material varied in their response. All species were inhibited initially by both lunar and earth materials. There was no significant difference in the response of any algal species to lunar and earth materials.

There appeared to be no effect of lunar and terrestrial materials on the percentage germination of seeds. Observational data taken 12 days after initiation showed that all challenge units had normal growth, pigmentation, and stem and root configuration. There was no evidence of toxicity.

Fern gametophytes which developed directly on or around the lunar and terrestrial materials showed a marked stimulation in growth.

Lunar material was not harmful to plant seedlings. All seedlings appeared healthy throughout the test period; Apollo 12 seedlings grew significantly better than those employed in Apollo 11 tests.

A number of changes were observed when tissue cultures were challenged with lunar and earth materials. A number of species of tissue cultures exhibited marked necrosis in those areas that contacted the crushed lunar rock. However, recovery occurred, and after 20 days these cultures appeared as well as, or better than, untreated controls. Since plant tissues were not subcultured during the test, most exhibited a general decline due to nutrient depletion.

Histological samples taken at random from 20 species were graded on characteristics of their cellular morphology. Results show clearly that there was no difference among the treatments. Lesions characteristic of an infectious process were not found.

Summary

Many effects of a physiological nature were observed when plant species were exposed to lunar materials. Most of the changes were beneficial; none could be associated with an infectious process. A few species were stimulated by the Apollo 12 lunar sample used in the botanical testing.

LIST OF PLANT SPECIES CHALLENGED WITH APOLLO 12 LUNAR MATERIAL ACCORDING TO EXPERIMENT TYPE

Challenge system	Species	Family
Algae	Anacystis nidulans (Richt) Drouet	Chroococcaceae
	Chlorella pyrenoidosa Chick	Oocystaceae
	Phaeodactylum tricornutum Bohlin	Phaeodactylaceae
	Porphyridium cruentum (Ag.) Naeg.	Bangiaceae
Seeds	Allium cepa L.	Liliaceae
	Brassica oleracea L.	Brassicaceae
	Capsicum frutescens L.	Solanaceae
	Lactuca sativa L.	Asteraceae
	Nicotiana tabacum L. var. Samson	Solanaceae
	Phaseolus aureus L.	Fabaceae
	Raphanus sativus L.	Brassicaceae
	Spinacia oleracea L.	Chenopodiaceae
Seedlings	Brassica oleracea L.	Brassicaceae
	Capsicum frutescens L.	Solanaceae
	Citrullus vulgaris Schrad.	Cucurbitaceae
	Citrus limonia L.	Rutaceae
	Cucumis melo L.	Cucurbitaceae
	Lycopersicum esculentum Mill.	Solanaceae
	Lycopodium cernuum L.	Lycopodiaceae
	Marchantia polymorpha L.	Marchantiaceae
	Onoclea sensibilis L.	Polypodiaceae
	Osmunda cinnamomea L.	Osmundaceae
	Phaseolus vulgaris L.	Fabaceae
	Pinus elliottii Engelm.	Pinaceae
	Raphanus sativus L.	Brassicaceae
	Sorghum vulgare Pers.	Poaceae
	Todea barbara (L.) Moore	Osmundaceae
	Triticum vulgare Vill.	Poaceae
	Zea mays L. var. everta	Poaceae
Tissue cultures	Glycine soja (L.) Sieb & Zucc.	Fabaceae
	Haplopappus gracilis (Nutt.) Gray	Compositae
	Helianthus annuus L.	Asteraceae
	Nicotiana tabacum L.	Solanaceae
	Nicotiana tabacum L. (Albino)	Solanaceae
	Oryza sativa L.	Poaceae
	Pinus palustris Mill.	Pinaceae
	Zea mays L.	Poaceae

SUMMARY

Approximately 75 pounds of lunar material originating from the moon's Ocean of Storms have been successfully returned to the earth and an aliquot of this material subjected to detailed biomedical evaluations. The results of these evaluations indicate that the presence of this material in a terrestrial biosphere does not represent a hazard to terrestrial life. In addition, the Apollo 12 astronauts who were directly exposed to this material have successfully completed their quarantine in the Lunar Receiving Laboratory's Crew Reception Area. Biomedical evaluations conducted during this quarantine period revealed that the crewmembers did not harbor any alien, hazardous life forms. Review of the detailed data included in this report will reveal that although the samples are not deleterious to terrestrial living systems, there is some species specific enhancement of certain biological parameters stimulated in the presence of the samples.