James G. Laume, M.D.

A B S T R A C T S

SYMPOSIUM - WORKSHOP

BIOLOGISTICS FOR SPACE SYSTEMS

1 - 3 MAY 1962

BILTMORE HOTEL, DAYTON, OHIO

CHAIRMAN:

ANDRES I. KARSTENS, Colonel, USAF, MC Commander Aerospace Medical Research Laboratories Wright-Patterson Air Force Base, Ohio LARGE AIGAL SYSTEMS. Dr. H. H. Bovee, Dr. A. J. Pilgrim, L. S. Sun, J. E. Schulbert, T. L. Eng, and B. J. Benishek. Bioastronautics Section, The Boeing Company, Seattle, Washington.

Of primary concern in manned space flight is the development of an oxygen producing system capable of supporting man in a closed respiratory loop. Emphasis has been directed toward a photosynthetic gas exchanger, utilizing the unicellular green plant, Chlorella pyrenoidosa. Boeing has fabricated an experimental, biological respiratory support system composed of thin plexiglass tanks sandwiched between layers of fluorescent lights. Experimentation with this apparatus has served to delineate several problem areas primarily in the area of design and component integration. Both manned and unmanned studies have been performed with the intact system. The results of these studies and the modification resulting therefrom will be presented and discussed.

DESIGN AND DEVE IOPMENT OF AN ENGINEERING MODEL PHOTOSYNTHETIC GAS EXCHANGER. H. Wallman, J. Dodson. Electric Boat Division, General Dynamics Corp., Groton, Connecticut.

A study was conducted to design, fabricate and test a highly instrumented photosynthetic gas exchange system capable of handling the carbon dioxide output of one man. The system designed consists of three major components:

(1) a multi-pass hight chamber containing thirty-six 96" fluorescent lamps, (2) a counter-current gas contacting tower, and (3) an instrumentation console for controlling and recording the important parameters. In addition, a centrifuge is provided for the harvesting of algae. Based on the growth rate of the algae, it was demonstrated that this system has the capability of supporting one man.

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SOLAR ILLUMINATED PHOTOSYNTHETIC GAS EXCHANGERS. Dr. Robert D. Gafford, Lt. Col. John D. Fulton (Assigned Arctic Aeromedical Research Labs), Space Flight Laboratory, Martin-Marietta Corp., Denver, Colorado.

Chlorella pyrenoidosa strain TX 71105 and the 52°C strain of Synechococcus lividus were cultured in thin films in hemispherical domes and solar oriented flat panels during the long Alaskan days of June and July 1961. Growth and photosynthetic rates were measured in cultures having film depths of 1, 2, and 4 cm under solar illumination. Comparative growth and photosynthetic data of the two species under the various conditions are presented. The maximum production rate observed in the flat panels was over 50 grams of algae and approximately 100 liters (STP) of oxygen per sq. meter of illuminated surface per day. Based on the observed data it is estimated that the illuminated area of algal suspension required for a one man gas exchanger will be 6 square meters or less. The maximum volume of the illuminator will be 60 liters. A small additional volume must be added for pumping and for gas exchange.

HIGHLY CONCENTRATED CARBON DIOXIDE AS A CARBON SOURCE FOR CONTINUOUS ALGAL CULTURES. Dr. Robert W. Tew, J. O. Sane, R. P. Geckler. Dept. of Life Sciences, Aerojet General Corp., Azusa, California.

Design of maximally efficient algae cultures will depend to a large extent on minimization of gas addition rates. Theoretical considerations show that this can be accomplished if high concentrations of carbon dioxide are used as the carbon source for photosynthetic gas exchangers. This paper comprises a detailed description of a laboratory system which allows continuous culture of thermophilic chlorella using pure carbon dioxide or nitrogen highly enriched in carbon dioxide. Reported in detail is an analysis of the sequence of events taking place during a long continuous run in terms of culture productivity and carbon dioxide equilibria under defined conditions. A discussion of the importance of this work as it relates to practical growth control and engineering for optimal gas exchange is included.

NOTES $f = K_{\nu} \left(.693 \right)$

THE PHOTOREACTIONS OF PHOTOSYNTHESIS. Dr. Bessel Kok. Research Institute for Advanced Studies, Martin-Marietta Corp., 7212 Bellona Ave., Baltimore 12, Maryland.

A brief survey will be given of the effects of light intensity and wavelength on the rates of photosynthesis.

The evidence for the concept of two light reactions and present thinking concerning the absorption, transfer and collection of light quanta in the trapping centers in photosynthesis will be discussed.

STRUCTURE OF THE PHOTOCHEMICAL APPARATUS. Mr. George Shidlovsky. Research Institute for Advanced Studies, Martin-Marietta Corp., 7212 Bellona Ave., Baltimore 12, Maryland.

A summary of our present state of knowledge on the morphology of photosynthetic organelles as revealed by electron microscopy and other techniques will be presented.

The evidence is that a lamellar type of organization of pigment and other molecules is necessary for aerobic photosynthesis in higher plant chloroplasts and algae. Some possible interpretations of this structural requirement will be discussed.

MECHANISMS OF OXYGEN EVOLUTION. Dr. Leo P. Vernon. Charles F. Kettering Research Laboratory, 150 E. South College St., Yellow Springs, Ohio.

Oxygen evolution is restricted to green plant photosynthesis, and distinguishes it from the bacterial type. After an adaptation period under a hydrogen atmosphere in the dark, some algae lose the ability to produce oxygen and practice a photosynthetic process similar to that of the bacteria. Such adapted algae readily revert to the regular plant photosynthesis at high light intensities, and thus possess alternate ways of utilizing the oxidizing equivalents produced under the influence of light in the chloroplast. In regular photosynthesis this oxidizing power is utilized to oxidize water and produce oxygen through a complex and unknown series of reactions. In the adapted algae, the oxidizing power goes to oxidize some other compound (hydrogen or endogenous hydrogen donors). Chloroplasts which have their oxygen-evolving system impaired by poisons resort to such an alternate oxidation of electron donors.

Manganese is required for the oxygen evolving system. Recent experiments indicate that oxygen evolution follows absorption of light by a pigment system different from that involved in production of reduced pyridine nucleotide. Various possible mechanisms of oxygen evolution will be discussed. REQUIREMENTS AND FUNCTIONS OF MICRONUTRIENTS WITH RESPECT TO PHOTOSYNTHESIS. Dr. Clyde Eyster. Charles F. Kettering Research Laboratory, 150 E. South College St., Yellow Springs, Ohio.

More than 15 elements are required by green plants for their nutrition and development. Those elements required in relatively large amounts in the range from 10-2 M to 10-4 M are macronutrients. Elements like Fe, Mn, Cu, Zn, B, Mo, Co, V, and Cl which are utilized in relatively small amounts are micronutrients. Fe is a constituent of cytochromes which are believed to serve in the transfer of electrons during photosynthesis. Mn is also a key element in photosynthesis and is required for the evolution of oxygen. Whole cells and sonicated cells of algae as well as green chloroplasts must have at least 10-/ M manganese in the culture medium for maximum oxygen production. Chloride has been shown to be essential for Hill reaction activity and non-cyclic photophosphorylation. A number of dehydrogenases are known to contain Zn.

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SELECTION OF BROADLEAF PLANTS FOR CLOSED ECOLOGICAL SYSTEMS. Dr. Samuel P. Johnson. Bioastronautics Section, The Boeing Company, Seattle, Washington.

The ultimate selection of broadleafed plants for use in space systems will depend on their ability to produce a nutritionally acceptable plant and to photosynthesize efficiently in the exotic environment of the space system. The exotic environment is defined as one having a high osmotic substrate. artificial light as a source of energy, temperatures averaging seventy degrees Fahrenheit, and an atmosphere composed of oxygen, carbon dioxide, and nitrogen but not necessarily the same composition normal to the plant. Nitrogen may be replaced by he lium or argon. In addition, the factor of weight lessness or near weightlessness must be considered. In order to restrict the number of plants to be considered, additional limitations are placed upon the prospective candidates. These are: the species should be non-nitrogen fixing, non-flowering, wholly edible if possible, a rossette mode of growth for conservation of space, and resistance to common diseases.

THE PERMANENT LUNAR BASE: DETERMINATION OF BIOLOGICAL PROBLEM AREAS. Dr. James C. Finn, Jr., and Oswald D. R. Brown. Dept. of Life Sciences, North American Aviation, Inc., 12214 Lakewood Blvd., Downey, California.

With the advent of Apollo, the requirement for a permanent lunar base is no longer academic. Whether the permanent base be established 4, 7 or even 10 years following the initial landing, the time remaining for solution of certain biological problems associated with definition of design parameters for the life support system is fast running out. This urgency argues for a comprehensive, yet flexible, national plan designed for timely solutions of the many biological problems which arise in connection with plant and food animal production, gas exchange and waste utilization. Some of these problems are brought into focus through a critical examination of the several variations of a biological subsystem which are suggested by analyses of criteria such as lunar environment, kinds and requirements of plants and food animals, and engineering considerations.

PARTIAL PRESSURE OF NITROGEN ON PLANT GROWTH.

Dr. F. Benjamin, W. Taufman, Space Environment and Life Sciences, Republic Aviation Corp.,
Farmingdale, L. I., New York.

Little work has been done on the influence of gravity, atmospheric pressure, and partial pressures of selected gases on higher plants. The results may well determine whether higher plants can be used effectively in a closed ecological system for space exploration. A series of experiments using a climbing type of vegetable plant (bush bean) showed a marked inhibition of growth under simulated zero gravity. However, even with a slight gravitational field (1/6 G) no significant effect was observed.

If atmospheric gas composition was maintained and pressure was lowered a proportional inhibition of growth was observed. In the next series of experiments the effect of absolute pressures with constant partial pressure of oxygen (200 mm Hg) and varied partial pressure of nitrogen on the growth characteristics of these same species of plants was examined. The experimental period was two weeks. Under these conditions bush beans have a lower rate of oxygen uptake during initial growth when the absolute pressure is low. Rhythmic variation of metabolic activity becomes apparent, which to our knowledge has not been observed previously. Partial pressure of nitrogen appears to affect plant growth and present results indicate that our earthly atmosphere is not necessarily the optimum condition for these plants.

HYDROPHYTE SEED PLANTS FOR GAS EXCHANGE SYSTEMS.

Dr. Syrrel S. Wilks, School of Aerospace Medicine,
Brooks Air Force Base, Texas.

Preliminary investigations with representatives of the family Lemnaceae (duckweed) indicate a justification for continued studies of these plants with respect to their photosynthetic gas exchange potentialities. These studies were carried out with representatives of the genera Wolffia, Lemna and Spirodela. Data were obtained on growth rates and gas exchange rates under varying conditions of light, temperature, nutrients, and concentration of 02 and CO2. In a cylindrical chamber 11 inches x 51 inches with approximately 10 ft2 of plant frond area, illuminated to an intensity of 500 foot candles with General Electric Power Groove, cool white fluorescent bulbs, a gas exchange rate of approximately 100 ml/hr/ft2 was observed. Total dry weight of plants in the system at the time of the rate indicated above was 50 grams.

Nutritional potentialities were indicated when four mice were sustained on 300 grams dry duckweed (compressed into small cylinders) for a period of 31 days - discontinued when food supply became exhausted.

The investigations suggest the possibility of greatly reducing the volume of the plant nutrient solution. Gaseous exchange occurs directly at plant-gas interphase. Harvesting equipment for duckweed system would be more simple than that for an algal system. The system would probably be more amenable to sub "G" environments than algal systems.

AEROBIC WASTE DISPOSAL SYSTEMS. Dr. James E. Moyer. School of Aerospace Medicine, Brooks Air Force Base, Texas.

The utilization of an aerobic microbiological process for the decomposition of wastes resulting from prolonged space probes has recently received considerable investigative attention. One of the methods currently being evaluated includes the use of a closed system employing activated sludge for the oxidation of organic wastes in combination with algae to produce the required oxygen for the process. The performance of a closed, illuminated activated sludge-algal disposal unit being maintained solely on solids resulting from fecal wastes will be discussed with regard to CO₂ - O₂ ratios, pH, total solids, and salt concentrations.

ANAEROBIC WASTE PROCESSING. Dr. Hugh L. Pote. Space Flight Laboratories, Martin-Marietta Corp., Denver, Colorado.

Methods for processing animal and plant wastes must be perfected before an effective regenerative life support system can be operated. An anaerobic thermophilic digester has been constructed and tested in this laboratory for the conversion of wastes to a suitable medium for plant growth. This digester has been fed with a combination of homogenized feces and plant material at a rate of 0.09 pound of volatile solids per cubic foot of digester capacity per day. Feedings were on a twelve times a day basis. Over twelve cubic feet of gas (70% methane) per pound of volatile solids was obtained. Reduction of volatile solids has been as high as 67%. Measurements of pH, volatile acids, percent total and volatile solids, alkalinity and evolved gas were frequently made. The effluent produced was centrifuged and the sludge and effluent were analyzed. The dark brown effluent was cleared with hydrogen peroxide. Samples of effluent were dried, incinerated and dissolved, serving as a portion of the nutrient medium for algae cultures.

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4. Rate of feeding powder to algae

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5. Odor of gasas coming from algae

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GAS PRODUCTION BY STORED HUMAN WASTES IN A SIMULATED MANNED SPACECRAFT SYSTEM. Robert B. Wheaton, John J. Symons, Norman G. Roth, Harold H. Morris. Life Support Dept., Whirlpool Corp., St. Joseph, Michigan.

Storage of untreated human wastes in sealed vessels has been suggested by some workers as a possible method for disposal of such wastes in manned spacecraft. Feasibility of this process was studied with human excrement sealed in metal containers under closely controlled conditions. Production of gas in the containers was studied as a function of storage time, head space, sample size and storage temperature. Chemical nature of the gases was determined together with the ratios of their occurrence. The micro-ecological flora responsible for gas production was studied. The application of these data to the design of spacecraft waste systems is discussed, with special emphasis on safety, feasibility, construction and trade-off advantages of sealed waste storage systems.

GASEOUS INTERCHANGE IN A CLOSED ECOLOGICAL SYSTEM. Dr. Sheldon A. London. Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio.

The biologistics of extended space missions necessitates the establishment of bioregenerating systems. The metabolic characteristics of microbiological entities are being studied to determine their applicability to closed ecological systems. This study was initiated to determine the feasibility of utilizing several microbiological forms to provide atmospheric control and nutritional support. Based upon empirical data, an interdependent relationship of a photosynthesizing algal culture (Chlorella pyrenoidosa), organisms responsible for aerobic sewage digestion and a high protein producing fungus (Linderina pennispora) was established. Ammonia, resulting from the digestion of sewage, was utilized by the fungal culture as a nitrogen source. Carbon dioxide, arising from the sewage and fungal growth chambers, served as a carbon source for the alga, which in turn, supplied oxygen for the aerobic organisms. Inclusion of animals in the system demonstrated the feasibility of atmospheric gaseous support.

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ANIMAL NUTRITION STUDIES WITH CHLORELLA 71105
AT GENERAL DYNAMICS/ELECTRIC BOAT. Joseph A.
Lubitz, R. J. Benoit. Electric Boat Division,
General Dynamics Corp., Groton, Connecticut.

Seventy-five pounds of freeze-dried material were produced in a pilot plant facility for mass culture of microalgae. The plant consists of two 600 gallon stainless steel tanks, each illuminated internally with sixty eight-foot high intensity fluorescent lamps, and accessory equipment: high-speed centrifuge, freezer, vacuum drier, laboratory mill, and dry mixer.

Chemical analyses of the algal flour yielded the following results: crude protein, 55.5%; crude fat, 7.5%; ash, 8.25%; moisture, 7.0%; total carbohydrate, 17.8%; crude fiber, 3.1%; and chlorophyll 2.68%. Chlorella 71105 as grown and processed at General Dynamics/Electric Boat contains all the essential amino acids. Available energy was 3.3 calories per gram.

Albino rats were fed various diets containing 92% or 21% Chlorella 71105 and no other source of protein. Control animals were fed diets of "lab ration" (24% crude protein) or diets containing egg protein or casein as the sole source of protein (10% protein). Food intake, feces production, and body weights were taken regularly for growth studies and estimation of coefficients of digestibility. The protein efficiency ratio (PER) for Chlorella 71105 was 2.19%, with 2% methionine the PER was 2.90, which compares favorably with literature values for casein.

Average weights after seven months on experimental diets were: casein - 579 gm, Chlorella plus methionine - 453 gm, Chlorella - 402 gm.

All animals, including two animals on 92% Chlorella grew satisfactorily and appeared normal except for a symmetrical alopoecia (loss of hair) in three animals fed Chlorella. Autopsy examinations have revealed no gross internal anomalies. Preliminary histopathological examination has revealed some provocative anomalies in some Chlorella fed animals, especially in salivary gland and pancreas.

THE NUTRITIONAL VALUE OF ALGAE CROWN UNDER STERILE CONDITIONS. John E. Vanderveen, 1/Lt., USAF, Eugene G. Sander, 1/Lt., USAF. Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio

In an attempt to determine the nutritional contribution made by bacterial contamination of mass cultured algae, digestion trials were conducted feeding aseptically grown algae. Both heterotrophically and autotrophically grown algae were fed to supply an adequate source of digestible protein for weanling male rats. Animals were housed in individual metabolism cages and feed was provided ad libitium. Data on feed consumption, body weight gain, and digestibility of the principal macro-nutrients will be compared with similar data obtained by feeding mass cultured, bacterially contaminated algae.

NUTRITIONAL VALUE OF DECOLORIZED ALGAE. Dr. Gerald Christensen, Dr. Irena Zommers. Bioastronautics Section, The Boeing Company, Seattle, Washington.

Any method which may be devised to make preparations of unicellular algae more desirable for human consumption must carry with it the assurance that no decriment results in the cellular content of essential nutrients.

To this end, composition studies have been carried out on preparations of <u>C</u>. <u>Pyrenoidosa</u> which have been decolorized by exposure to intense light under a variety of conditions. The effect of this treatment, which results in a significant increase in palatability, on cellular amino acids and fat soluble vitamins has been measured. The decolorization results in virtually complete destruction of the carotenes. The only change of significant magnitude noted in the amino acid pattern was the complete loss of hydroxy-proline following decolorization. The details of these and other nutritional studies will be discussed.

FUNCI AS A NUTRIENT SOURCE. Dr. William D. Gray, Dept. of Fotany & Plant Pathology, Ohio State University, Columbus, Ohio.

Concomitant with arrival of the space age and its associated problems there has appeared in sharper focus the age-old problem of feeding an expanding population. Although more attention is being directed toward this problem it is too frequently termed an approaching food shortage. A few calculations reveal that what is erroneously described as an approaching food shortage is in reality an approaching protein shortage.

In all probability there is no single solution to the problem of augmenting the world's protein supply but research in our Mycology Laboratories has revealed that significant contributions can be made by using certain Imperfect Fungi. The problem is being approached from the standpoint of converting carbohydrate to protein by microbial means, and it has been found, for example, that excess sugar (as blackstrap molasses) produced annually in Puerto Rico alone can be converted to sufficient protein to supply the annual needs of one million people.

BIO-ECOLOGY OF DIGESTION. Dr. Lorraine Gall. Republic Aviation Corp., Farmingdale, I.I., N.Y.

The digestive processes and nutrition of the host are often influenced by the predominating microorganisms in their digestive tracts. For example, in ruminants, the billions of anaerobic microorganisms in the rumen convert unavailable nutrients in the ration into a form available to the host, and synthesize essential B vitamins as well. In turn, ration influences the type of predominating rumen organisms and, therefore, the efficiency of digestion and the nutrition of the host. This is also true to a lesser extent with intestinal flora of mice and other simple stomached animals. Data from these studies and current work will be presented and discussed.

These animal studies suggest questions, such as: 1. Will the astronaut's diet influence his intestinal flora? 2. Will any such change be nutritionally significant? 3. Can bacteria used in a closed ecological system in processing waste contribute to the nutrition of the astronaut?

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ENZYMATIC DIGESTION OF AIGAL CELLS. Mr. Alan Shefner, Maurice E. King. Life Sciences Research Section, Armour Research Foundation, Chicago 16, Illinois.

Untreated algal cells are incompletely digested in the digestive tract of man. We have been investigating a variety of enzymes with a view to developing an enzyme supplement to an algal ration which would increase the nutritive value of the algae.

Such an enzyme additive would function through direct degradation of algal cells, and/or by making the cells more susceptible to the action by normal digestive enzymes.

Enzymes have been evaluated in vitro for two hours in artificial gastric juice followed by four hours in artificial intestinal juice.

Typical commercial cellulases have been without effect in our system. Favorable results have been obtained with enzyme systems derived from the snail, Helix pomata and from Myrothecium verrucaria. Pectinase also has been effective, both alone, and in combination with the snail and mold enzymes.

These results are consistent with the studies of Northcote and others on the composition of the Chlorella cell wall.

THE NUTRIENT CONTENT OF VARIOUS ALGAE AND THE

AMINO ACID ADEQUACY FOR GROWTH OF RATS & CHICKS.

Dr. Gilbert A. Leveille, Dr. Howarde E. Sauberlich,
Lt. Col. Marion E. McDowell, USA (MC). U. S.

Army Medical Research and Nutrition Laboratory,
Fitzsimons General Hospital, Denver, Colorado.

The content of certain nutrients in three samples of algae was determined by analysis and by feeding studies. The algae were analyzed for total, amide, ammonia and humin nitrogen, amino acids, several vitamins (thiamine, riboflavin, pantothenic acid, pyridoxine, niacin and ascorbic acid) total and chromogen-free lipid, ash and calories. The analytical data obtained from the three algae tested, 1) a mixture of Scenedesmus obliquus and Chlorella ellipsoida, 2) Chlorella pyrenoidosa and 3) Spongiococcum excentricum indicate a high nutritive potential.

Feeding studies with both rats and chicks in which the three algae were fed as the sole source of dietary protein showed all of the algae to be deficient in certain amino acids. Animals fed

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(continued)

THE NUTRIENT CONTENT OF VARIOUS ALGAE AND THE AMINO ACID ADEQUACY FOR CROWTH OF RATS & CHICKS (continued)

the mixture of algae (S. obliquus and C. ellipsoida) grew better and demonstrated higher protein efficiency ratios than did animals fed diets containing either of the other two algae. Similar results were obtained with both chicks and rats.

Amino acid supplementation studies showed all three algae to be deficient in methionine and that the mixture of S. obliquus and C. ellipsoida was also deficient in glycine for the growing chick. The algae C. pyrenoidosa was deficient in histidine for the growing rat. Results were obtained which appeared to indicate the existence of an amino acid imbalance; however, the data are not adequate to demonstrate this conclusively.