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CURRENT RESEARCH ON REGENERATIVE SYSTEMS

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SUMMARY

Multiple studies directed toward the development of a regenerative life support system have shown that easily synthesized organic compounds and microbiological materials are potentially capable of being used as foods for long duration space missions. Animal feeding studies have supported these views. The organic compounds presently believed to offer the greatest potential are glycerol, simple glycerol derivatives such as triacetin, and formose sugars. Laboratory studies indicate that glycerol can be synthesized from formaldehyde which in turn is obtained by the direct catalytic oxidation of methane, a by-product of the Sabatier reaction used in the spacecraft atmosphere control system. Formose sugars are derived from the self condensation of formaldehyde. Mixtures of glycerol and triacetin have been shown to be suitable as a major component of diets fed to weanling rats for prolonged periods. These compounds do not exist as stereoisomers and therefore offer advantages over the formose sugars. Hydrogenomonas eutropha is the microbiological system under investigation. An automated system for the continuous autotrophic production of Hydrogenomonas bacteria is in operation, and the nutritional requirements for growth in the system using urea as a nitrogen source are being studied. Nutritional evaluation of Hydrogenomonas bacteria has shown they are capable of supplying the total protein requirement of growing rats for prolonged periods. The potential and problems of these regenerative systems and the prospects for the accomplishment of a totally regenerative food system will be discussed.

Introduction. Development of a system capable of regenerating at least part of the crew's food requirements during missions of very long duration has been shown to be desirable (1,2). A variety of schemes to utilize metabolic waste as a source of food have been proposed and two of the approaches are under investigation here.

Regenerative systems can be of two types. Either some biological system is involved or else only physicochemical reactions are used. Examples of the former are systems utilizing algae, bacteria, duckweed, or some other living organism. The physicochemical approach has been relatively unexplored. Its usefulness will depend upon finding organic compounds which can be synthesized relatively easily from metabolic products or other surplus material on the spacecraft and which can be safely consumed as food for extended periods.

A search has been conducted to find organic compounds which can be tolerated as a major component of the diet (3). Currently, investigations are in progress to develop diets which contain virtually all the calories as purified organic compounds. It is postulated that the essential compounds of the diet such as the vitamins, essential amino and fatty acids, and trace elements which together comprise only a minor proportion by weight of the total food requirements will be carried along during the very long missions and physicochemically regenerated compounds will comprise the bulk of the diet. The compounds which appear to have the greatest promise are the formose sugars, glycerol, propylene glycol and some of their derivatives.

An alternative and/or complementary system involves the possible utilization of Hydrogenomonas bacteria. These bacteria which can utilize metabolic waste for autotrophic growth have been shown (4,5) to contain high quality protein. If an apparatus could be designed to produce this material during space flight, an even greater reduction in the amount of food which would otherwise have to be carried along would result. It is unlikely that Hydrogenomonas can be made a major component of the diet since it apparently contains too large an amount of nucleic acids to be tolerated by humans (5). However, as will be shown subsequently, it may serve as a very useful food supplement.

Nutritional Studies. The nutritional qualities of the formose sugars have not been investigated extensively. In the only previously reported study, Akerloff and Mitchell found (6) that their product was toxic to rats. The distribution of sugars present in the final product after the self condensation of formaldehyde is dependent upon the conditions of the reaction (7). Further, many of the sugars may be present as the formal derivative and liberate formaldehyde when exposed to the acidity of the stomach. We have removed the residual formaldehyde from a product produced continuously in a plug-flow reactor and, by means of gas chromatography of the silyl derivatives, have shown that our product contained predominantly pentoses and hexoses (8). It was possible to incorporate up to 20% by weight of this material into rat diets without major detrimental effect (3). A backmix reactor is now being used to better control the reaction and hopefully to yield a product which does not contain the materials which have previously limited its potential usefulness (8).

Compounds such as glycerol and triacetin offer advantages over the formose sugars. They do not exist as stereoisomers and therefore can be totally metabolized whereas formose sugars contain L-isomers which in most part will not be utilized by the body.

There have been a number of human feeding studies with glycerol and it has been shown to have medicinal value (9,10). Here, an effort has been made to determine the maximum amount of materials such as glycerol and triacetin which can be tolerated by growing rats. In growing rats (which must clearly be differentiated from the situation which obtains in mature animals), we have observed the following during a number of three month feeding studies:

(1) There is negligible reduction in weight gain when 40% glycerol replaces conventional carbohydrate in the diet.

(2) The animals can tolerate up to 20% triacetin well. Greater amounts cause a decrease in weight gain.

(3) A large loss in weight and considerable mortality is associated with diets containing either glycerol or triacetin as 60% of the diet.

(4) A diet containing a mixture of 40% glycerol and 20% triacetin is tolerated fairly well (also see Table I). Thus, the toxicity of these two compounds is not additive.

(5) The type and quantity of the protein present in the diet has an influence upon the weight gain in the presence of glycerol and triacetin.

(6) Excess vitamins are of benefit to the animals consuming these diets.

The results of a feeding study to determine whether a mixture of H. eutropha, glycerol and triacetin could be made a major proportion of the diet of weanling rats is shown in Table I. The H. eutropha was obtained as a single uniform lot (Grain Processing Corporation, Muscatine, Iowa) which had been grown heterotrophically for 24 hours on a medium containing salts, casein hydrolysate, protopeptone, yeast extract and sucrose. The cells were killed by heating at 90°C for 15 min., washed three times with distilled water and used as a frozen paste containing 21.6% by weight dry cells and contained 144 mg N/gm dry weight. The casein used for the control diets contained 140 mg N/gm dry weight. In addition to the components shown in Table I, all diets contained 5% USP XIV salt mixture (plus 16.5 mg $ZnSO_4$/100 gm diet), 5% safflower oil, 2% α-cellulose, 1% vitamins, 1% agar and a total of 2 parts water to each part dry weight. After formulation, the diets were a thick paste which was stored at 4°C and fed fresh daily. There were eight male Sprague-Dawley rats in each group; the animals were caged as pairs and weighed daily.

It can be seen from Table I that 12% protein in the form of either casein or H. eutropha is not adequate for maximum growth of the animals. Substitution of 60% of the starch in the diet with a mixture of glycerol and triacetin reduced the growth of the animals. When the concentration of protein was increased to 24%, maximum growth occurred and in this case, substitution of the starch with glycerol-triacetin in the diet containing H. eutropha did not result in decreased growth. However, when casein was the protein, the growth was significantly ($P < 0.05$, t test) lower. Growth of the animals was essentially the same with diets containing 48% protein in the form of either casein or H. eutropha, whether or not the carbohydrate component of the diet was starch or the glycerol-triacetin mixture. The differences shown are not statistically significant.

These data show that over 90% of the calories of the diet of growing rats can be composed of a mixture of H. eutropha, glycerol and triacetin

without detrimental effect on the growth of the animals. Rats excrete allantoin as the catabolic product of nucleic acid purines rather than uric acid which is the excretion product of man. Consequently, one would not expect to observe in rats the undesirable effects which are associated with excess uric acid in man.

Systems Development. It is of no value to demonstrate the potential usefulness of materials such as H. eutropha, glycerol and triacetin as food unless satisfactory systems can be developed for their production from metabolic waste during missions of long duration. Accordingly, we have been conducting research to determine the weight, volume and power requirements of regenerative systems.

Synthesis of formose sugars and glycerol will require the intermediary synthesis of formaldehyde. A contractor of the NASA (General American Transportation Corp., Niles, Illinois) has been successful in developing a method for the direct catalytic oxidation of methane to formaldehyde. The methane is produced as a by-product of the Sabatier process in the spacecraft atmosphere control system. The recycle system currently being investigated uses a reactor composed of Berl saddles coated with sodium tetraborate held at 675°C. Conversions of about 30% are obtained when the feed is composed of 40% methane, 36% oxygen, 23% nitrogen and 0.5% nitric oxide. Addition of reactors to convert by-product carbon monoxide and carbon dioxide back into methane will result in a system capable of effecting the quantitative conversion of carbon dioxide to formaldehyde. The product is obtained as its solid polymer and can be volatilized by gentle heating for use in subsequent steps.

The conversion of formaldehyde to glycerol is being investigated for us by the Esso Research and Engineering Corporation, Linden, New Jersey. They have shown that it is possible to control the self condensation of formaldehyde to yield predominantly the three carbon sugars, glyceraldehyde and dihydroxyacetone. Our feeding studies have shown that neither of these compounds per se is suitable as food. However, the mixture can be catalytically reduced with hydrogen to produce glycerol.

The bacterium, Hydrogenomonas eutropha, when grown in the autotrophic mode requires salts, hydrogen, oxygen, carbon dioxide and a nitrogen source such as urea or ammonia. An experimental laboratory model of an

automated system suitable for spacecraft application was developed by Battelle Memorial Institute for NASA (11). Using the same sensors and controls, the culture volume can be either two liters or twenty liters. The automatic oxygen sensor control utilizes a Beckman polarographic detector (Clark type electrode) in either the liquid or gas phase. Carbon dioxide tension is regulated by a Beckman carbon dioxide sensor (a modified pH electrode). Hydrogen constitutes the remainder of the gas phase and is controlled by a total pressure regulator. The hydrogen concentration is always greater than 80%. In addition, a thermistor temperature controller and a pH recorder-controller system connected to reservoirs of acid and base are present in the system. A modified autoanalyzer arrangement is used to monitor and control the cell density (turbidity) and the urea concentration in the culture.

The primary goal of investigators working with continuous culture H. eutropha systems has been to optimize cultural conditions so that maximum growth rates at high population densities can be maintained reliably for an indefinite period. Such a culture would reduce the volume and weight requirements of the system to a minimum.

With our apparatus, it is possible to attain stable cell population densities in the range of 4.1 gm dry weight/L at growth rates of 0.25/hr (i.e., population per liter increases by 25% each hour). The same high growth rate in cultures containing 6.1 gm/L has been reported by L. Bongers. Growth rates as high as 0.35/hr have been observed occasionally as well as population densities of 10 gm/L. Thus, the genetic limitations on growth and cell density have not yet been reached. At present stable production rates, it is estimated that 12 liters of culture is sufficient to convert the entire inventory of one man's carbon dioxide output to cell mass. However, it is unlikely that H. eutropha will comprise all of the astronaut's diet and a smaller culture volume will suffice to supply his protein requirements.

Unlike the situation with carbon dioxide conversion, the efficiency of the organism in converting urinary nitrogen to protein nitrogen has not been studied in any detail. Our studies are being performed with purified urea. In batch culture H. eutropha can utilize nearly all the major

nitrogenous components of urine when supplied individually and the intermediates of degradation appear to be ammonia and/or urea. The effect that human urine will have on the growth rate and carbon dioxide assimilation in continuous culture remains to be determined.

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TABLE I

GROWTH OF MALE WEANLING SPRAGUE-DAWLEY RATS ON DIETS CONTAINING H. EUTROPHA, GLYCEROL AND TRIACETIN

DIET (% BY WEIGHT)					ANIMAL WEIGHT ± SEM	
CASEIN	H.EUTROPHA	GLYCEROL	TRIACETIN	STARCH	ORIGINAL	18 DAYS
12% PROTEIN						
13.7	0	0	0	73.3	79.1 ± 2.9	152.8 ± 4.5
13.7	0	30	30	13.3	78.0 ± 3.4	117.4 ± 4.3
0	13.4	0	0	73.6	77.0 ± 5.0	148.6 ± 6.3
0	13.4	30	30	13.6	77.0 ± 3.9	129.4 ± 6.3
24% PROTEIN						
27.4	0	0	0	59.6	76.3 ± 2.9	186.9 ± 4.9
27.4	0	29.8	29.8	0	84.1 ± 2.6	166.8 ± 6.9
0	26.8	0	0	60.2	76.5 ± 4.2	182.9 ± 3.1
0	26.8	29.8	29.8	0.2	74.9 ± 4.0	178.8 ± 3.4
48% PROTEIN						
54.8	0	0	0	32.2	79.3 ± 1.7	181.0 ± 3.3
54.8	0	16.1	16.1	0	78.6 ± 4.1	178.1 ± 5.1
0	53.6	0	0	33.4	78.4 ± 3.2	204.3 ± 13.1
0	53.6	16.7	16.1	0	83.3 ± 3.1	191.5 ± 8.3