Biological reactors as artificial organs

Concept and preliminary in vitro study

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Artificial organ design generally attempts to provide a substitute for the most basic physiologic functions; for example, an artificial kidney can balance electrolytes and remove excess solutes and water. However, artificial organs are only a compromise and do not completely duplicate natural functions. If we measure success in survival years or compare the condition of the patient on an artificial kidney with his condition before renal failure, we see that the substitute is far from ideal. When present concepts of the artificial kidney are examined, it is obvious that all fall short of duplicating the total function of the natural kidney in (1) adaptation to individual needs through continuous purification, (2) selective transport mechanisms, (3) in situ use, and (4) aiding other physiologic processes. Most artificial organs associated with metabolic or catabolic functions have limitations which prevent their continuous use in situ. New concepts must be explored to achieve any major improvement in substituting organ function.

General concept

At present, we do not understand complete organ function nor the inadequacies of the substitutes now in use. Because a comprehensive knowledge of all uremic toxins is lacking, we do not know whether these toxins are being

adequately removed.[1] Realizing the shortcomings of present technology, we should reconsider the workings of nature and their application to our specific purpose. The use of living microorganisms as substitutes for normal physiologic functions was thus conceived, and this study was undertaken to apply the concept to renal support.

Microorganisms are biological reactors serving as an intermediate step in the cycle of matter. They also play a vital part in normal physiology, and are known to exist in situ. Therefore, the choice of microorganisms appears to be logical if one considers the normal course of events in the cycle of matter. Many vitamins are synthesized within the intestine, presumably by bacteria.[2] The importance of this synthesis in man, at least of nicotinic acid and vitamin K, has been emphasized by the onset of deficiency symptoms following the use of antibacterial agents. Microorganisms serve a vital function in nature by acting on matter to form compounds useful in producing food and energy utilized by man.[3] In applying this concept, an attempt can be made to bring man and his undesirable metabolic products into more intimate contact with the organisms capable of treating his metabolic deficiency or abnormality, thereby shortening the sequence of events and facilitating mass transfer.

This concept has many applications. In some cases microorganisms themselves could be used, and in other cases the microorganisms could serve to develop and culture the appropriate enzyme systems, which could then be extracted and utilized.

Application to renal support

In applying this concept, the primary interest is the development of an alternative means for treating chronic renal failure. Nearly 14,000 patients in the United States are maintained on chronic hemodialysis. Generally, the patient is treated two or three times a week for 3 to 10 hours each time. This is far from ideal. Much effort is presently directed at altering the duration and frequency of dialysis and regulating operating conditions to obtain varying removal rates for the various molecular sizes known or hypothesized to exist as uremic toxins.[4] In the proposed concept, it is necessary only that the uremic toxin be an ingredient of the diet of the microorganism. Numerous compounds must be removed from a uremic patient and the effectiveness of a single microorganism to perform the task is most unlikely; a group of microorganisms would probably be necessary. The specific strains necessary to enter into a symbiotic relationship with the uremic state would probably have to be cultured.

Preliminary study

To apply this concept, the proper mixture of microorganisms and reactants must be brought together. A logical source of reactants is normal urine. Although the patient's metabolic byproducts may not be the same as those of normal subjects, normal urine is readily available for study and contains the bulk number and types of constituents to be removed. As a source of microorganisms, activated sludge containing a variety of organisms was procured from a sewage treatment plant.

All urine was supplied from the same individual on a normal diet, with fluid intake restricted to concentrate the urine. No drugs were taken. Only morning urine samples (~300 ml volume) were used to further concentrate the solutes.

To start the reactor, 670 cc of activated sludge solution procured from the Easterly Sewage Disposal Plant in Cleveland was added to 560 cc of urine in a 3-liter vessel. Additional volumes of urine, 950 ml and 220 ml, were added on the 5th and 6th days. Further additions of urine were made only after concentrating the urine by evaporation under 60C.

Table 1. Bacteriologic analyses made during study period

Sample	Organisms	Organisms/ml
Sludge	*Pseudomonas* species	Few
	Bacillus species (3 strains)	Few
	Diphtheroid bacillus	Few
	Alpha streptococci	Many
Reactor (day 34)	*Pseudomonas alcaligenes*	Many
	Diphtheroid bacillus	Many
	Alpha streptococci	Many
Reactor (day 60)	Sterile	

A volume of 5,520 ml of urine concentrated to 3,495 ml was added during the next 30 days. The reactor was aerated 8 hours a day for 5 days a week and was maintained at ambient conditions over a 2-month period. The reactor was continually vented during the study period. Starting with mixed organisms and strains of Pseudomonas, bacilli, diphtheroid bacilli, and alpha streptococci, three dominant organisms were cultured: (1) *Pseudomonas alcaligenes,* (2) *Diphtheroid bacillus,* and (3) alpha streptococci (*Table 1*). These organisms showed susceptibility to general antibiotics. The reactor was allowed to run down after the last urine was added and was found to be sterile at the end of the study period.

Table 2 shows the analysis of a typical urine specimen, compared with analyses taken from the reactor at different times. During this period significant consumption of urea nitrogen, creatinine, uric acid, chloride, potassium, and sodium was shown, with an increase in ammonia and sludge mass. Gas chromatography was carried out to compare major organic acid constituents of the concentrated urine with those in the urine reacted upon by the microorganisms. The *Figure*

Table 2. Typical urine analyses and estimated quantity of removal

Solute	Concentration in typical urine mg/100 ml	Concentration at day 44 mg/100 ml	Concentration in sterile reactor mg/100 ml	Minimal estimated removal, g
Urea nitrogen	>800	4	800	34.0
Creatinine	>50	0.7	0	3.6
Uric acid	>60	14	8.6	4.1
Cl^-	>700	94	1,000	20.0
Na^+	>460	600	1,050	1.9
K^+	>235	360	365	6.0
NH_3	>60	...	≈900	...
pH	≈5.5	...	8.9	...

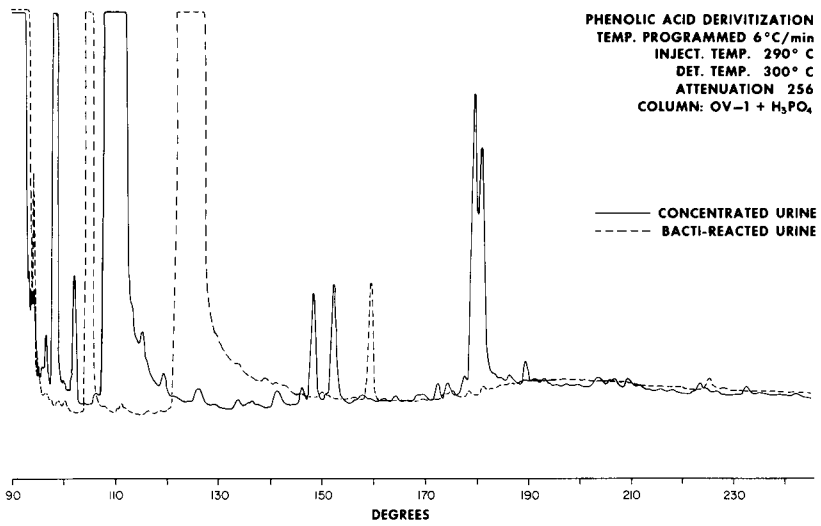

Figure. Gas chromatograms showing phenolic acid differences between concentrated urine and bacti-reacted urine.

depicts noteworthy qualitative differences. Although complete identification was not made, it is obvious that consumption of the urine components by the microorganisms results in specific by-products in addition to increased biomass. These by-products could be toxic in themselves. However, they could also possibly be utilized by the patient as a food supply,[5] or could be in forms more amenable to removal from the system by conventional means, such as absorbents and ion exchange resins than are the original uremic constituents.

Discussion

Most important is how to utilize the concept. Possibly a free system of microorganisms might not be best suited for easy application. In such cases the system could be contained by semipermeable membranes encapsulated in polymers or gels, or incorporated into the construction of standard devices such as shunts, dialyzers, or reactors. For in situ application a most likely location of such a biological reactor (solution of microorganisms) would be in the intestines. During the early stages of the development of dialysis, the intestine was used. Clark et al[6] have reported on the use of isolated intestinal loops in the treatment of renal failure. Their 2-year follow-up of a patient has shown the potential and advantages of this method. In previous work[7] the removal of metabolites in the isolated intestinal loop containing activated carbon was demonstrated. Therefore, a combination of the isolated intestinal loop with microorganisms appears to be feasible. The microorganisms would consume uremic toxins through the intestinal wall in an active and continuous reaction on a 24-hour per day basis.

Applying microorganisms (biological reactors) as artificial organs is presented for consideration as a renal support device. This concept, it is

believed, is different from that of therapy with present artificial kidneys, and is an approach to research in perfecting devices for metabolic assist. Preliminary data presented in application to renal support indicate the possibility of applying the concept and raise many points for discussion; however, it is hoped that metabolic assistance by this method would be more physiologic and that in situ application would be possible. The concept is general and would have many applications for replacing physiologic function. Liver and kidney diseases and errors in metabolism might be treated with the proper choice of reactor design. In addition, a metabolic medium and microorganisms could be used to cultivate necessary enzyme systems for incorporation into devices for treatment.

Summary

The use of living microorganisms as substitutes for normal physiologic functions was conceived and a preliminary in vitro study applied to renal support carried out. Urine was acted upon by activated sludge (mixture of bacteria) for 2 months. Three dominant organisms were cultured in the reactor. Decreases of common urinary constituents were noted, with increases in ammonia and sludge mass.

References

1. Malchesky PS: Patient oriented dialyzer selection and design. Presented at symposium on Current Status in Artificial Organs, Cleveland, Ohio, October 19, 1973. Biomater Med Devices Artif Organs **2:** 249–266, 1974.
2. Pimparkar BD: Diverticulosis of the small intestine, Chap 47, *in* Bockus HL: Gastroenterology, v 2, ed. 2. Philadelphia, WB Saunders, 1964, pp 138–175.
3. Baker JJW, Allen GE: The Study of Biology. Ed 3. Reading, Mass., Addison-Wesley Publishing Company, 1968, pp 478–483.
4. Babb AL, Popovich RP, Christopher JG, et al: The genesis of the square meter-hour hypothesis. Trans Am Soc Artif Intern Organs **17:** 81–91, 1971.
5. Spicer A: Proteins from carbohydrates. Chem Britain **9:** 100–103, 1973.
6. Clark JE, Templeton JY III, Cohn HE, et al: Isolated small bowel dialysis in 23 patients. Trans Am Soc Artif Intern Organs **11:** 18–21, 1965.
7. Nosé Y, Tajima K, Nakazono M: Intracorporeal artificial kidney. Proc Fifth Annual Contractors' Conf Bethesda, National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, 1972, p 45.