Hemodialysis Culture of *Serratia marcescens* in a Goat-Artificial Kidney-Fermentor System

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Hemodialysis was employed to simulate in vivo conditions for growth in mammalian blood, but without phagocytosis, by using the goat and *Serratia marcescens* as a host-parasite model. The blood stream was shunted surgically via prosthetic tubing from a carotid artery through the hollow-fiber membranes in an artificial kidney hemodialyzer and back into a jugular vein. The dialysate solution concurrently was pumped from a modular fermentor through the hemodialyzer jacket outside of the membranes and back into the fermentor. Hemodialysis between the two circuits was maintained continuously. When equilibrium was attained, bacteria inoculated into the dialysate circuit multiplied first exponentially at the maximal rate and then arithmetically at a lesser rate equally well under aerobic or anaerobic conditions. When a population of about 10^9 viable bacteria/ml was exceeded, the goat reacted acutely with signs of general toxemia, pyrexia, and leukopenia, apparently because of dialyzable toxic material produced by the culture. The maximal molecular size of the toxic material was defined relative to a rigid globular protein of 15,000 in molecular weight and 1.9 nm in hydrodynamic radius or to a flexible fibrous polyglycol of 5,500 in molecular weight and 2.6 nm in hydrodynamic radius, based on determinations of the membrane porosity threshold for dialysis.

We have devised a new way to grow organisms by in vivo dialysis, with the experimental rationale derived from the fact that the interior milieu in animals is maintained essentially by circulation of the blood. Dialyzers connected directly with the circulatory system are in common clinical use as artificial kidneys for humans. It appeared that such a hemodialyzer could be used to establish continuous communication between the blood stream of an animal and a fixed volume of dialysate solution inoculated with an organism. In this way, dialyzable molecular constituents of the blood would diffuse into the culture and so feed it, yet the blood cells and macromolecules would be too large to diffuse through the membrane so that phagocytosis and immunological reactions against the culture would be prevented. Conversely, metabolic products of small molecular size from the culture would diffuse through the membrane barrier into the blood and thus relieve the feedback inhibition that often limits growth in a closed culture system. The dialyzable culture products, including toxins, could also exert effects on the host. Membranes of different porosity could be employed in the system to distinguish between molecules of different size in the blood components and culture products. The system would be analogous to the batch fermentor-continuous reservoir mode of operating an in vitro dialysis culture (see Fig. 8 in reference 11), but with the blood supply of an animal used as the nutrient reservoir.

In vivo dialysis culture was first attempted in 1896 by Metchnikoff, who grew cholera bacteria in a collodion membrane sac implanted within the peritoneal cavity and thereby demonstrated the production of a diffusible toxin (6). Subsequently, dialysis chambers were implanted in the peritoneal cavity and elsewhere for a number of purposes by using a number of different organisms, including animal cells and tissues (see review by Schultz and Gerhardt [11]). However, the use of implanted chambers is limited by the environment in the peritoneal fluid being different from that in the blood, by the occluding growth of macrophages on the membrane surface, by the restricted size, and by the difficulty in sampling.

We conceived the use of hemodialysis to offset...
these limitations and essentially to transpose the blood milieu extracorporeally. The feasibility of hemodialysis culture was tested with the domestic goat and *Serratia marcescens* as the host-parasite model. Described below are the methodology, the unusual growth characteristics of the bacterial culture, the acute toxemic response of the host, and the maximal molecular size of the dialyzable toxic material.

**MATERIALS AND METHODS**

The hemodialysis culture system is depicted and diagramed in Fig. 1. Blood from an experimental animal was circulated by the heart through one side of an artificial kidney hemodialyzer (the blood circuit), and dialysate culture from a modular fermentor was circulated by a pump through the opposite side (the dialysate-culture circuit).

The goat was selected as the experimental animal because of its convenient size, long neck for accessibility in vascular surgery, placid disposition, and hardiness. The goat has disadvantages in its relative sensitivity to anaesthesia and hemolysis (2). Short-haired domestic goats of mixed breed were used, a total of 15 in this study. All were females, 1 to 3 years of age and 50 to 100 lb (22 to 45 kg) in weight. They were maintained indoors in stalls, unrestrained except for a rear-leg hobble. During dialysis trials the goats were partially harnessed into a caged platform but were free to stand, lie, eat, drink, and excrete wastes. The body temperature was measured with a rectal Fahrenheit thermometer as frequently as needed.

A permanent external prosthetic shunt was established between a carotid artery and jugular vein in the lower neck by vascular surgery, based on the techniques of Quinton et al. (8, 9). Vessel tips, connectors, and infusion T's were made of medical-grade Teflon, and the tubing was made of Silastic (Cobe Laboratories, Inc., Denver, Colo.).

Sodium heparin (Upjohn Co., Kalamazoo, Mich.) was used to prevent the blood from clotting, with subcutaneous injections of 15,000 to 20,000 USP units/24 h. During dialysis, about 1,500 USP units/h were continuously introduced into the arterial shunt by means of an infusion pump (model 240, Sage Instruments, Inc., White Plains, N.Y.). Ethyl isobutylzine hydrochloride was administered as a tranquilizer in early experiments but later was obviated by training the animal. Neither drug affected growth of the test bacterium. The possibility of blood clotting in the hemodialyzer was monitored visually and by a thermometer in the effluent tubing, where the temperature went down if the flow was reduced by clotting.

A given goat became usable for hemodialysis experimentation about a week after surgery and remained so for about 1 to 3 months, when accidental exsanguination or the formation of a clot in an artery or vein (despite heparinization) caused termination.

A Cordis-Dow hollow-fiber artificial kidney was selected as the hemodialyzer (model 2 or 3, Cordis Corp., Miami, Fla.). The jacketed cylindrical unit measures 7.0 cm in diameter and 21.6 cm in height and contains a bundle of 11,000 hollow-fiber membranes fabricated from regenerated cellulose, through which the blood is circulated. Each fiber is 13.5 cm in length and 225 μm in inside diameter, and the bundle provides about 1 m² of total membrane surface area. The dialyzer was sterilized with 1.5% formaldehyde and rinsed thoroughly with water before use. The dialysate culture was circulated outside the hollow-fiber membranes, through the dialyzer jacket.

Beaker chemical dialyzers with a looped bundle of hollow-fiber membranes (Dow Chemical Co., Midland, Mich.) also were employed as hemodialyzers. The beaker dialyzers measure 7 cm in diameter and 14 cm in height and provide a total membrane surface area of about 0.1 m². They were used with three different porosities identified in terms of the nominal molecular weight (MW) of retained molecules: MW 300 (Osmolyzer, model b/HFO-1); MW 10,000 (Dialyzer, model b/HFD-1, with the same porosity as the artificial-kidney dialyzer); and MW 30,000 (Ultrafilter, model b/HFU-1). The beaker dialyzers that were first tested caused hemolysis and toxemia and therefore were unsatisfactory for use as hemodialyzer.

![Fig. 1. Hemodialysis culture system. The major components of the experimental system shown in the photograph (top) are identified in the tracing (bottom). An artificial kidney is shown, but beaker dialyzers also were employed as the hemodialyzer.](http://iai.asm.org/)
ers. However, the embedding material used to secure the fiber bundles subsequently was changed in manufacture, and more recent products proved satisfactory as hemodialyzers. In differential dialysis trials, three beaker dialyzers were inserted in series into the dialysate-culture circuit in addition to the artificial kidney as the hemodialyzer. Consequently, three separate samples of dialyzable culture product were obtained simultaneously with hemodialysis.

A modular 1-liter glass fermentor with control of temperature, agitation, and aeration (Microterm model 102, New Brunswick Scientific Co., Inc., New Brunswick, N.J.) was used to contain the dialysate-culture suspension. Anaerobic conditions were achieved by continuously sparging the fermentor contents with sterile argon, which was scrubbed free of oxygen by passage over hot copper wire kept reduced with a stream of hydrogen gas. Aerobic conditions were achieved by continuously sparging the fermentor contents with filter-sterilized air at a rate of 2.4 liters/min and by driving the impeller at a rate of 300 rpm. Unless otherwise stipulated, the culture in the fermentor was aerated and stirred.

A Maisch sterilizable gear pump (model 53DFC, Tuthill Pump Co., Chicago, Ill.) was used to circulate the dialysate culture from the fermentor through tubing to the dialyzer and back at a rate of 400 ml/min. Thick-walled rubber tubing was used for the dialysate-culture circuit. Medical-grade Tygon or Silastic tubing was used for the blood circuit.

The dialysate-culture circuit was charged with 800 ml of a glucose-salts solution approximately balanced in makeup to that of goat blood (2). The solution was constituted as follows (per 100 ml): glucose, 50 mg; Na+ (as NaCl and sodium acetate), 310 mg; K+ (as KCl), 15 mg; Ca++ (as CaCl2), 11 mg; and Mg++ (as MgCl2), 3.7 mg. The solution volume represented about 40% of the total volume of blood of a goat.

S. marcescens strain 8UK was selected as the test organism because of its previous use in developmental studies on in vitro dialysis culture (3, 4). This gram-negative species occurs mostly as single cells, grows rapidly on synthetic or natural media under either aerobic or anaerobic conditions, and is red pigmented when grown on most media at about 30 C. S. marcescens is quite capable of parasitic growth, produces endotoxin, and may cause serious diseases in man. The inoculum was prepared from an aerated exponential culture at 35 C in Trypticase soy broth (Baltimore Biological Laboratory, Cockeysville, Md.), which was sedimented, and the cells were suspended in the dialysate solution to an appropriate concentration.

Hemodialysis was initiated, and the system was allowed to equilibrate for 1 h prior to introducing the inoculum. The culture was maintained at 39 C (approximately the normal temperature of goats) and consequently was not pigmented. Samples of the culture were removed periodically by syringe and needle through a self-sealing rubber diaphragm positioned in the circuit tubing. Cell populations were measured by optical density and by viable cell counts, which were performed by surface plating on Trypticase soy agar.

Cell-free samples of the culture for biochemical analyses were obtained by membrane filtration. Samples of the blood were collected in ethylenediaminetetraacetic acid for cell counts or were allowed to clot for serum, which was immediately stored at −20 or −70 C until assayed. Assays for the following routinely were made on the serum samples with a flame photometer and autoanalyzer (model SMA 12, Technicon Corp., Tarrytown, N.Y.) by use of standard procedures: sodium, potassium, calcium, magnesium, inorganic phosphate, glucose, urea nitrogen, uric acid, total protein, albumin, bilirubin, cholesterol, alkaline phosphatase, lactate dehydrogenase, and glutamic-oxaloacetic transaminase. Leukocyte and erythrocyte counts of the blood samples were made by use of microscope counting chambers, and differential cell counts were made by use of Wright-stained smears.

The membrane porosity threshold of the artificial kidney was determined with the distribution analysis method of Scherrer and Gerhardt (10). A polyethylene glycol (PEG) sample of number average molecular weight (Mn) 3,350 was dialyzed against a PEG sample of Mn 17,500, with the concentrations adjusted to maintain osmotic balance across the membrane. Dialysis was continued for 4.5 h at 23 C. The change in concentration of the PEG sample of Mn 3,350 was measured by refractometry and dry weight, and its molecular weight distribution before and after dialysis was determined by column chromatography with Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.). The mass of polymer in each elution fraction (1.5 ml) was measured by dry weight.

RESULTS

Hemodialysis without culture. During control hemodialysis with the artificial kidney, the dialyzable materials that were measured in samples of the blood and the dialysate solution (sodium, potassium, calcium, magnesium, inorganic phosphate, glucose, and urea nitrogen) reached equilibrium within 1 h and then remained within normal ranges of concentration for goat blood. Large molecules in the blood (total protein, albumin, bilirubin, cholesterol, alkaline phosphatase, lactate dehydrogenase, and glutamic-oxaloacetic transaminase) did not measurably diffuse into the dialysate solution and remained at normal concentrations. Body temperature of the goat (Fig. 2A) and blood erythrocytes (Fig. 2C) also were unaffected. However, total leukocytes in the blood increased in number during the first few hours of control hemodialysis and then remained essentially constant at the higher level (Fig. 2B). The two most numerous types of leukocytes (neutrophils and lymphocytes) were not affected selectively.

Growth characteristics of hemodialysis culture. The glucose-salts solution originally charged into the dialysate circuit was shown to
be inadequate to support growth of \textit{S. marcescens}, primarily because of the absence of a nitrogen source. Even after attainment of equilibrium with blood, the dialysate solution supported only a slight amount of bacterial multiplication when samples of the dialysate were withdrawn into separate culture tubes and inoculated.

When hemodialysis was maintained continuously for an extended period with the artificial kidney, an inoculum of \textit{S. marcescens} in the dialysate circuit multiplied extensively and in a characteristic bimodal pattern, with a short lag phase (Fig. 2D, 3A, 3C, 4A, and 5A) or without one (Fig. 5D). The multiplication at first progressed in the usual way as an exponential function of time. The rate of exponential multiplication in hemodialysis culture, about 2.0 generations/h, was essentially as great as the maximum that had been attained with this bacterium by other means of culture or types of medium.

When a population density of about $10^{9.5}$ viable cells/ml was reached, the multiplication changed from an exponential to an arithmetic (linear) function and progressed at a rate about one-sixth of that before (Fig. 2D, 3A, 3C, 4A, 5A, and 5D). This second mode of multiplication was verified by plotting multiples (rather than logarithms) of bacterial numbers versus time, with a resulting straight-line progression (Fig. 3B and 3D). The shift in growth mode was explained by the bacterial population reaching a density for which the nutrient demand exceeded the steady-state supply of nutrients from hemodialysis. Because diffusion proceeds as a direct function of time, the bacterial multiplication became limited similarly. The bimodal pattern of multiplication fulfilled theoretical predictions (see Fig. 25 in reference 11).

When an inoculum of $10^{9.5}$ viable cells/ml was employed (near the critical density for change
highly aerobic conditions in the fermentor because of knowledge that oxygen diffuses slowly through a cellulose dialysis membrane (3, 11). However, strictly anaerobic conditions in the fermentor resulted in the same bimodal growth pattern and unexpectedly supported nearly the same exponential and arithmetic rates of multiplication as with aerobic conditions (Fig. 5D versus 5A). Apparently the limitation on growth rate of *S. marcescens* by oxygen supply (3, 4, 12) occurs only in artificial media or at population densities in excess of those attained with hemodialysis culture.

The artificial kidney was available with only one membrane porosity, which retains molecules larger in nominal molecular weight than about 10,000. However, chemical beaker dialyzers constructed similarly with a bundle of hollow-membrane fibers and available with three different porosities of membrane were found satisfactory for use as a hemodialyzer. The

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**Fig. 4.** Effects of goat hemodialysis culture of *S. marcescens* (A) on glucose concentration in the dialysate and in the blood (B).

in multiplication mode with a smaller inoculum, the multiplication soon became arithmetic (Fig. 3D) and progressed in this mode for several hours. A maximum was reached at about $10^{15.8}$ viable cells/ml (Fig. 3C), followed by a slight decline in the population (Fig. 3D).

The effects of the bacterial multiplication on glucose concentrations in the dialysate and in the blood are shown in Fig. 4. After an initial rise, the dialysate glucose decreased rapidly and eventually became undetectable. The pattern was similar for glucose in the blood, with greater hyperglycemia initially and lesser hypoglycemia subsequently than in the dialysate.

The cultures were managed usually with

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**Fig. 5.** Comparison of aerobic (left) with anaerobic (right) hemodialysis culture conditions on the bacterial growth curves (A and D) and on the host body temperature (B and E) and blood leukocytes (C and F).
growth responses of *S. marcescens* with the beaker dialyzers are shown in Fig. 6. The MW-300 beaker dialyzer did not support a significant change in bacterial population over an extended time period (Fig. 6A) although dialysate glucose decreased slightly during the trial, indicating metabolic activity of the culture. In vitro dialysis with the MW-300 beaker when using Trypticase soy broth medium in the reservoir also failed to support growth, so essential nutrient molecules apparently are prevented from passage through this membrane. The MW-10,000 beaker, which is constructed with the same membrane as in the artificial kidney, and the MW-30,000 beaker both supported a bimodal pattern of growth (Fig. 6D and 6G, respectively) similar to that with the artificial kidney. The membranes in both beakers regulate the passage of much the same size class of small molecules, and both retain serum proteins. Representative rates of bacterial multiplication during exponential and linear growth, respectively, were 1.7 and 0.2 generations/h with the MW-10,000 beaker and 1.8 and 0.3 generations/h with the MW-30,000 beaker.

**Host responses to hemodialysis culture.** When an inoculum of *S. marcescens* was introduced into the dialysate solution after the equilibration period with the artificial kidney, the goat seemed quite normal for a number of hours, depending on the size of the inoculum. The body temperature (Fig. 2E), the blood erythrocyte count (Fig. 2G), and the serum biochemistry for the most part remained within normal ranges. The blood glucose concentration (Fig. 4B) and the white blood cell count (Fig. 2F) both increased in the initial hours of hemodialysis culture, although the latter reaction also occurred in hemodialysis without culture (Fig. 2B).

After this uneventful incubation period, an acute episode of illness developed in the goat with dramatic suddenness, apparently because of dialyzable toxic materials produced by the culture. The animal evidenced general signs of toxemia with violent shivering and lethargy that persisted for 2 h or more. In some cases bloody urine was passed; in others urine output ceased. Feeding and drinking also ceased during an episode.

The time courses of some host responses measured in a representative trial are shown in Fig. 2, together with control responses during hemodialysis without culture. The body temperature (Fig. 2E) started to rise after about 7 h of hemodialysis culture, which coincided with the time when a population of $10^8$ viable cells/ml was exceeded (also see Fig. 7A) and when multiplication changed from an exponential to a linear rate in the culture (Fig. 2D). An acute reduction in the number of white blood cells was a second main sign of host toxemia (Fig. 2F). The number of red blood cells remained within the normal range (Fig. 2G). Among the other host parameters monitored, only the concentration of blood glucose changed significantly, with the development of moderate hypoglycemia (Fig. 4B).

General pictures of the host pyrexia and leukopenia responses are shown in Fig. 7, in which the average changes in six trials were plotted as functions of the number of viable bacterial cells in the hemodialysis culture. It was apparent that both responses ensued after a population of about $10^9$ viable cells/ml was attained.

The fever and leukopenia observed with the aerated hemodialysis cultures were less apparent with cultures maintained under strictly anaerobic conditions, although the cultures multiplied similarly in the two situations (Fig. 5). The reason for the difference in host responses was not apparent.

Substitution of each of the three beaker dialyzers in place of the artificial kidney in the hemodialysis culture system resulted in predictable host responses (Fig. 6). Because the MW-300 beaker as a hemodialyzer failed to support appreciable multiplication of the inoculum.
lum (Fig. 6A), no significant responses were observed in the host (Fig. 6B and 6C). The MW-10,000 or MW-30,000 beaker as a hemodialyzer supported similar patterns of culture growth (Fig. 6D and 6G) and host responses (Fig. 6E, 6F and 6H, 6I) as did the artificial kidney (cf. Fig. 2), although the fever and leukopenia responses with the two beakers occurred earlier in the time course of growth.

The three beaker dialyzers were then inserted in series into the dialysate-culture circuit to collect three different size classes of culture product simultaneously with artificial kidney hemodialysis. When the goat evidenced acute toxemia, the three differential dialysis samples were removed. Injection of the MW-10,000 beaker-dialysate sample into another goat (3 ml, intravenously) evoked pyrexia but not measurable leukopenia, thus partially duplicating the results obtained by direct hemodialysis culture with either the artificial kidney or the same beaker dialyzer even though the amount of dialyzable toxic material was much less than that transferred over a period of time in continuous hemodialysis. Similar results were obtained with the MW-30,000 beaker sample. However, the MW-300 beaker sample (and also the original salts solution, employed as a control) did not evoke any measurable host response.

It might be thought that the host responses were caused by some nonspecific effect of bacterial growth, such as the depletion of blood glucose. This was shown unlikely by the observation that goat hemodialysis culture of *Bacillus anthracis* (the avirulent Sterne vaccine strain) did not result in a toxemic host response even though massive bacterial growth and host hypoglycemia occurred with either aerobic or anaerobic conditions in the fermentor. The anthrax cells grew in long chains but did not form spores.

The argument also might be advanced that the results were caused by intact bacterial cells passing through an undetected opening in the membrane of the hemodialyzer. In the control hemodialysis trials, smaller particles (blood proteins) did not pass into the dialysate circuit. Furthermore, in dialysis culture in vitro with the artificial kidney and a reservoir of nutrient medium, the reservoir remained sterile for at least 24 h. When the differential dialysis system was employed, the dialysate solutions in the beaker dialyzers also remained free of the bacteria. Moreover, during the hemodialysis culture trials bacteria were not detected by routine aerobic and anaerobic culturing of blood samples.

**Molecular size of the dialyzable toxic material.** The results with the beaker dialyzers enabled an estimation of the maximal molecular size of the dialyzable toxic material relative to the molecular weight of proteins that reportedly just diffuse through or are just excluded by the membrane in the artificial kidney and MW-10,000 beaker dialyzer (Bulletin no. 175-1187-71, Dow Chemical Co.). Of the proteins reported, cytochrome *c* (presumably of bovine heart origin, molecular weight 13,370 [13]) lies closest within the dialysis threshold of the membrane. An equivalent Einstein-Stokes hydrodynamic radius ($r_{hs}$) of 1.88 nm was calculated, based on a diffusion coefficient of $1.4 \times 10^7$[13]. Myoglobin (presumably of horse heart origin, molecular weight 16,890 [13]) lies just outside the dialysis threshold of the membrane, from which an $r_{hs}$ of 1.90 nm was calculated based on a diffusion coefficient of $1.3 \times 10^7$ (13). Consequently, the size of a molecule just able to dialyze is intermediate in equivalent size between these rigid globular
proteins, i.e., a molecular weight of 15,000 and an \( r_E \) of 1.89 nm.

The dialyzable toxic material from \( S. \) marcescens might be more fibrous and flexible than globular and rigid in its molecular configuration. Consequently, its size might be better related to the dialysis of a molecule like that of a PEG which in solution coils randomly and loosely into a form that is large relative to its molecular weight. Scherrer and Gerhardt (10), by using polyglycol samples, have devised a distribution analysis method to determine the porosity threshold of bacterial cell walls. By use of this method, the dialysis threshold of the artificial kidney membrane was successfully determined with a polydisperse PEG sample of \( M_n \) 3,350, whereas smaller (\( M_n \) 1,540) and larger (\( M_n \) 9,500) polyglycol samples proved unsatisfactory. The results (Fig. 8) showed that the size of a molecule just able to dialyze, determined from the intersection point of the two distribution curves, was equivalent to a quasi-monodisperse PEG with a \( M_n \) of 5,500 and an \( r_E \) of 2.6 nm.

Thus, the maximal molecular size of the dialyzable toxic material from \( S. \) marcescens was defined relative to two different types of molecules. If its molecular configuration was like a rigid globular protein, the toxic material was less than a molecular weight of 15,000 and an \( r_E \) of 1.9 nm. If it was like a flexible fibrous polyglycol, it was less than a molecular weight of 5,500 and an \( r_E \) of 2.6 nm.

**DISCUSSION**

The results demonstrated the feasibility of employing hemodialysis and a goat-artificial kidney-fermentor system to grow organisms. Selection of the goat and \( S. \) marcescens as the host-parasite model was made less for intrinsic interest and more for convenience in developing the new system and studying its operation. In its potential, this prototype hemodialysis culture system appears to have broader applicability, but within certain limitations. Animals other than the goat appear adaptable, provided that they are sufficiently large and can be held quietly. Organisms other than \( S. \) marcescens, including ones having more complex nutritional requirements, appear capable of culture provided that they have a comparably short generation time so as to multiply adequately within the period (e.g., about 15 h) that an animal can be held quietly and attended constantly. The milieu in the dialysate appeared to be much the same as that in the blood to the extent that small dialyzable molecules are involved, but macromolecules such as serum proteins do not permeate the most porous membrane used, and it is questionable whether dialysis exchange of \( \text{CO}_2 \) and \( \text{O}_2 \) is sufficiently rapid. Consequently, the in vivo milieu of the blood was only partially duplicated. This was evidenced when \( B. \) anthracis grew in the system asporogenously as in vivo, but with the formation of long chains as in vitro. The hemodialysis culture system appears especially useful for examining the effects of small microbial products on a host, and of small host molecules on a parasite population, in a situation where the microbial cells can attain a mass comparable to that in severe infections and produce relatively large amounts of products. The system also appears promising for studying the effects of antibiotic drugs on microbial cultures.

The results also demonstrated methods for sampling culture products by differential dialysis and for accurately determining the maximal size of a dialyzable product relative to different molecular configurations, without prior chemical extraction or treatment. These methods probably could be extended to membranes of coarser porosity and molecules of larger size and appear useful especially for the study of polydisperse products.

The nature of the dialyzable toxic material demonstrated by goat hemodialysis culture of \( S. \) marcescens is unknown. One possibility was that of an endotoxin, which is a known product of the organism and was suggested by the fever, leukopenia, and general physiological response of the animal. Native endotoxin usually is considered to consist of a polydisperse macromolecular complex of lipid, polysaccharide, and protein with a nominal molecular weight of about \( 10^4 \) to \( 10^6 \), and molecules of this size could not permeate the membranes of any of the dialyzers used in this study. The smallest frag-
ment of endotoxin that yields some or all of the typical biological reactions is undefined (14, 15), but the state of dispersion of endotoxin fractions is known to affect certain biological reactions (7, 15). Continuous hemodialysis conceivably might allow the passage of small active fragments or of small inactive ones which then could aggregate or become adsorbed to blood constituents and thereby acquire endotoxic-like activity.

The dialyzable toxic material from S. marcescens might also be attributed to an ordinary metabolic product that is deleterious to the host. An example of this situation occurs among the complex of toxins produced by Vibrio cholerae, in which the dialyzable and heat-stable type 3 toxin eventually was identified as ammonia (1). However, a similar situation with the dialyzable material from S. marcescens seems unlikely because of the range and severity of host reactions and because the effects were less marked under anaerobic culture conditions where the accumulation of intermediary metabolic products would be greatest.

Finally, a toxin different from any of the above might account for the dialyzable toxic material from S. marcescens. This possibility deserves consideration in view of emerging clinical and experimental evidence that factors other than endotoxin may be more critical than endotoxin in determining lethal outcome of severe sepsis due to gram-negative organisms (5).

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LITERATURE CITED