ENHANCEMENT OF CARDIAC OUTPUT BY THE DRAG-REDUCING AGENT, SEPARAN AP-273, A LINEAR POLYELECTROLYTE OF EXTRAORDINARY LENGTH

A Thesis
Presented to the
University of Manitoba

In Partial Fulfillment of
The Requirements for the Degree of
Master of Science

By

Paul Bernard Coleman

1985

Department of Physiology
Faculty of Medicine
Winnipeg, Manitoba

ENHANCEMENT OF CARDIAC OUTPUT BY THE DRAG-REDUCING AGENT, SEPARAN AP-273, A LINEAR POLYELECTROLYTE OF EXTRAORDINARY LENGTH

BY

PAUL BERNARD COLEMAN

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

MASTER OF SCIENCE

TO MY WIFE AND PARENTS

ACKNOWLEDGEMENTS

I express my gratitude to Dr. P.I. Polimeni who supervised my Master's Programme. His assistance and enthusiasm played a major role in the development of this thesis.

I am indebted to Mr. B.T. Ottenbreit, whose expert surgical contributions were crucial to the success of many experiments, to Mrs. M.D. Otten for advice and assistance in the preparation of polymer solutions, and Mr. Henry Kroker for technical advice.

To my parents I express my appreciation for their continued encouragement.

Finally, I thank my wife who helped me return to complete this thesis.

The opportunity to pursue this study was made possible by a Graduate Student Scholarship from the Manitoba Health Research Council, which also supported the research together with the Manitoba Heart Foundation through grants to Dr. Polimeni.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

^{o}C	Degree Celsius
cm	Centimetre
cP	Centipoise
DNA	Deoxyribonucleic acid
dP/dt	First Deriviation of pressure as a function of time
ECG	Electrocardiography
EDTA	Ethylenediamine tetraacetate
F	Flow
g	gram
h	Hour
HEPES	H-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid
Hz	Hertz
i.p.	Intraperitoneal
i.v.	Intravenous
kg	Kilogram
l	Litre
LV	Left ventricule
v	Velocity
M	Mole
mg	Milligram
min	Minute
ml	Millilitre
mmHg	Millimetre mercury
mM	Millimole
msec	Millisecond
n	Normal
N	Number
P	Probability
ppm	Parts-per-million
r	Radius
Re	Reynolds number
RGGu	Rhamnagalactogalacturonan
sec	Second
t	Time
η	Viscosity coefficient
ΔP	Pressure gradient
μm	Micrometre
π	Pi, the constant 3.1416...
ρ	Density
$>$	More than
$<<$	Much less than
$<$	Less than
$\leq$	Less than or equal to
$=$	Equal to
$\simeq$	Approximately equal to
$\sim$	Approximately

Subscripts:

a	Solvent containing additive
s	Solvent without additive

ABSTRACT

A review of the literature shows that frictional forces generated in turbulent flow may be dampened by the addition of linear polymers of high molecular weight (> 50,000 daltons). This effect, sometimes called the "Toms phenomenon" or "polymer drag reduction" is believed to be due to the alignment of the macropolymers parallel to the axis of flow, which tends to laminarize the dynamic structure of the flow. The phenomenon occurs in virtually all types of flow with both organic and aqueous solutions. Although most of the research conducted in this field involves flows at supracritical Reynolds numbers (Re), recent studies indicate that disturbed flows can be increased under appropriate conditions when the critical Re is extremely low.

Four macropolymers -- a poly(ethylene oxide), a polyacrylamide, a calf thymus deoxyribonucleic acid, and a polysaccharide extracted from okra -- have been reported to decrease the pressure gradient in constant blood flows through straight pipes at supracritical Re. These linear macropolymers are all characterized by extraordinary molecular weights and lengths. One of these polymers, the okra extract rhamnogalactogalacturonan (RGGu), was previously reported to markedly enhance cardiac output in the rat.

The aim of the present study was to test the hypothesis that in vivo blood flow can be increased significantly by injecting into the bloodstream stiff linear macromolecules of lengths spanning the diameters of at least several erythrocytes. Separan AP-273 -- a linear, polydisperse, anionic polyacrylamide with a molecular weight ranging from 10^5 to $5 \cdot 10^7$ daltons -- was tested for its hemodynamic effects in the rat. The variables recorded were aortic blood flow, left ventricular blood pressure and its first derivative as a function of time, carotid blood pressure, and the electrocardiogram. Results show that Separan, like RGGu, is capable of markedly increasing

cardiac output in association with a profound fall in total peripheral resistance and minimal changes in heart rate and mean blood pressure. The hemodynamic effects of Separan disappear when the macropolymer is broken into shorter fragments by shearing forces, a result similar to that previously observed with RGGu.

Blood flow through straight plastic tubing interrupted by two loops increased when a Separan solution was added to the blood, whereas addition of an equal volume of saline had no discernible effect on flow. This finding differs from previous *in vitro* results with blood containing drag reducing polymers insofar that Re for the blood flow in our study was subcritical. Since neither vasodilation nor hypoviscosity can account for these results, polymer drag reduction is considered to be the likely explanation for the *in vitro* findings.

The fact that two macropolymers of different chemical composition but similar molecular conformation and linear dimension cause similar hemodynamic effects, suggests that a physical mechanism is primarily responsible for these effects. This interpretation is further supported by a more recent preliminary finding in our laboratory that a poly(ethylene oxide) averaging $2 \cdot 10^6$ daltons molecular weight, Polyox WRSN-60K, also enhances cardiac output. Consideration of the results obtained *in vitro* with Separan and *in vivo* with sheared Separan and RGGu supports the thesis that blood flow in the living organism may be increased by a mechanism related to polymer drag reduction.

I. INTRODUCTION

When confronted with the task of improving cardiac output in the sick heart, the most efficient means known to the scientific community is by decreasing the force against which the heart must work (afterload). The heart will respond favourably to reduced afterload under almost all conditions, but this is especially true for the diseased heart. Hypoviscosity and vasodilation are the best known methods for increasing cardiac output by decreasing afterload, but the enhanced flow is usually minimal with vasodilating drugs because a simultaneous increase in vascular capacitance tends to decrease venous return. For this reason, until recently, vasodilators have been used sparingly as a means of increasing cardiac output. It now appears that several vasodilator drugs are being produced that can markedly increase cardiac output. The difference between these new vasodilator drugs and the earlier less effective ones may be that the new ones act predominantly on the arterial vasculature.

Hypoviscosity, the other method of increasing cardiac output by decreasing afterload, has in the past usually been synonymous with hemodilution. Although small decreases in viscosity produced large increases in cardiac output, the hemodynamic advantage of this increase

is partly diminished by the fact that oxygen capacity decreases in diluted blood. More recent drugs found to be hemorheologically active are able to decrease viscosity without hemodilution either by reducing the plasma level of fibrinogen or by rendering the erythrocyte plasma membrane less rigid.

The purpose of this thesis is to propose a third means of increasing cardiac output by decreasing peripheral resistance, although not necessarily the afterload, namely, via the introduction of drag reducing stiff macromolecules -- particularly polyelectrolytes -- into the circulation capable of laminarizing the dynamic structure of flow. "Polymer drag reduction," a subject that has been studied primarily by hydrodynamicists, is relatively unknown among those involved in study of the heart and its vasculature.

A. Polymer Drag Reduction or the "Toms Phenomenon"

Polymer drag reduction was apparently first observed by B.A. Toms in 1947 in the flow of a mixture of polymer and organic solvent. Although Toms did not understand the nature of his discovery, he did understand that the observation was extraordinary (Toms, 1949) and the polymer effect on flow was later given the rubric "Toms phenomenon". Since 1949, fewer than two thousand papers have been published on the phenomenon, the large majority of which are found in engineering and physics journals. After Toms' initial discovery, there was a lag period until the phenomenon was rediscovered and became of greater

interest during the 1960's. It was during this period and the next decade that the greatest number of publications were produced.

Great expectations were placed on using the Toms phenomenon for practical purposes, but few of these ideas actually succeeded. One of the few applications of the phenomenon that did prove useful was a 24% increase in oil flow through the Trans-Alaskan pipeline by use of drag reducing agents without any change in energy requirements (Burger et al., 1980; Holt, 1981). Drag reduction in the pipeline was found to become increasingly effective with greater flow velocity, decreasing pipe diameter, and decreasing viscosity (Burger et al., 1980). Other successes include the use by the New York City Fire Department of a polymer to increase water flow through fire hoses and the addition of polymer drag reducing agents to the sewers of several English towns with inadequate pipe diameters during periods of excess sewer flows. We shall see below that in the 1970's several studies were undertaken to observe the effects of polymer drag reducing agents on in vitro and in vivo blood flows. The results of these studies demonstrated that a few of these polymers produced large hemodynamic changes that in principle could prove therapeutically useful.

What then is "polymer drag reduction"? Normally one observes a decreased rate of flow in a pipe upon the addition of a high molecular weight polymer to a fluid. However, Toms (1949) discovered that steady flow of the solvent monochlorobenzene through a pipe under a constant pressure gradient could be enhanced by as much as 40% by the addition of 100 mg/liter of polymethylmethacrylate as long as the Reynolds

number (Re) was in excess of about 1,000, i.e., supracritical. Initially it was thought that this phenomenon was due to a non-Newtonian relationship between viscosity and shear rate. Later, however, it was found that flow could be increased at such low polymer concentrations that changes in viscosity were not detectable.

By an extraordinary coincidence, about the same time that Toms made his discovery in England, Mysels made a similar discovery upon the addition of napalm to gasoline and he received the first patent (Mysels, 1949) based on polymer drag reduction. Unlike many other simultaneous discoveries in science, where there are parallel developments culminating in a major finding, these two discoveries were entirely serendipitous.

After Toms reported his findings, it was almost 15 years before further work was carried out. By the early 1970's more than twenty polymer drag reducing agents had been identified (Hoyt, 1971). Several more polymer-organic solvent systems were discovered demonstrating the Toms phenomenon (Table 1), including polymethyl methacrylate in toluene (Hershey & Zekin, 1967), polystyrene in benzene (Hunston & Reischman, 1975), and polyisobutylene in kerosene (Ram et al., 1967), toluene (Rodriguez et al., 1967), and benzene or cyclohexane (Hershey & Zakin, 1967).

Among the drag reducing polymers effective in aqueous systems were poly(ethylene oxide) (Virk et al., 1967; White & Hoyt, 1969, Patterson & Abernathy, 1970; Hansen & Little, 1971; Taylor & Middleman, 1974; Keller et al., 1975), deoxyribonucleic acid (Hoyt, 1966; White and

Solute	Solvent	Reference
Napalm	Gasoline	Mysels, 1949
Polymethyl methacrylate	Monochlorobenzene	Toms, 1949
" "	Toluene	Hershey & Zakin, 1967
Polyisobutylene	Benzene	Hershey & Zakin, 1967
"	Cyclohexane	" " "
"	Kerosine	Ram et al., 1967
"	Toluene	Rodriguez et al., 1967
Polystyrene	Benzene	Hunston & Reischman, 1975

Table 1. Compilation of polymer-solvent combinations reported to demonstrate polymer drag reduction in the flow of organic solutions.

Hoyt, 1969), polyacrylamide (Barnes et al., 1969; White, 1969; Green, 1971), carboxymethylcellulose (Brandt et al., 1969), polysaccharides of bacterial (Kenis, 1968), algal (Hoyt & Soli, 1965, and vegetable (Savins, 1964; Gilbert & Ripken, 1969; Gutstein et al., 1970; Castro & Neuwirth, 1971; Castro, 1972; Hoyt, 1972) origin (see Table 2 for some typical drag reductions reported).

Some polymers have been shown to produce drag reductions (defined as $100\,[1 - (\Delta P_a / \Delta P_s)]$ in constant flow, where subscripts a and s represent polymer additive in solvent and the solvent alone, respectively; see Fig. 1) of as much as 70% or more using 100 ppm or less of polymer additive in turbulent flow. This demonstrates how much of an effect these agents can have on flow. It should be noted that for a constant pressure gradient, a 70% reduction in drag produces approximately a three-fold increase in flow.

B. Generalities of Polymer Drag Reduction

From work carried out over the past twenty years, researchers have come to some general conclusions about polymer drag reduction and drag reducing polymers (Lumley, 1969; Bark et al., 1975). These polymers are basically of molecular weights between 10^5 to 10^7 daltons. The higher the molecular weight, the greater the effectiveness of the DRA. Polymers with molecular weights under 50,000 daltons do not demonstrate drag reducing effects at all. The length of the molecule's axial structure, however, as opposed to its molecular weight per se, seems to

Drag Reducing Agent	Conc. (PPM)	D.R. (%)	References
Polyethylene oxide	8	58	White, 1969
	10	67	Hoyt, 1972
	20	65	Taylor & Middleman, 1974
Polyacrylamide	32	58	White, 1969
Bacterial polysaccharide	200	62	Kenis, 1968
Algal polysaccharide	-	60	Hoyt & Soll, 1965
Vegetable polysaccharides			
Guar gum	200	68	Savins, 1964
Karaya gum	850	67	Hoyt, 1972
Okra gum	25	40	Castro & Neuwirth, 1971
Deoxyribonucleic acid	-	68	Hoyt, 1966

Table 2. Compilation of polymers reported to demonstrate drag reduction in aqueous flows. The concentrations (Conc.) are given in parts per million (PPM) and drag reduction (D.R.) is expressed as a percentage.

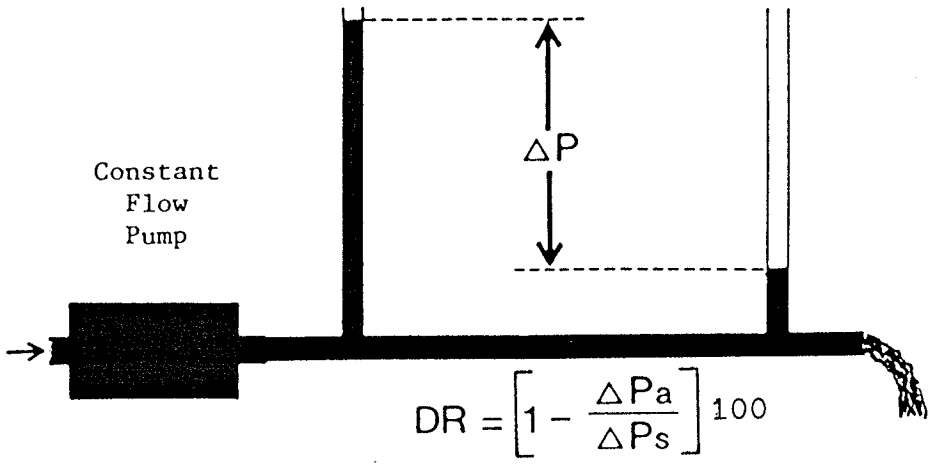

Figure 1. Drag reduction by polymer additives. Drag reduction (DR) in a constant flow through a pipe may be calculated by determining the ratio of pressure gradients of polymer solution (ΔP_a) and solvent alone (ΔP_s).

be the determining factor as to how much drag reduction is to be expected.

Drag reduction is more closely related to the concentration of the largest, rather than an average, molecular weight polymer (Van der Meulen, 1974; Hunston & Reischman, 1975). Reduction of drag is decreased with an increase in the number and lengths of the polymer's side chains. Drag reducing polymers work best when the polymer-polymer interactions in the solvent are minimal, and the polymer-solvent interactions are maximal. These interactions are generally optimized when anionic sites are distributed along the entire length of the polymer molecule. These distributed charges favor an extended molecular conformation, because electrostatic repulsion tends to impede molecular folding (Molyneux, 1984). Although early studies had suggested that flexible polymers tend to be better drag reducers than stiff polymers, this view is discounted in the more recent literature (Little, 1973; Frommer et al., 1974). Because molecules in linear conformations tend to align themselves parallel to the axis of flow, molecular entanglements are minimized.

Drag reduction is proportional to polymer concentration in dilute solutions. However, at high polymer concentrations the drag reduction is offset by increasing viscosity and flow eventually decreases after attaining its peak. Polymer drag reduction has been shown to be enhanced upon the addition of salt (Yamashita et al., 1983), but this does not appear to be a general phenomenon (Little, 1973). Solvent power, viscosity, and temperature have been found to be important

factors in drag reduction effectiveness in some systems (Peyser & Little, 1971).

Depending on the strength of the molecular bonds in any polymer and the rate of shear, polymer drag reduction may cease within a few minutes or it may last many years. Loss of drag reduction during flow may be due to mechanical, chemical or thermal degradation of the polymer molecule. Polymers of very high molecular weight are particularly susceptible to mechanical degradation (Gampert & Wagner, 1982). According to Dunlop & Cox (1973) molecular aggregates are a common feature of polymer solutions and some types of aggregation enhance drag reduction. This might account for some variability of experimental results in drag reduction studies, for there appears to be an optimal point in the rate and duration of mixing these polymers by mechanical agitation (Stenberg et al., 1977). This is especially true with the shear stresses associated with the flow velocities usually applied to demonstrate the Toms phenomenon, where Re is 1000 or much greater.

Some researchers have concluded that pipe diameter plays a part in the amount of drag reduction (Elata, 1966; Hansen & Little, 1971; Rudd, 1972; Sellin & Ollis, 1983), but others claim otherwise (Virk et al., 1967; Patterson & Abernathy, 1970). Gadd (1971) reported that drag reducing polymers are more effective at a given concentration and Re as tube diameter decreases. Sharma et al. (1979a) have proposed an equation predicting the onset Re of drag reduction which depends on the type of polymer and its concentration, pipe diameter, and the flow

velocity gradient near the wall. The equation indicates that for a given concentration of polymer the onset of drag reduction should occur at lower Re as pipe diameter decreases. Gadd (1971) also reported that drag reduction can only take place during turbulent flow, when the shear stress at the tube's inner wall passes a critical value. While this is usually the case, we shall see below that under certain conditions polymer drag reduction occurs in the absence of turbulence.

Along with the standard study of drag reducing polymers in flow through straight tubes with circular cross-section, these polymers have been studied in many other types of flow (Table 3), including oscillatory flow (Barnes, 1969; Voitkounsky et al., 1972; Driels & Ayyosh, 1976), orifice flow (Giles, 1969; James & Saringer, 1980), flow through a nozzle (Green, 1971; Hoyt & Taylor, 1977a,b), flow on a flat plate (Granville, 1967; Wu, 1969) or rotating disc (Fabula et al., 1963; Gilbert & Ripken, 1969), flow of a submerged jet (Gadd, 1965), cylindrical Coullette flow (Elata & Tirosh, 1964; Keller et al., 1975), annular flow (Tiu & Chee, 1979), screw helicoidal flow (Kuo & Kovasznay, 1981), and flow through a column of sand (Noselevich et al., 1979).

It was known, long before the Toms phenomenon was demonstrated, that particles suspended in fluid, such as dust, asbestos, and nylon fibers, reduce drag in turbulent flows of both air and liquids. Surprising results were obtained when the suspended macroscopic particles and drag reducing polymers were combined, namely, the resulting drag reductions were greater than the sum of the drag

1. Straight pipe turbulent flow (Toms, 1949)
2. Flow between rotating disks (Fabula et al., 1963)
3. Flow between rotating cylinders (Elata & Tirosh, 1965)
4. Flow of submerged jet (Gadd, 1965)
5. Flow over flat plate (Granville, 1967)
6. Oscillatory flow (Barnes et al., 1969)
7. Orifice flow (James & Saringer, 1980)
8. Screw pump flow (Kuo & Kovasnay, 1981)

Table 3. Compilation of some hydrodynamic conditions in which the polymer drag reduction phenomenon is observed. While polymer drag reduction most commonly occurs in turbulent flow, the phenomenon is sometimes demonstrated in unstable or disturbed flows. Most recently polymer drag reduction has been demonstrated in capillary-like flow through a column of sand at very low Reynolds number.

reductions obtained by the two methods alone (Lee et al., 1974, Sharma et al., 1979,b). This effect is most noticeable near the tube's wall, where the viscous sublayer thickens when the second drag reducing system is added. Of even greater surprise is the finding that this effect may be seen under conditions in which the polymer alone may have little or even no drag reducing effect.

C. Speculations on the Mechanism of Polymer Drag Reduction

It is a commonly known fact in hydrodynamics, that initially randomly positioned linear molecules align themselves in a direction parallel to flow. The reason for this orientation is that isotropic particles position themselves to produce the least hydrodynamic resistance (Eisenberg, 1976). The most common explanation for the Toms phenomenon is that linear polymers inhibit turbulence by lining up parallel to the flow, and thus impart a dynamic laminar structure to the flow. Another view is that polymer molecules act to "buffer" the energy normally lost to turbulence. These very long molecules act as energy sinks by bending during turbulence, and by doing so, inhibit heat dissipation normally associated with the friction of turbulence (Kohn, 1973).

Although a number of theories have been proposed by hydrodynamicists to explain the Toms phenomenon (Fabula et al., 1966; Virk et al., 1967; Ruckerstein, 1971; Hansen, 1973; Kohn, 1973;), none have become universally accepted. Savins has identified at least five

different types of polymer drag reduction (Savins, 1969), leading him to believe there are possibly many more yet to be discovered.

In steady flow through a pipe drag reducing polymers do not increase flow at Re <1000, and might even decrease it slightly at higher polymer concentrations. But the presence of these polymers tends to maintain the slope of the pressure-flow curve beyond the supracritical Re (Fig. 2), thereby suggesting that a dampening of turbulence occurs upon the addition of polymer. This dampening or laminarizing effect can be visualized by injecting dyes into the flow (Gadd, 1965; Taylor & Middleman, 1974; Hoyt & Taylor, 1977a,b). When drag reduction is plotted against flow as a function of polymer concentration, it is apparent that whereas the greatest increases in flow occur at high polymer concentrations in the turbulent regime, the greatest flow increments take place in the transitional zone at relatively low polymer concentrations (White, 1969), i.e., for a given polymer concentration drag reducing agents are most efficient in disturbed flow, wherein turbulence is not fully developed.

Along with continuing questions about the mechanism, the site where drag reduction takes place also remains obscure. Some investigators believe that the site of action is at the viscous sublayer next to the tube's inner wall (Brandt et al., 1969; Rudd, 1972), while others feel it is at the turbulent core (Metzner & Park, 1964; Taylor & Middleman, 1974). In more recent times, it has been generally concluded that drag reduction takes place throughout the entire flow, but with special emphasis at the wall layer. One hypothesis that has been definitively

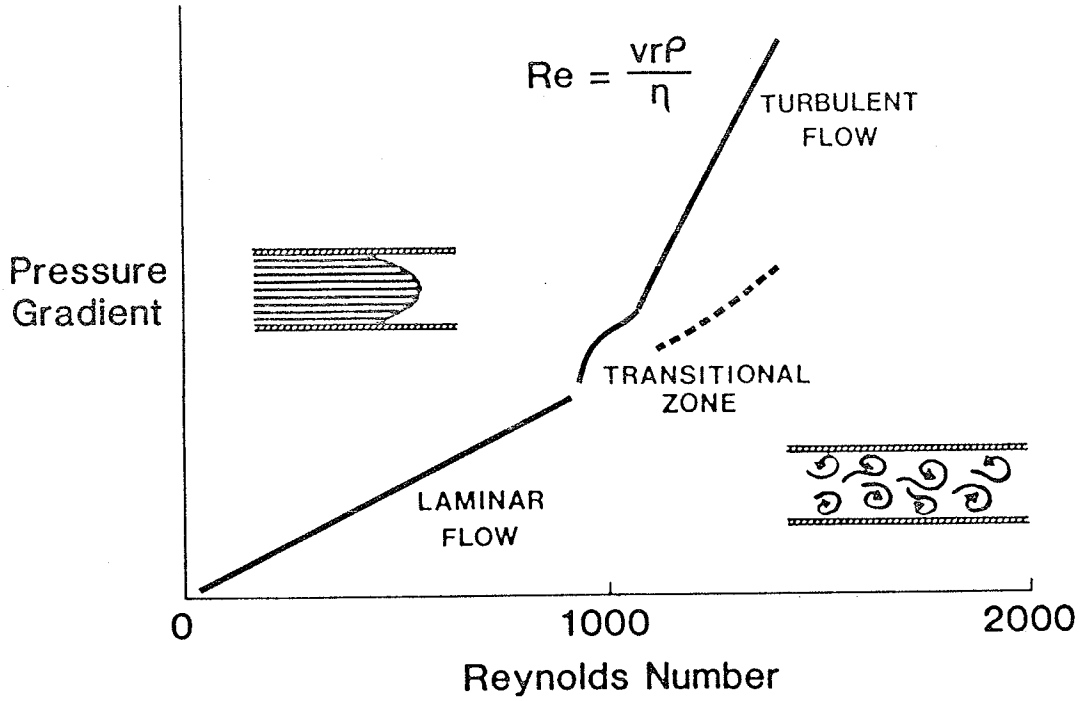

Figure 2. Relationship between pressure gradient (ΔP) and Reynolds number (Re). The slope $\Delta P/Re$ is constant in the laminar regime ($Re < 1000$), but the slope increases in the turbulent regime ($Re > 1000$). The addition of drag reducing agents to the flow causes the $\Delta P/Re$ curve at a slope expected for laminar flow to extend well into the turbulent regime, i.e., turbulence is dampened. Re is defined by the ratio of the product $vr\rho$ and η, where v is the mean velocity of the fluid in the pipe, r is the radius of the pipe, and ρ and η are the density and viscosity of the fluid, respectfully.

excluded, at least with regard to poly(ethylene oxide), is that the mechanism might involve absorption of the polymer to the pipe wall surface (Grigoryan et al., 1979).

D. Polymer Drag Reduction in Blood Flow: The Background

1. In vitro blood flow

In vitro studies of the Toms phenomenon have also proved successful using blood. Only four polymers have been shown to reduce drag in blood flow thus far (Table 4), viz., a deoxyribonucleic acid derived from calf thymus (Hoyt, 1966), the poly(ethylene oxide) "Polyox" (White & Hoyt, 1969; Stein et al., 1972), a polysaccharide extract of okra (Castro, 1972), and the anionic polyacrylamide Separan AP-30 (Greene et al., 1970b, 1978). It is noteworthy that all of these polymers are characterized by linear lengths among the highest range known to science, approaching 100 micrometres! It is difficult to discern from the literature whether or not drag reducing polymers in the 1 to 10 μm range have been examined in pipe blood flows. Had such polymers been tried in blood and failed to demonstrate any drag reducing effects, such negative results probably would not have been reported.

2. Can polymer drag reduction be applied to in vivo blood flow? Some considerations

The next logical question was, what effects do polymers reducing drag in blood flow in vitro have in vivo? More to the point, could these polymers produce a change in the flow pattern of blood so that

Drag Reducing Agent	Conc. (PPM)	D.R. (%)	References
Polyethylene oxide	200	48	Stein et al., 1972
Polyacrylamide	40	30	Green et al., 1974
Deoxyribonucleic acid	122	50	White & Hoyt, 1969
Okra gum	25	50	Castro, 1972

Table 4. Compilation of artificial and natural polymers reported to demonstrate drag reduction when added to blood flowing in vitro at high Re. Polymer concentrations are given in parts per million and drag reduction is expressed as a percentage. The drag reducing polymers listed here, all characterized by extremely great molecular lenghts, appear to be the only ones reported to be effective in blood flows.

total peripheral resistance would drop, thereby causing an increased cardiac output without proportional increase in myocardial energy expenditure?

Decreasing viscosity is known to cause a disproportionate increase in cardiac output (Dormandy, 1970; Fowler & Holmes, 1975), but when drag reducing polymers are added to blood the blood becomes slightly more viscous than normal or remains unchanged. Both hypoviscosity and polymer drag reduction involve decreasing friction forces. However, hypoviscosity involves the lowering of friction between laminar layers, whereas polymer drag reduction decreases the friction associated with obliquely directed flow vectors produced by flow disturbances or turbulence. There is little relationship between myocardial oxygen consumption and external cardiac work, but there is a directly proportional relationship between oxygen consumption and both heart rate and mean systolic ventricular pressure. This relationship demonstrates that, theoretically, cardiac output can be increased without an increase of biochemical energy expenditure, because myocardial oxygen consumption depends mostly on myocardial fiber tension instead of flow volume (Sarnoff, 1958). Sarnoff's classic work demonstrated this point clearly. While maintaining cardiac output and heart rate constant, a 175% increase in external work, associated with an increased aortic pressure, produced a 178% increase in oxygen consumption. When external work was increased 696% by increasing flow subsequent to reduced resistance, holding aortic pressure and heart rate constant, oxygen consumption rose only 53%.

One major concern in research involving polymer drag reduction *in vivo*, is that previous *in vitro* studies with drag reducing agents have usually been at Re well above the critical level for turbulence. Within the circulatory system flow normally is at subcritical Re, except above the semilunar valves of the aorta, where Re reaches about 5000 during peak ventricular systole (Caro, 1978; Dinner, 1981).

When speaking of fluid movements in the laminar regime, one normally refers to a constant steady flow. When dealing with pulsatile flow, as is the case *in vivo*, the definition of laminar flow becomes unclear. Truly laminar flow is not obtained in pulsatile flow, and large artery energy losses are more appropriately evaluated in terms of turbulent friction relationships (Streeter *et al.*, 1963).

One might predict that polymers would have little *in vivo* effect, because the greatest flow resistance in the vasculature occurs at the level of the arterioles where Re is well below critical. It should be borne in mind, however, that when it is stated that turbulence occurs at Re >1000, one assumes a steady flow of a Newtonian fluid through straight rigid tubes. In tubes with bifurcations, convergences, curvatures, and other geometric complexities such as occur *in vivo*, the critical Re is markedly lower (*e.g.*, Roach *et al.*, 1972).

When flow fluctuates (Ku & Giddens, 1983), or when the diameter is not constant (Meisner & Rushmer, 1963), turbulence and eddy formation take place at Re well below 1000 in tubes simulating the vasculature. Also, secondary rebounding waves generated at elastic vascular walls impede flow by resistive effects. Elastic tubes produce surface waves

that disrupt the viscous sublayer next to the tube's wall (Silberberg, 1976) and convert kinetic energy to frictional energy dissipated as heat. It is possible that upon the addition of extended linear polymers part of the energy associated with secondary waves may be absorbed by the molecules for eventual reconversion to kinetic energy. In this way, flow may be increased for a given driving pressure.

Secondary transient flow patterns should be markedly altered by pulsation (Zalosh & Nelson, 1973). Such secondary transient flows and large stagnant areas are known to occur distal to bifurcations even in non-pulsatile flows (Lefort, 1976). Disturbed flow can be predicted anywhere along the flow path where boundary layer separation occurs. This can be at bifurcations, bends, sinuses, aneurysms and places distal to stenotic or coarctated areas (Greene et al., 1970a). In vivo high-speed cinematography (McDonald, 1960) and cinefluorography (Ohlesson, 1962) of the thoracic aorta has shown systolic disturbances to be present. Pulse rate and maximum flow velocity appear to be the two major determinants of flow disturbances occurring under normal physiological conditions (Nerem & Seed, 1972). Flow has been shown to be turbulent or at least disturbed in vessels much smaller than the large arteries. In fact, flow disturbances have been seen in vivo in vascular junctions at Re under 100 (Gutstein et al., 1970).

When compared to plasma of equal viscosity and density, whole blood is more susceptible to turbulence. This greater susceptibility is due to the random movements of suspended red blood cells observable in vivo by high-speed cinematography. Such gyrating movements of the

erythrocytes have been seen in blood vessels down to the level of the microvasculature (Block, 1968).

Hoyt (1974) suggested that drag reducing agents could be used in any type of flow as long as a finite rate of change of shear rate with time was present. This view is supported by the theoretical considerations of Hansen (1973) and Kohn (1973), but more importantly poly(ethylene oxide) has been shown to be an effective drag reducing agent in pulsed laminar flow *in vitro* at Re below 100 (Voitkounsky *et al.*, 1972). Poly(ethylene oxide) also functions as a drag reducing agent when fluid oscillates in a semicircular monometer tube at $Re < 1000$ (Driels & Ayyash, 1976). Using injected dye to demonstrate an apparently laminar flow in a helicoidal flow system, Kuo and Kovasznay (1981) demonstrated drag reduction in this type of flow. Polyacrylamide produced a better drag reduction in oscillatory flow compared to steady flow (Barnes, 1969). Yamashita *et al.* (1983) have recently reported that a polyacrylate increased the rolling velocity in a rolling ball viscometer, where $Re \simeq 100$. However, perhaps the findings of Noselevich *et al.* (1979) are the most important with respect to a possible biomedical application of the polymer drag reduction principle. These investigators found that a poly(ethylene oxide) of $2 \cdot 10^6$ daltons enhanced aqueous flow through a column of sand at $Re < 1$. The dimensions and geometric complexity of the interstices, as well as the operative pressure gradient, are comparable to those of the microcirculation.

E. Early Findings in Animals Suggesting the Occurence of an *In Vivo* Hemodynamic Phenomenon Related to Polymer Drag Reduction

Bove *et al.* (1969) showed that a 50% Hypaque radiopaque solution increased flow by 40% under a constant pressure gradient when 30 ppm of poly(ethylene oxide) was added to this solution. This appears to be the first biomedical application of polymer drag reduction. However, the study of drag reducing polymers in living animals was initiated in the mid-1970's independently in two laboratories, one at the University of Akron and the other at the University of Chicago.

1. The University of Akron group: Studies on post-stenotic flow disturbances and atherosclerosis using Separan AP-30.

In Akron, Ohio, Green, Mostardi and colleagues apparently assumed that *in vivo* flow could only be increased with polymers by dampening the energy loss occurring in turbulent regions of the vasculature. With this in mind, they produced a dog model of aortic stenosis and demonstrated a lessening of turbulence distal to the stenosis after the intravenous injection of the drag reducing agent, Separan AP-30, an anionic polyacrylamide with an "average" molecular weight of about $4 \cdot 10^6$ daltons. Based on their hot film anemometry work, Mostardi *et al.* (1976) concluded that flow disturbances in the vasculature decreased with the addition of a long-chain anionic polyacrylamide.

Because it was widely acknowledged that atherosclerotic plaques tended to develop at vascular sites adjacent to turbulent or disturbed flows, and there was likely to be some causal relationship between the

two, the Akron group designed experiments to test the hypothesis that Separan AP-30 might be a protective agent against the development of atherosclerosis. Rabbits on high cholesterol diets injected with the polymer tri-weekly were reported to show markedly less fatty plaque formation along the length of the aorta than rabbits fed the same diet not receiving injections. Although the protective effects of the drag reducing agent appeared to be striking, little information was given of a quantitative nature nor was it clear upon what basis the photographs of the open and stained aortae were selected (Mostardi et al., 1978).

2. The University of Chicago group: Studies on cardiac output using an okra extract, rhamnogalactogalacturonan

In late 1974 preparations were begun in Chicago to test the effects of an okra gum extract, previously shown to be a drag reducing agent when added to blood in vitro (Castro, 1972), on canine and rodent hemodynamics.

Castro had shown in pipe flow that an okra extract, presumed to be a polysaccharide by the researcher, reduced drag in the flow of various aqueous solutions and of blood at supracritical Re. Flow was also shown to increase under constant pressure gradients when the extract was added to fluids passing a pipe constriction where flow is separated. These effects occurred at additive concentrations too small to make detectable increases in viscosity. Although other polymers had been reported to enhance in vitro blood flow (see Table 4), it was decided that okra extract would be tried in vivo soon after the

appearance of the report by Voitkounsky et al. (1972), who claimed improved flow at low Re in oscillatory flow. Unknown to the Chicago group, an okra extract had been injected into bled dogs over two decades earlier (Benjamin et al., 1951) for reasons unrelated to drag reduction. This study had been made in an attempt to find an inexpensive and abundant plasma substitute to replace dextran, which at that time could be produced only in limited quantities. It is of interest that Benjamin, who was a surgeon with considerable experience in the use of plasma expanders, described the effects of the okra extract as therapeutically "startling." It might be conjectured that what had startled Benjamin was the additional drag reducing effect of the extract in addition to the volume expansion. Inspection of the extraction protocols used by Castro, Benjamin et al., and later Polimeni et al., arrived at independently, suggests that the same polysaccharide was the active ingredient in each case.

The first crude extracts used in Chicago in dog and rat studies yielded encouraging results, but the results were not consistent and indicated that harmful side-effects were produced. Further purification was necessary. Using the rat hemodynamic model as a bioassay system to guide continued purification, a small amount of purified material was finally produced with the advice and assistance of J.A. Cifonelli, a polysaccharide specialist. Using an electromagnetic flow probe on the ascending aorta, cardiac output was demonstrated to increase markedly upon injection of 5 mg/kg of this material in the methohexital-anesthetized rat model (Fig. 3). Heart

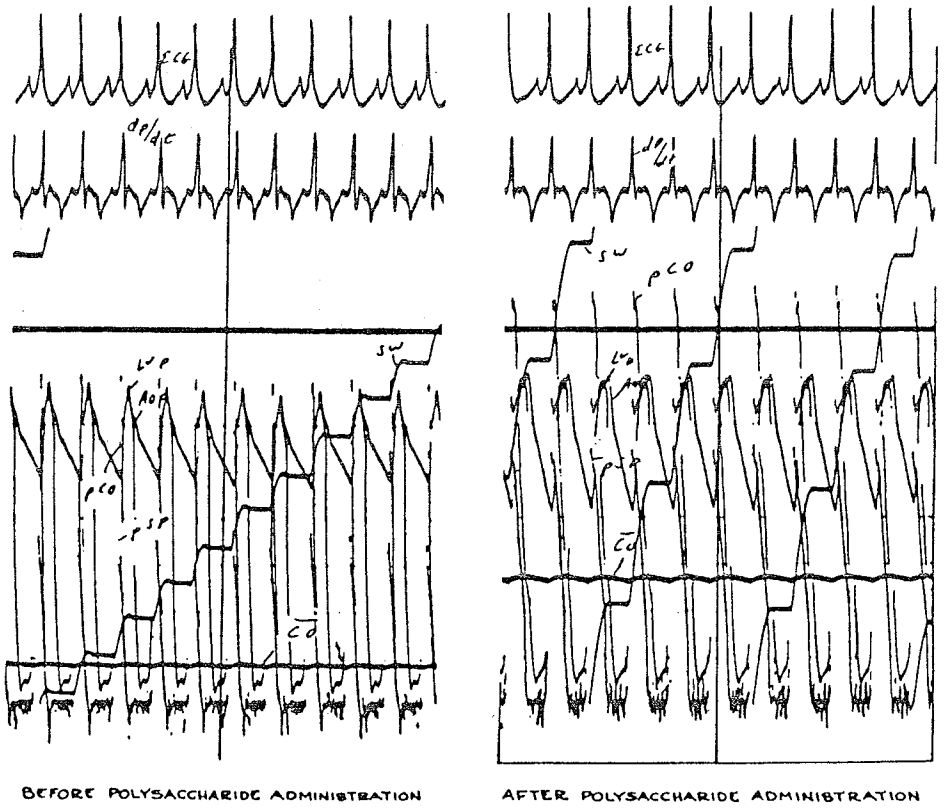

Figure 3. Hemodynamic response to rhamnogalacto-galacturonan (RGGu) in barbiturate-anesthetized, open-chest rats. The hemodynamic variables shown are the electrocardiogram (ECG), first derivative of left ventricular pressure (dP/dt), left ventricular pressure (LV_p), aortic pressure (Ao_p), peak (pCO) and mean ($\overline{CO}$) cardiac output, peak stroke power per beat (pSP), and stroke work per beat (SW). Cardiac output increased about three-fold at a dose 8 mg/kg. Modified from Polimeni et al., 1979.

rate, electrical activity, left ventricular dP/dt, and peak left ventricular and aortic systolic pressures remained near normal, but end-diastolic blood pressure fell markedly (Polimeni *et al.*, 1977, 1978, 1979). In another variation of the *in vivo* model, the polymer was injected into a rat with a low cardiac output due to cardiogenic shock. In this case, cardiac output, left ventricular dP/dt, peak left ventricular and aortic systolic pressures markedly rose (Fig. 4). In the hypotensive rat the change in LV dP/dt is presumed to be due to improved coronary blood flow concommitent with the enhanced cardiac output and elevated blood pressure, not to a positive inotropic effect.

In similar experiments on hypertensive rats, or rats with artificially produced ascending aortic stenosis, the only difference between results obtained in control and test animals was an expected small increase in left ventricular pressure (Table 5). In order, to negate the possibility that cardiac output was increased simply by the volume of polymer solution being added to the blood, similar experiments were done with volumes of saline larger than those used for the polymer. These experiments produced only small and transient changes in the rat model.

Analysis of the hemodynamic data clearly indicated that the most important variable altered by the okra extract was peripheral resistance. The crucial question in attempting to elucidate the mechanism of action was whether or not this reduced resistance was caused by a hitherto unknown *in vivo* drag reducing effect, or by the well-established mechanisms of vasodilation or hypoviscosity. Blood

SHOCK

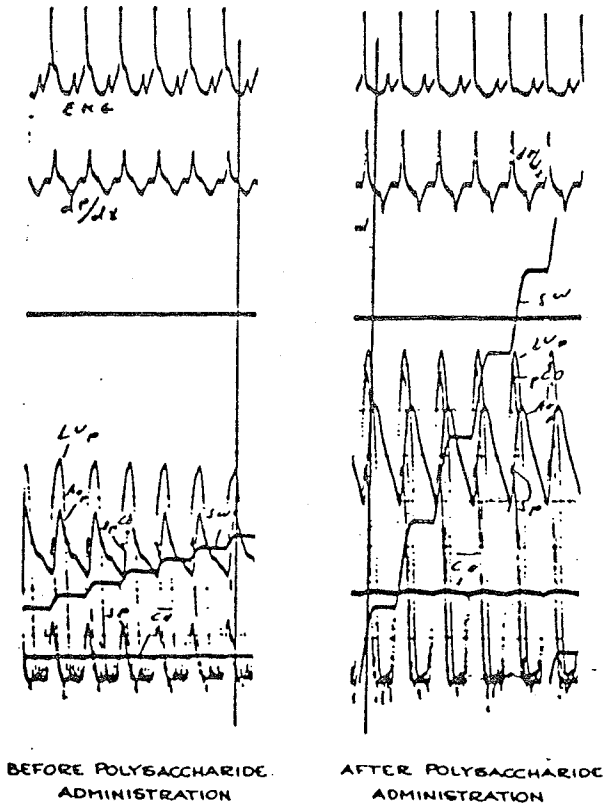

Figure 4. Hemodynamic response to rhamnogalactogalacturonan in barbiturate-anesthetized, open-chest rat in cardiogenic shock. From Polimeni et al., 1979.

SUBSTANCE AND ANIMAL STATE	FUNCTION								
	HR	LV_p	dP/dt	$\overline{CO}$	pCO	pSP	SW	dF/dt	Imp
Polysaccharide									
Normal rats	0	0	0	3	2	2	2	2	−1
L.V. Banded rats	0	1	0	3	2	2	2	1	−1
S.H.R. rats	0	1	0	3	2	2	2	2	−1
Phenoxybenzamine									
Normal rats	0	−1	0	1	2	3	1	3	*
S.H.R.	0	−1	0	0	1	0	−1	1	−1

*Not measured

SUBSTANCE	FUNCTION							
	HR	LV_p	dP/dt	$\overline{CO}$	pCO	pSP	SW	dF/dt
Propanolol	−1	1	−1	0	1	1	0	−1
Polysaccharide	0	0	0	2	2	2	2	2
Methoxamine	0	1	0	0	−1	2	2	0
Polysaccharide	0	0	0	2	3	2	2	2

Table 5. Relative effects of RGGu on certain circulatory functions of normal (barbiturate-anesth., open-chest), aorta-constricted (L.V. banded), and spontaneously hypertensive (S.H.R.) rats. The abbreviations HR and dF/dt refer to heart rate and flow acceleration, respectively; "Imp" refers to impedance and all other abbreviations are as in Fig. 3. The numbers -1, 0, and 1 through 3 refer to a small diminution, no change, and minor, modest, and marked increases, respectively. Modified from Polimeni et al., 1979.

viscosity effects were excluded as an explanation of the hemodynamic data in studies of blood viscosity before and after injection of the purified okra extract over a wide range of shear rates. On the contrary, a small increase in viscosity was detected with a Wells-Brookfield cone/plate viscometer (Polimeni and Al-Sadir, unpubl. obs.). Vasodilation could not be then excluded, but the hemodynamic effects of phenoxybenzamine, an adrenergic blocker and vasodilator, differed from those of the okra extract in showing a much smaller increase in cardiac output associated with lowered LV peak pressure. More extensive studies on the effects of vasodilators on rat cardiac output (e.g., Vetterlein et al., 1979) have shown the same thing, viz., vasolidators increase the rodent cardiac output only moderately (25%) at best.

In two experiments carried out in the laboratory of R. Replogle, the effects of the okra extract on the mesentery microvasculature were studied by microscopy with electronic measurement of vessel diameters. Upon injection of the okra extract venular diameters increased about 30% while blood flow velocity obviously increased, but arteriolar diameters were not altered. The venular response was attributed to a passive dilatation secondary to increased inflow, and this appeared to occur mainly in the vicinity of the capillaries (Polimeni et al., unpubl. obs.)

Castro's in vitro studies and the hemodynamic effects observed in Chicago were compatible with the hypothesis that the okra extract acted in vivo in a manner related to polymer drag reduction. But to maintain

this argument it was necessary to show that the molecule had the physical characteristics required to reduce drag. That is, the molecule had to be extremely long, with relatively few side chains, and in the extended conformation in solution.

It was clear from the chemical literature that the composition and conformation of okra gums varied, depending on the source of the okra. When one batch of okra was ineffective in augmenting cardiac output, the problem was traced to a change in the source of the vegetable that was normally obtained from the Tennessee region (Polimeni, personal communication). When the extracted material was eventually purified and subjected to gas chromatography, the components of the molecule were found to be the sugars rhamnose, galactose, and galacturonic acid. Electrophoretic studies indicated that the molecule had negative charges, attributed to the galacturonic acid moiety. The rhamnogalactogalacturonan (RGGu) could not pass a molecular sieve which permitted passage of globular molecules less than one million daltons in weight. Electron microscopy (Fig. 5) showed the polymer to be linear, spanning the diameters of at least several erythrocytes, and lacking side-chains. The fact that negative charges were distributed along the length of the molecule made it highly unlikely that the observed extended conformation was merely an artifact of fixation. After the active substance in okra was identified, it was realized that the polymer had been previously described by Whistler and Conrad (1954), who, working in Indiana, may have obtained their okra from the same regional source as the Chicago group.

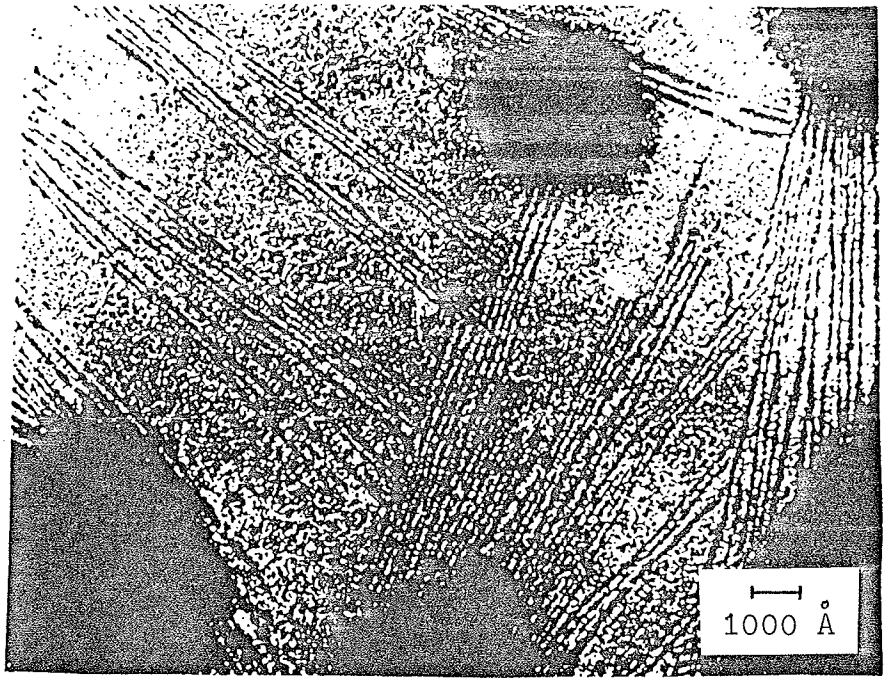

Figure 5. Electron micrograph of RGGu molecules or molecular aggregates prepared by phosphotungstate negative staining technique and magnified 140,000-fold (K. Levine and P.I. Polimeni, unpubl. obs.). The linear conformations of the molecular structures are apparent; the large circular figures are staining artifacts.

Several other known polymer drag reducing agents were tested *in vivo* by the above researchers in the hope of finding additional hemodynamically effective polymers. Of those tested, including a deoxyribonucleic acid derived from roe, poly-methyl-L-glutamate and the gums tragacanth, guar, locust bean, karaya and xanthan, none increased cardiac output. However, these negative findings do not argue against an *in vivo* polymer drag reduction effect, because of the agents tested only RGGu had the extraordinary long length that appears to be necessary for effective *in vivo* drag reduction.

A commercial pharmaceutical laboratory, Arnar-Stone Laboratories in Illinois, undertook preliminary steps to test RGGu further. Extraction of RGGu was successfully carried out by R.J. Gorczynski, who confirmed the hemodynamic effects of RGGu, but unfortunately Arnar-Stone too was unsuccessful in producing RGGu in quantities sufficient for large animal studies. Thus, the problem of increasing the yield was assigned to Pfanstiehl Laboratories, also of Illinois, as this company had considerable experience with polysaccharides. After a year's work with no end to the problem in sight Arnar-Stone ceased further investigation. Along with the fact that few natural linear polymers exist with the extraordinary lengths apparently required for *in vivo* flow enhancement, the main roadblock to adequate sources of these polymers is the great difficulty of preventing denaturation during the extraction process. Extraction of large polymers from natural sources usually results in molecular degradation and tertiary deformation (MacGregor & Greenwood, 1980).

F. Recent Animal Studies with Renatured Xanthan, Separan AP-30, Separan AP-273, and Polyox WSRN-60K

Polimeni attempted a study of one last natural polymer, a "renatured" xanthan. Previous work with native xanthan had been unsuccessful, but the renatured xanthan gave hope for success since it was much longer while retaining its linear form (Holzwarth & Prestridge, 1977). Polimeni was unable to completely dissolve the renatured xanthan supplied by Holzwarth. In collaboration with G.P. Sharma an opaque solution was injected into the porcine femoral artery, hoping that the microvasculature would filter larger particles. These experiments were done at the end of unrelated experiments. Predictably the results were not consistent, but cardiac output was increased enough to suspect xanthan was augmenting flow (Fig. 6, panel A).

Until this time, Polimeni had not attempted *in vivo* studies with artificial polymers. Although they were by far the major agents used for drag reduction in industry, it was suspected that chemicals produced on such a large scale for industrial use would be toxic when administered *in vivo*. The Akron group had claimed, however, that chronic intravenous injections of the linear anionic polyacrylamide, Separan AP-30, into rabbits had resulted in no obvious toxic sequelae.

Several informal experiments were conducted with AP-30, measuring cardiac output in dogs by a thermodilution method (courtesy of R.M. Prewitt). In some but not all cases, cardiac output increased significantly. The results were marked by the same inconsistency that had plagued the renatured xanthan work (Polimeni, personal

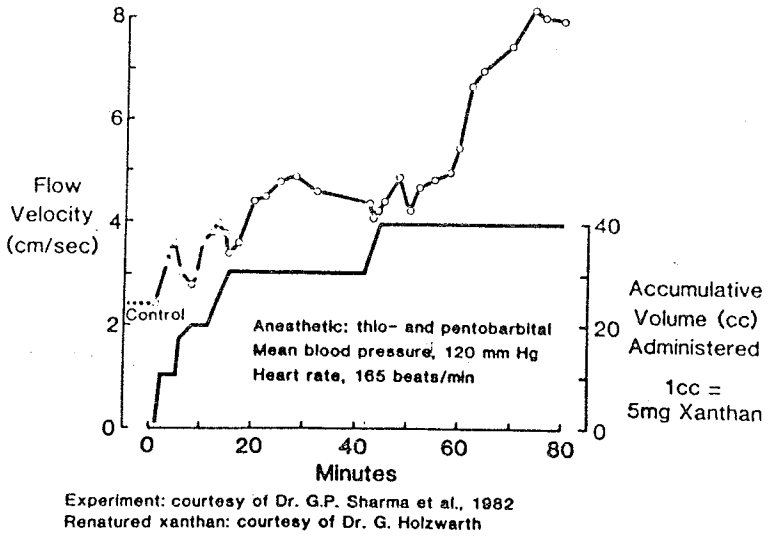

Figure 6A. Increased aortic blood flow velocity in dog after administration of renatured xanthan.

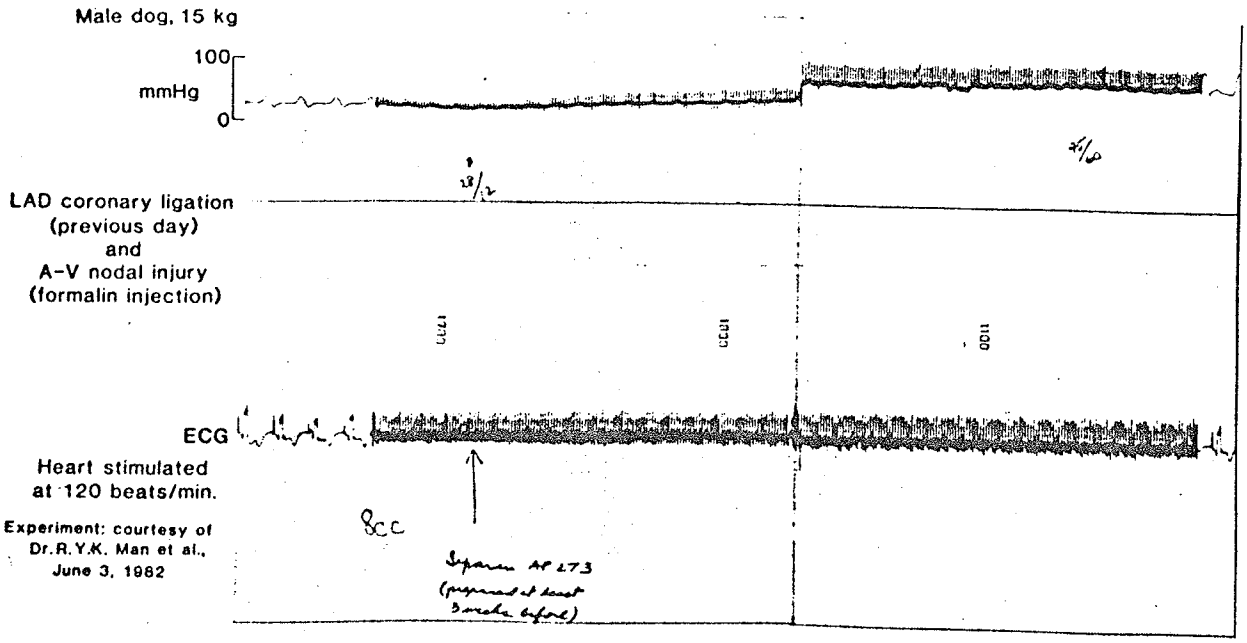

Figure 6B. Elevation of arterial blood pressure in canine cardiogenic shock using 10 ml of 0.02% Separan AP-273 solution.

communication). However, the fact that marked increases in cardiac output were sometimes seen with two polymers other than RGGu was intriguing and not easily explained. The negative results might simply be due to an inadequate understanding of the conditions that are necessary for the polymers to be effective in vivo.

Another anionic polyacrylamide of great length, Separan AP-273, was tried. The polymer was injected into dogs at the end of unrelated experiments in which the left anterior descending coronary artery was ligated and formalin had been injected into the A-V node. Blood pressure, but not cardiac output, was measured in these informal studies. At the time AP-273 was injected, the animals were normally in low output shock. Soon after injection, however, blood pressure would sometimes rise. This was best illustrated in one experiment when blood pressure rose from systolic/diastolic pressures (mmHg) of 28/12 to 100/60 during a one hour period (Fig. 6, Panel B). Never did blood pressure rise from its low output shock status without administration of Separan.

The preliminary results with Separan AP-273 were encouraging, if negative experiments could be ignored as being due to a poor understanding of some critical conditions that had to be met. Given the conformational requirement for polymer drag reduction to be possible, it did not seem far-fetched to consider the failures to be due to subtleties of drug preparation and administration. The first problem to be tackled, was to prepare a polymer solution that gave consistent results. Only when this goal were achieved, would it be

possible to study putative in vivo polymer drag reduction. The second stage was to establish the hemodynamic effect of Separan AP-273 in an operative sense, comparing these effects with those of RGGu. Because any agent capable of markedly augmenting cardiac output has obvious therapeutic possibilities, particularly if such augmentation can be produced while diminishing cardiac afterload, there are numerous practical questions that can be raised concerning possible drug toxicity. It must be emphasized, however, that the primary goal is to demonstrate that a second polymer drag reducing agent can improve flow while reducing total peripheral resistance. Given the extremely low probability that two macropolymers of similar physical properties -- but totally dissimilar chemical composition -- would cause similar hemodynamic effects unrelated to their physical structures, the demonstration of similar hemodynamic responses to the two polymers would be strong evidence in favor of our basic thesis: Some drag reducing polymers cause marked improvement of in vivo blood flow by some mechanism related to the Toms phenomenon. We shall see below (Discussion section) that most recently a third linear macropolymer previously shown to reduce drag in in vitro blood flow, poly(ethylene oxide), has now been shown to markedly increase cardiac output (Polimeni and Ottenbreit, personal communication).

II. MATERIALS AND METHODS

A. Materials

1. Preparation of 0.2% Separan AP-273 Solutions

Rat cardiac output is postulated to increase when blood flow disturbances are dampened by the presence in blood of linear polymer chains of sufficient length to provide drag reduction (Polimeni et al., 1977). The paramount problem to overcome in this project was to prepare a solution of Separan AP-273 (Dow Chemical Co., Midland, Michigan) at a polymer concentration suitable for i.v. injection that would increase cardiac output. To achieve this end many "trial and error" experiments had to be performed, and thus the rat hemodynamic model became, in effect, a bioassay system to test the efficacy of any changes made to the polymer solution preparation. It was, therefore, critical to prepare a solution of Separan AP-273 in which:

a) the solvent used to solubilize the solid polymer is a vehicle suitable for i.v. injection,

b) the viscosity of the polymer solution is not unduly high,

c) the linear structure of the polyacrylamide chains is maintained without polymer entanglement,

d) a sufficient fraction of the linear polymer chains are long enough to induce drag reduction in blood.

2. Solubilizing Separan AP-273

This anionic polyacrylamide is a highly hydrophilic substance, dissolving readily in 145 mM NaCl (0.9% saline, Fisher Scientific Co., Fairlawn, New Jersey). However, the process of polymer dissolution is complex, the dry polymer powder initially swelling in the saline solution to form partially wetted powder lumps. Once these lumps are dispersed they continue to swell and form a gel in which the polymer chains remain in contact with one another, forming a loose structure within which the solvent moves freely (MacGregor & Greenwood, 1980). It is necessary to reduce these polymer-polymer interactions with further addition of the solvent and some form of agitation so that solvent-solute interactions are favoured, i.e., each polymer molecule should be surrounded by a layer of solvent molecules. At this point the polymer is truly in solution with homogeneous macro-ion dispersal.

A method recommended by Dow (Dow product bulletin #192-841-1281) was used to prepare the polymer solution: the investigator blew into a chilled glass 250 ml beaker to form a layer of condensate onto which a preweighed 0.2 g sample of Separan (lot # MM81072 ONI) was sprinkled. The chilled beaker was rotated several times. Premeasured diluent, 100 ml 145 mM NaCl, was poured down the sides of the beaker to minimize lump formation.

The need to agitate the partially dissolved polymer presents the investigator with a dilemma. Separan AP-273 is a polydisperse polymer with molecular weights ranging from 10^5 to over $5 \cdot 10^7$ daltons and a weight-average molecular weight of $6 \cdot 10^6$ daltons (see Fig. 7).

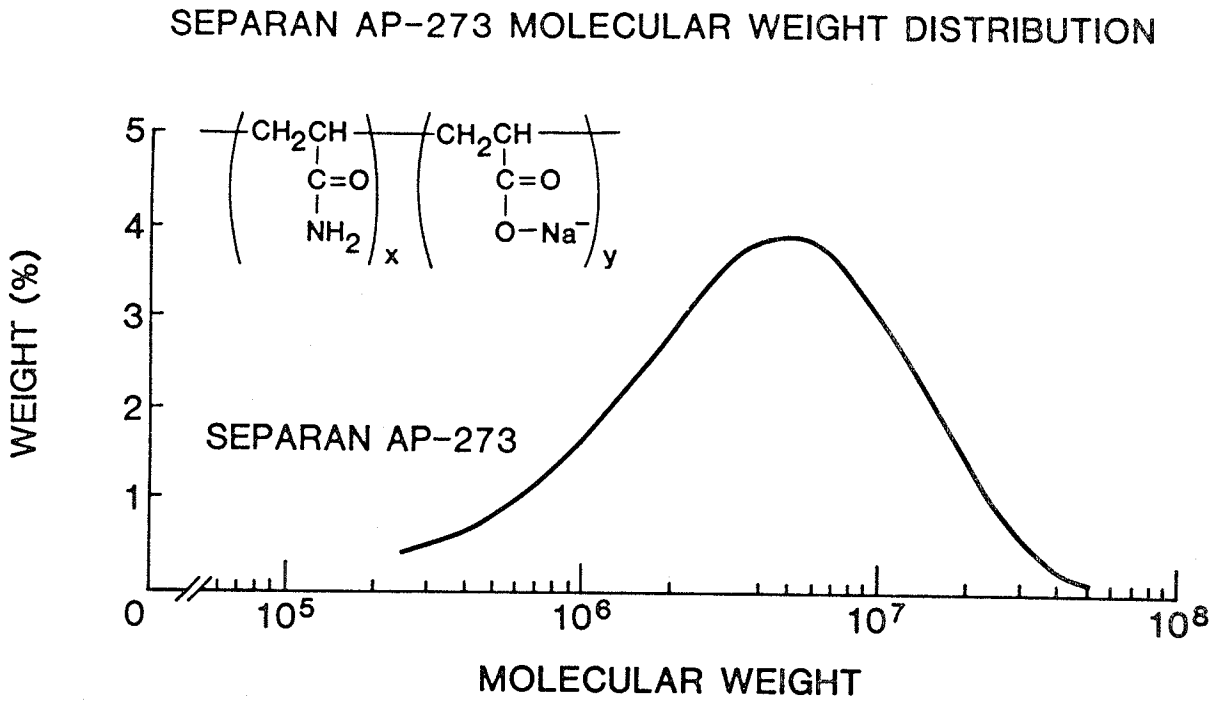

Figure 7. Separan AP-273 chemical composition and molecular weight distribution. Modified from "Separan Polymers," Dow Chemical Co., 1981.

These molecules, especially those at the higher end of the molecular weight distribution are particularly susceptible to shear degradation. Unfortunately, it is precisely these longer molecules that are believed to be the most effective drag reducing agents. It was determined with the hemodynamic bioassay method that orbital shaking (Labline Instruments Inc., Melrose Park, Illinois) for 5 days at 70 rpm at ambient temperature was suitable. In the earlier work the polymer solution was protected against light, but later it became apparent that this was an unnecessary precaution.

3. Removal of contaminants from the Separan solutions: Protocol I

It was considered that Separan AP-273, produced in mass quantities for industrial use, might be contaminated with heavy metals and other unknown substances. In an attempt to remove any contaminants that might be present, the 0.2% polymer solutions were subjected to exhaustive dialysis in the presence of the heavy-metal chelator Na_2-EDTA and $CaCl_2.2H_2O$ (Sigma Chemical Co., St. Louis, Missouri), the latter used to stabilize the macromolecular structure. The entire 100 ml of 0.2% Separan was poured into a washed cellulose dialysis bag (Spectrapor 2, 12,000 to 14,000 M.W. cut-off, Spectrum Medical Industries Inc., Los Angeles, California), the bag was tied and immersed in 2 liters of a solution containing 145 mM NaCl, 5 mM Na_2-EDTA, and 6 mM $CaCl_2.2H_2O$; the pH was adjusted to 7.40 (Fisher Accumet model 620 pH meter). The dialysate, stirred continuously, was replaced twice and dialysis was assumed to be nearly

complete after 7 days. With this procedure, assuming complete equilibration between the dialysate and polymer solution, any contaminate present in the polymer retentate would be reduced to 0.0125% of its original concentration. Separan solutions dialyzed in this manner consistently increased rat cardiac output.

4. **Removal of contaminants from the Separan solution: Protocol II**

It is critical, for drag reduction to occur, that long polymer molecules maintain their linear conformation in solution and not become entangled or assume a spherical or random coil shape. To this end, the pH of the solvent is important to optimize polymer-solvent interactions and reduce polymer-polymer entanglements by maintaining anionic charges dispersed along the entire length of the Separan molecule. Linear macromolecules of like charge will not only repel one another, but they also tend to assume rigid, rod-like shapes in solution. For these reasons, a more basic pH of 7.8 was chosen and seemed to yield good results.

Dialysate pH was adjusted with 10 mM HEPES (N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid, United States Biochemical Corp., Cleveland, Ohio). This substance is considered to be one of the least toxic pH buffers known (Shipman, 1969; Good, 1980). Eventually use of the EDTA chelator was discontinued, and calcium concentration was reduced to 1 mM. Thus the dialysate (and polymer vehicle) composition became 145 mM NaCl, 10 mM HEPES, 1 mM Ca at pH 7.8.

Separan AP-273 solutions, including those used for injection, are characterized by high viscosity and pituitousness, i.e., the solution is "thready". The viscosity of the Separan solution just prior to dialysis is 16 cP at a shear rate of 120 sec^{-1}, but falls to about half that within a week of dialysis (Polimeni and Otten, unpubl. obs.). The solution is non-Newtonian. In aqueous and particularly in saline high molecular weight polymers show "aging" effects, defined as a steady decrease in viscosity with time. These effects are generally believed to be physical, relating to the disentangling or disaggregation of the long polymer chains (Narkis & Rebhun, 1966; Shyluk & Stow, 1969). Buffered Separan solutions as old as one year gave consistent increases in cardiac output in our laboratory. Little or no chemical decomposition is believed to occur in buffered polymer solutions at slightly basic pH. Polyacrylamide solutions are particularly resistant to microbial degradation and thus no antibacterial agents need be added.

5. Preparation of ultrafiltrated Separan solution

In an attempt to bias the molecular weight distribution towards the heavier (i.e., longer) macromolecules, and filter out any residual acrylamide or oligopolymer fragments, several experiments were conducted with a Separan solution circulated through a Minitan system filter (Fig. 8). The major component of the system is an array of molecular filters, sandwiched alternately with thin-spaced separators, packed between two acrylic manifolds held together by end stainless

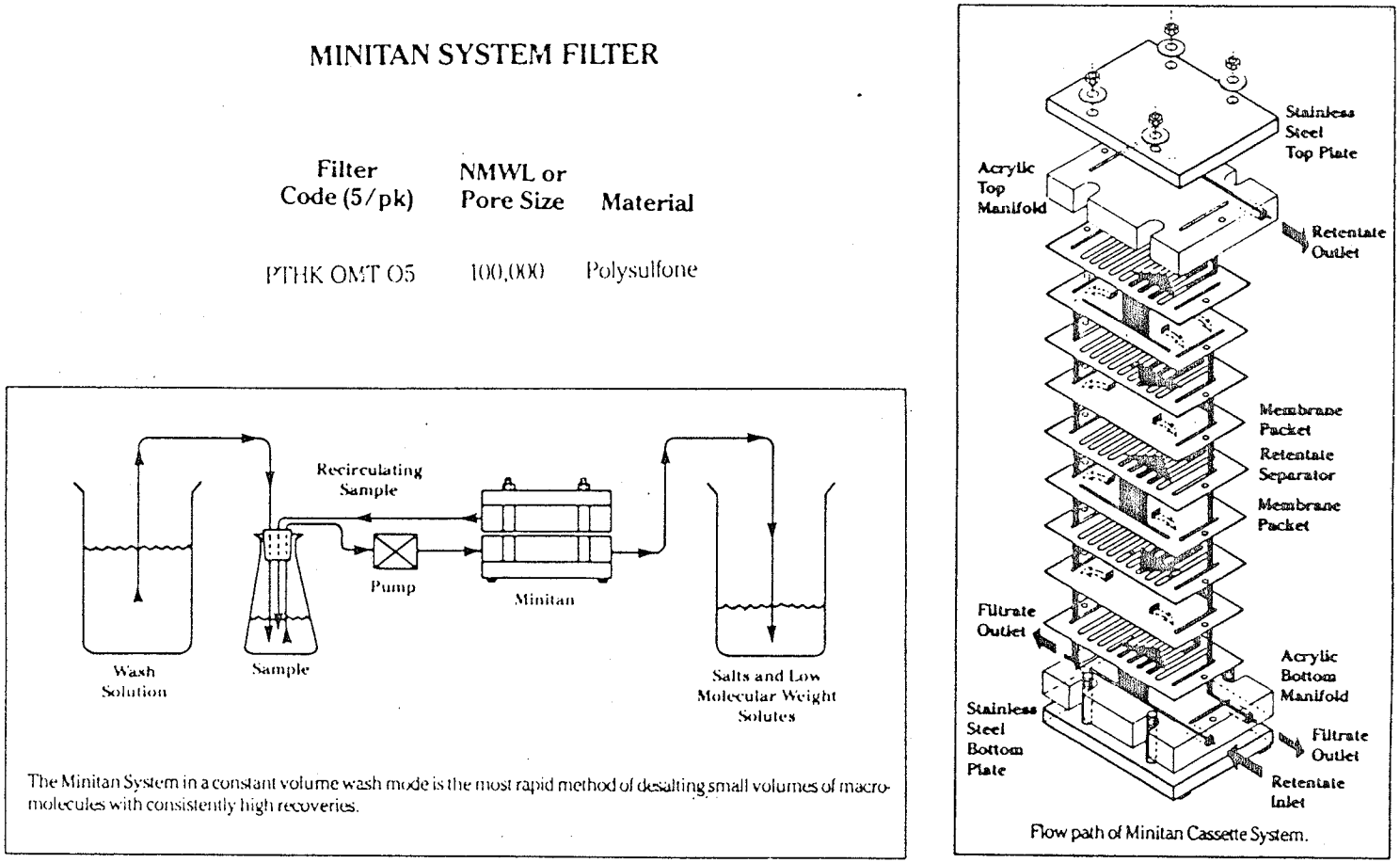

Figure 8. Minitan system filter system. The molecular filtration system is in the constant volume washing mode using a membrane with a nominal molecular weight limit rated at 100,000 daltons. Modified from "Separation and Concentration Applications with Millipore Ultrafiltration Systems," Millipore Ltd., 1983.

steel plates. The system of membrane filters were configured in a manner that resulted in a serpentine pathway for the retentate. After trying several filters, the filters eventually chosen had a nominal molecular weight limit of about 100,000 daltons. Polymer solution was pumped from an Erlenmeyer flask into the filter cassette under a pressure of 0.7 kg/cm^2. The retentate was returned to the flask and the filtrate discarded. The level of polymer solution in the flask was maintained with an inflow of wash (i.e., polymer vehicle) solution. Although a limited number of experiments were performed with the filtered solutions and some insights were gained, on the whole this aspect of the research was disappointing. The theoretical and practical difficulties will be discussed in the Results section.

B. Methods I: Instrumentation and Calibration

1. Hemodynamic recording system: Instrumentation

An eight channel direct writing recorder (model 4568C, Hewlett Packard, Waltham, Massachusetts) fitted with an oscilloscope monitor (model 1308A) had eight channels arranged as follows: Channels #1 and #2 were kept free to use as reference markers for peak and trough aortic blood flows registered on channel #6 (amplifier model 8809A); channel #3 (model 8805C) was assigned for left ventricular pressure; channel #4 (model 8805C) was used for arterial (carotid) pressure; channel #7 (model 8811B), for the electrocardiogram; and channel #8 (model 8813A), for the first derivative of left ventricular pressure with time. Signals from interfaced probes were visualized on the

oscilloscope monitor and recorded on photorecording paper (#9270-0914 paper, developed with Hewlett Packard Rapid Developer solution #8500-0894). All hemodynamic signals were recorded prior to infusion of either Separan AP-273 or solvent solutions for "control values." Additional tracings at a film speed of 200 mm/sec were made at various post-infusion time intervals, depending on the experiment. A final recording for each experiment was made for 5 sec at a film speed of 10 mm/sec, then 1 ml saturated KCl solution was injected directly into the left ventricular chamber to establish the "zero" baseline for all recorded variables. Each rat was weighed after death to confirm that no significant weight loss had occurred during the experiment.

A schematic representation of the hemodynamic recording system is given in Figure 9.

2. Calibration of aortic flow probe

Cardiac output was measured using an electromagnetic flow probe (model EP-100) with a 2.0 mm internal lumen diameter attached to a square-wave electromagnetic flowmeter (model 501, Carolina Medical Electronics Inc., King, North Carolina). The flowmeter was calibrated to determine the "probe factor" and to match flowmeter gain and probe sensitivity. The details of the procedure are given in the instrument manual (CME Electromagnetic Flowmeter, Model FM501, 1979). Briefly, blood was pumped at a constant rate through a segment of rat thoracic aorta with its intercostal branches tied closed. A flow probe was attached around the vessel, which was immersed in a saline bath at

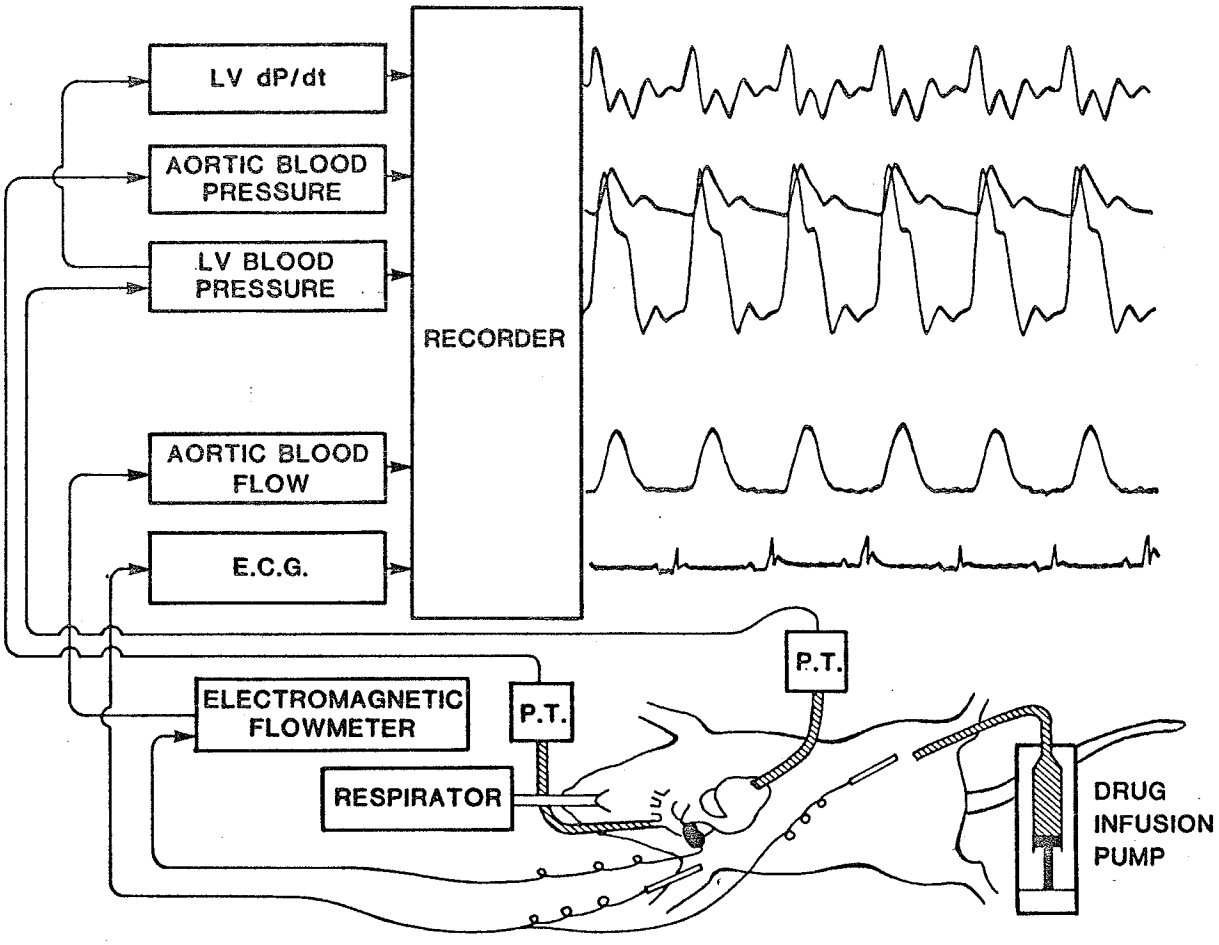

Figure 9. Rat hemodynamic recording system. The anesthetized animal was placed on a respirator through a trachial incision. Right carotid and LV blood pressures were measured via polyethylene catheters attached to pressure transducers (PT) and amplifiers. A standard differentiator converted the LV pressure signal to a first derivative pressure reading (LV dP/dt). The ECG was registered from standard RA-LL leads. Cardiac output was approximated by placing an electromagnetic flow probe with a 2.0 mm internal diameter on the ascending aorta, connected to a signal coupler via a square-wave flowmeter. The hemodynamic variables were followed on a monitor during the experiment and registered photographically. Separan solution or solvent were infused into the left femoral vein with a syringe pump.

ambiant temperature. Calibrations made at about 25°C are valid for 37°C. The rate of flow was verified volumetrically. In one calibration session blood flows with and without Separan present were compared to exclude the possiblity of a polymer-induced artifact. It was considered that anionic sites on the macro-ion might exaggerate the probe response as these sites traversed the electromagnetic field. A small (~5%) increment was in fact observed, but no correction is made for this effect.

Recorder channel #6 received the signal from the flowmeter via a signal-coupler. When not in use, the probe was cleaned and stored in 0.9% NaCl. Before each use the flow channel was balanced and nulled as follows: With the mode dial on "standby" air bubbles trapped in the probe lumen were first tapped free. The mode dial was then set at "balance" and the flowmeter analogue scale was adjusted to read zero. With the mode dial set to "null" the null control dial was rotated until the flowmeter scale read 0.5 ml/min. After calibration, the mode dial was returned to the "standby" position.

3. Calibration of the arterial and left ventricular pressure transducer probes

The arterial pressure and the left ventricular pressure transducer (model P23 GD and model P23 ID, respectively, Gould Instruments International, Oxnard, California) were interfaced to the recorder and calibrated with a mercury manometer. Zero pressure was set on the monitor and recorded on film with each transducer open to the

atmosphere. With the "USE/OFF/CALIBRATE" dial on each amplifier set at "USE" the beam was examined for deflection throughout its entire range. Adjustments were made at each range to ensure that the beam was zeroed. With a preferred range chosen ("25") each transducer was subjected to 100 mmHg pressure and this span produced by the manometer was set to scale on the monitor and on film. The probes were calibrated and ready for surgical implantation.

C. Methods II. Surgical Procedures and Implantation of Hemodynamic Probes

1. Anaesthesia

Sprague-Dawley female rats (N=98) between 200 and 300 g were kept isolated for two weeks after arriving at the University of Manitoba's Central Animal Care facility to adjust to their environment. After weighing, each rat received an initial intraperitoneal injection of sodium pentobarbitol (lot # 6812, Allen and Hanbury, Toronto, Ontario), 40 mg/kg body weight. The barbiturate was prepared as a 40 mg/ml solution by dissolving 4 g sodium pentobarbitol in 40 ml propylene glycol (Sigma Chemical Co., St. Louis, Missouri), 10 ml 95% ethanol, and 50 ml 0.9% NaCl. Pentobarbitol was the anaesthetic of choice because anaesthesia could be maintained for a long duration with minimal attention and pentobarbitol is a cardiodepressant well suited to the type of hemodynamic model that was desired.

A constant level of anaesthesia was maintained throughout each experiment with abdominal subcutaneous injections (0.2 ml) of a 10 mg/ml solution of sodium pentobarbitol at 15 min intervals 75 min after the first flow reading was obtained. The volume of anaesthetic injected was sufficient to maintain fluid balance. The rate of body fluid loss, which is relatively high in the open-chest animal, was previously determined by weighing each rat before and after experiments.

2. Respiration

The respirator (Harvard Apparatus rodent respirator model 680, Ealing Scientific Ltd., St. Laurent, Quebec) was adjusted at a frequency of 80 strokes/min with approximately a 3 ml stroke volume. To minimize dehydration of the rat during the experiment, the respirator's intake duct was connected to a 100 ml Erlenmeyer flask half-filled with distilled water. Another tube kept an open flow between the inside of the flask and the atmosphere, thus humidifying the air.

A midline incision extending from the level of the diaphram to the base of the neck was made through the skin on the rat's ventral side. A small piece of plastic sheeting (Saran Wrap) was placed over the incision to minimize evaporation. A tracheotomy was performed and the rat was attached to the respirator with a polyethylene cannula inserted into its trachea. The lungs and heart were then exposed by mid-line thoracotomy, and with the respiration rate held constant, adjustments were made in stroke volume to optimize respiration.

3. Aortic flow probe implantation

Once respiration was assured, the pericardium was removed from the heart and the thymus was deflected to expose the ascending aorta. Using blunt surgical procedures, the ascending aorta was isolated, the lumen of the aortic flow probe was slipped around the vessel, and the probe was secured by fixing the probe lead to the surgery board.

4. Implantation of arterial (carotid) pressure transducer probe

The carotid artery, isolated from the vagus nerve, was cannulated with a length of PE 50 tubing filled with heparinized 0.9% NaCl. The cannula was attached to the arterial pressure transducer coupled to the channel #4 amplifier.

5. Insertion of pressure transducer probe into left ventricular chamber

A 20 gauge teflon catheter was slipped through a puncture in the chest wall and inserted 3 to 5 mm into the apex of the heart so that the catheter tip was within the left ventricular chamber. Once the ventricular pressure of the beating heart forced blood to the distal end of the teflon catheter it was immediately attached to the left ventricular pressure transducer (model P231D, Gould Inc., Oxnard, California) filled with heparinized saline.

6. First derivative of left ventricular pressure with time (LV dP/dt)

The signal for left ventricular pressure was converted by a standard differentiator to a first derivative pressure reading (LV dP/dt, channel #8).

7. Electrocardiography

The ECG leads (14148A, Hewlett Packard, Waltham, Massachusetts) were interfaced to the channel #7 amplifier, one lead was inserted subcutaneously into the rat's right foreleg, one lead was inserted in a similar manner into the left hind leg, and the third lead connected the right leg to ground via a nearby metal object.

8. Femoral vein cannulation for infusion

An incision exposed the left femoral vein, which was isolated and covered with plastic sheeting before the other surgical procedures to implant the hemodynamic probes were complete. Once several stable cardiac output readings were recorded from the rat, a 25 gauge butterfly needle was inserted into the vein. The needle was connected via a length of polyethylene tubing to a 5.0 ml syringe filled with the polymer solution or the solvent control solution. Solutions were infused using a syringe pump (model 341, Sage Instruments of Orion Research Inc., Cambridge, Massachusetts) at an infusion rate of 0.25 ml/min.

D. Methods III. *In Vitro* Separan AP-273 Experiments

It is well known that very high molecular weight polyacrylamides, such as Separan AP-273, are among the few polymers capable of reducing drag in blood flow through straight pipes. The four polymers -- including Separan -- hitherto applied to demonstrating the Toms phenomenon in blood all were used under conditions of supracritical Re. But can Separan, and presumably some other polymers, reduce drag in blood flows well below the critical Re when the pipe geometry is not straight? This is the question that the experimental apparatus and procedures described below were designed to address. There appears to be no answer to this question in the physics and engineering literature, where the overwhelming bulk of work on polymer drag reduction is carried out.

1. *In vitro* blood flow apparatus

To test the hypothesis that Separan AP-273 can reduce the drag of blood flowing at subcritical Re through a conduit system of even minimal geometric complexity, a two-loop tubular system was constructed as illustrated in Figure 10. The column of blood was 104 cm high, and assuming that the density of blood is 1.05 g/cm^3, the pressure gradient along the conduit was 148 mmHg. The level of blood was kept constant and each flow run, five runs for each blood sample, lasted precisely 10 sec. Application of a wire clamp permitted rapid starts and stops. The blood was collected in a tared beaker and weighed after each run. Preliminary trials established that runs could be repeated

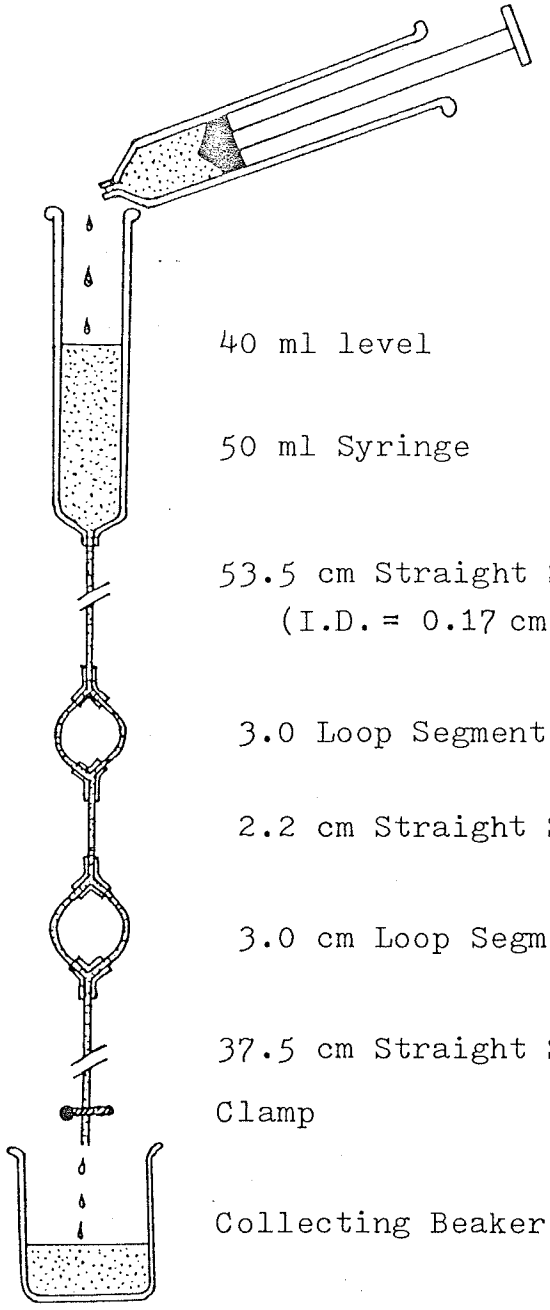

Figure 10. *In vitro* blood flow apparatus. See text for details.

to within 0.02 g. Blood samples were well mixed just before each run to avoid sedimentation, which would have resulted in heterogeneity of hematocrit and therefore viscosity. Care was taken to thoroughly clean the conduit system before changing blood samples.

2. Calculation of Reynolds number

The Reynolds number for blood flow (F) through this conduit system is approximated in the following manner. $Re = v \rho r/\eta$, where v is the mean flow velocity, ρ is blood density (assumed to be 1.05 g/cm^3), r is the tube radius (0.085 cm), and η is the blood viscosity coefficient, which is assumed to be 0.04 Poise at shear rates above 100 sec^{-1} (Whitmore, 1968). For flow through the cylindrical tubes $v = F/\pi r^2$, where F = 0.4 cm^3/sec. Therefore $Re = F \rho/\pi r \eta$, or $Re \leq 39$. Because blood is non-Newtonian and η increases at low shear rates, the calculated Re is an upper limit. It should be noted that although $Re \ll 1{,}000$, this does not mean that the flow is laminar throughout the conduit system. Reynolds' rule for the onset of turbulence is applicable only for steady flows through straight tubes. Considerable flow disturbance can be generated in the regions of the tube loops despite the low Re, and flow resistance at these sites can accordingly be expected to be greater than predicted for straight tube flow.

3. Preparation of blood-Separan mixtures

The blood used for <u>in vitro</u> flow experiments was human whole blood to which 1.0 ml heparin (Heparin sodium, 1000 USP/ml, Allens and

Hanbury, Toronto, Ont.) was added to each 100 ml blood. The blood was divided into three aliquots. One aliquot received a small volume (see below) of 0.04% Separan solution and another received the same volume of polymer vehicle solution alone. After the solutions were added to blood, the blood samples were placed on an orbital shaker and rotated at 60 rpm for 20 min at ambient temperature.

The concentration of Separan in blood just used in trial runs was approximately equal to that calculated for the in vivo concentration. Assuming that 8% of the rat's body weight represents blood of density equal to 1.05 g/cm^3, the rat's blood volume is 76 cm^3/kg body weight. Therefore, an in vivo injection dose of 2.0 mg Separan/kg rat is equivalent to the addition of 5.0 ml of a 0.04% Separan solution to 76 ml blood, or a concentration of 6.2 ml 0.04% Separan solution per 100 ml blood. This concentration of Separan decreased in vitro blood flow, as might be expected of the addition of a fluid more viscous than blood itself. But trial and error indicated that at a concentration one-sixth the equivalent in vivo dose, i.e., 1.0 ml Separan solution per 100 ml blood or 5 mg Separan/liter blood, flow through the conduit system increased. Thus the in vitro experiments were performed with blood and blood containing 1.25 ml 0.04% Separan-buffer solution or 1.25 ml buffer solution per 100 ml blood.

4. In vitro flow protocol

Blood, or blood with either Separan or buffer, was poured into the 50 ml reservoir syringe up to the 40 ml mark. Blood was run through

the conduits to cleanse the system and discarded before experiments were performed. The wire clamp at the lower end of the tubing was opened and blood collected in the tared beaker for ten seconds before the clamp was again closed. During the ten second flow period the blood level was maintained at the 40 ml mark by continually adding blood from a freely held syringe. The collection beaker was weighed to determine how much blood had passed through the tubes during the ten second flow period. Before each run blood in the reservoir syringe was gently stirred with a rod to maintain hematocrit homogeneity. Five runs were performed for each of the three samples, all obtained from a single bag of blood, and the resulting flow values averaged. A total of five bags were used exclusive of preliminary trials. Between experiments with the different blood or blood solutions the tube system was first flushed with n-saline solution and then with the samples.

E. Methods IV. Statistics

All data is described as mean $\pm$ standard deviation unless otherwise indicated. The significant differences between means were tested with the student's paired t-test with $P < 0.05$.

RESULTS

A. Effect of Intravenous Injection of Saline Solution on Aortic Blood Flow

Experiments were designed to demonstrate that simple addition of saline to the blood stream in the same volume as that used to inject Separan would not in itself cause an increase in aortic blood flow. Cardiac output was recorded and saline infusion begun only after the output was constant for at least 1 hour.

Cardiac output was considered to be stable when four consecutive aortic blood flow readings taken every fifteen minutes were within ten percent of each other.

Initially one hour of testing after infusion of saline was thought to be sufficient, but later it was discovered the open-chest rats remained stable for at least two hours. Therefore the final few rats were monitored over 120 minutes post-infusion.

Upon placement of the aortic flow probe cardiac output usually fell 20-30% within fifteen minutes, and then leveled off for the remainder of the experiment. Thus the output was generally determined within ninety minutes of thoracotomy.

Rats were placed on a respirator and fitted with an aortic flow probe. After equilibration of outflow, one set of rats were infused with 4 ml/kg saline at an infusion rate of 1.25 ml/min. Another set of rats was simply monitored for outflow after equilibration had been achieved.

Fig. 11 shows that no significant change in aortic flow occurred with the addition of saline to the rats over a two hour period. Thus simple infusion of a fluid under such conditions would not alter flow.

The rats that were not infused showed no apparent change in flow after the initial decrease immediately following thoracotomy. The only noticeable change in the saline-infused rats was a small, but not statistically significant, increase of output in the first period measured immediately after infusion (t = 15 min).

In a few experiments mentioned below, the rat was fitted with the aortic flow probe and monitored for up to six hours. In these long duration experiments the rats were carefully weighed before and after their six hour flow analysis. By noting the weight loss due to fluid evaporation from an open chest, respiration, etc., it was determined that 0.2 ml of fluid was lost every fifteen minutes. Also, the anaesthetic began to lose effect after one hour. Thus, in all further experiments, 0.2 ml saline was injected intraperitoneally (i.p.) every fifteen minutes for the first hour the rat was open chested, and 0.2 ml sodium pentabarbitol-saline solution containing 1 mg/ml anaesthetic was infused every 15 min. for the remainder of an experiment. These long duration experiments established the stability of cardiac output in the rat model, under favorable conditions, over a six hour period. However, such long duration experiments could not be repeated consistently after Separan injections when the left ventricular pressure cannula was in place, because blood frequently seeped out of the ventricle at the cannula insertion site. This phenomenon, which

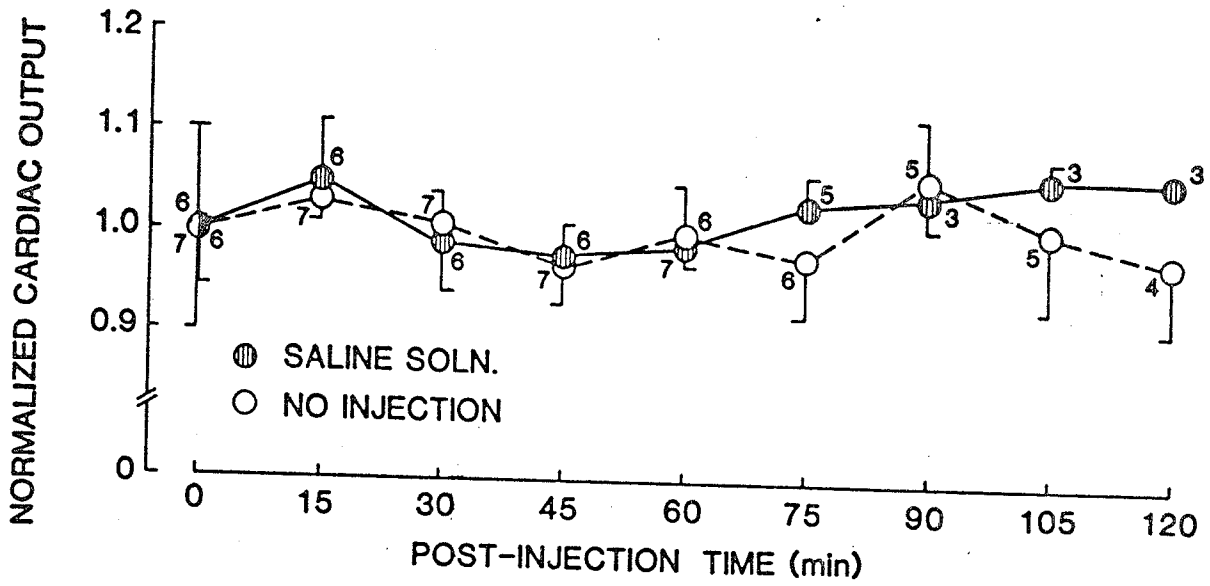

Figure 11. Effect of intravenous injection of n-saline on cardiac output. After at least one hour of stable (± 10%) cardiac outflow, one group of rats was injected with 4 ml/kg n-saline at an infusion rate of 0.25ml/min (striped circles); another group received no fluid other than that in the initial anesthetic (open circle). The numbers adjacent to the circles indicate the number of recordings made for that point.

was not seen in the absence of Separan, is perhaps indicative of a "slippery blood" characteristic reminiscent of the phrase "slippery water" sometimes found in the engineering literature.

B. Preliminary Experiments with Separan

When this thesis study of Separan began, the effect of several different factors known to generally influence pharmacological findings were unknown. Therefore, there was an initial period of trial and error.

By examining the effect that these factors had on blood flow, the rat in effect became a bioassay for the Separan. Some variables that might affect cardiac output include polymer concentration, dose, infusion rate, pH, buffer composition, dialysis membrane cutoff, temperature, and light. From the above, it is evident that a large number of experiments had to be conducted during the trial and error period to narrow the significant factors in our hemodynamic study.

These exploratory experiments had no rigid protocol and were too varied and numerous to describe. Infusion of the polymer always altered the configuration of the instantaneous flow curve by increasing the baseline-to-peak amplitude and, with rare exceptions, the width of the curve. Four general configurations were observed in these experiments, shown in panels A-F of Figure 12. In panel A, the peak increased after infusion of the polymer (curve p) compared to that of the control (curve c). The control baseline represents zero flow,

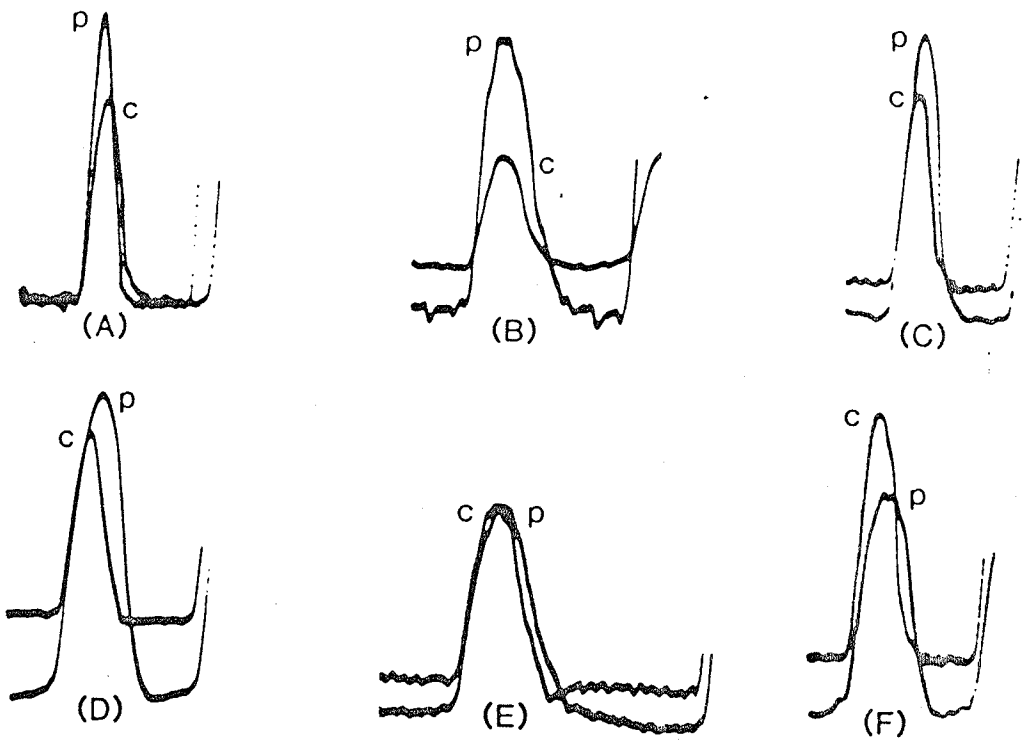

Figure 12. Instantaneous aortic blood flow recordings made in control (c) and after injection of Separan polymer (p) under various conditions in preliminary experiments. See text for details.

confirmed at the end of experiments when cardiac activity was stopped abruptly with an injection of saturated KCl directly into the heart. The few occasions when the configuration in panel A was seen were after infusion of low polymer doses. This result shows that an increased flow can occur without a baseline shift, as was usually the case in the Chicago study of RGGu. In the next section it will be shown that in some experiments the baseline may return to its original position and the meter still indicates that blood flow is markedly enhanced after polymer injection.

The most characteristic configurations showed a marked lengthening of the amplitude due to a rise of the peak, a widening of the bell-shaped curve, and a downward shift of the baseline (panels B-D). More rarely the peak remained unaltered (Panel E) or fell (Panel F), the latter configuration generally being associated with high polymer concentrations. A baseline shift in the aortic flow curves was a constant feature of Separan at higher doses. Although the reason for this base-line shift remains elusive, some facts are known.

When the heart was infused with KCl to terminate an experiment, the aortic flow baseline always returned to its original level before infusion of the Separan. Thus the baseline shift does not appear to represent a change in the flow meter itself. When blood was pumped at a constant rate through an isolated vessel, along with a dose volume of polymer vehicle, for a given time period and the output volume was measured volumetrically, the flow probe was calibrated. If blood containing a volume of Separan solution equal to the polymer vehicle

volume used before, was again pumped through the vessel at the same flow rate as before, there was less than a 5% increase in flow displayed on the flow meter. This shows that the baseline shift is not caused by some dynamic interaction between anionic polymer sites on the Separan and the electromagnetic flow probe as the macro-ion traverses the magnetic field.

C. Hemodynamic Effect of 2.4 mg/kg Dose of 0.04% Separan AP-273 in the Rat

Experiments were conducted to test the effect of an infusion of 2.4 mg/kg (6 ml/kg) Separan AP-273 injected at a rate of 0.25 ml/min. Infusion was preceded by a one hour period of constant flow ($\pm$ 10%) as before to ensure equilbration of the model. The control hemodynamic parameters were recorded just prior to infusion.

In the first technically successful experiment recorded, shown in Figure 13, large increases were found in aortic blood flow, LV and aortic pressures, and dP/dt; however, little change was seen in heart rate until the sixth hour, when it became markedly lower.

At t=0.25 hours, the recorded blood flow had increased 3.5 times that of the control and mean aortic pressure was 1.4 times greater. Hence the calculated systemic peripheral resistance (mean aortic blood pressure/aortic blood flow) had dropped to 40% of its control value.

Aortic flow and the other variables remained well above the control value until the sixth hour in this experiment, demonstrating the long-lasting effect that the Separan could have on the cardiovascular

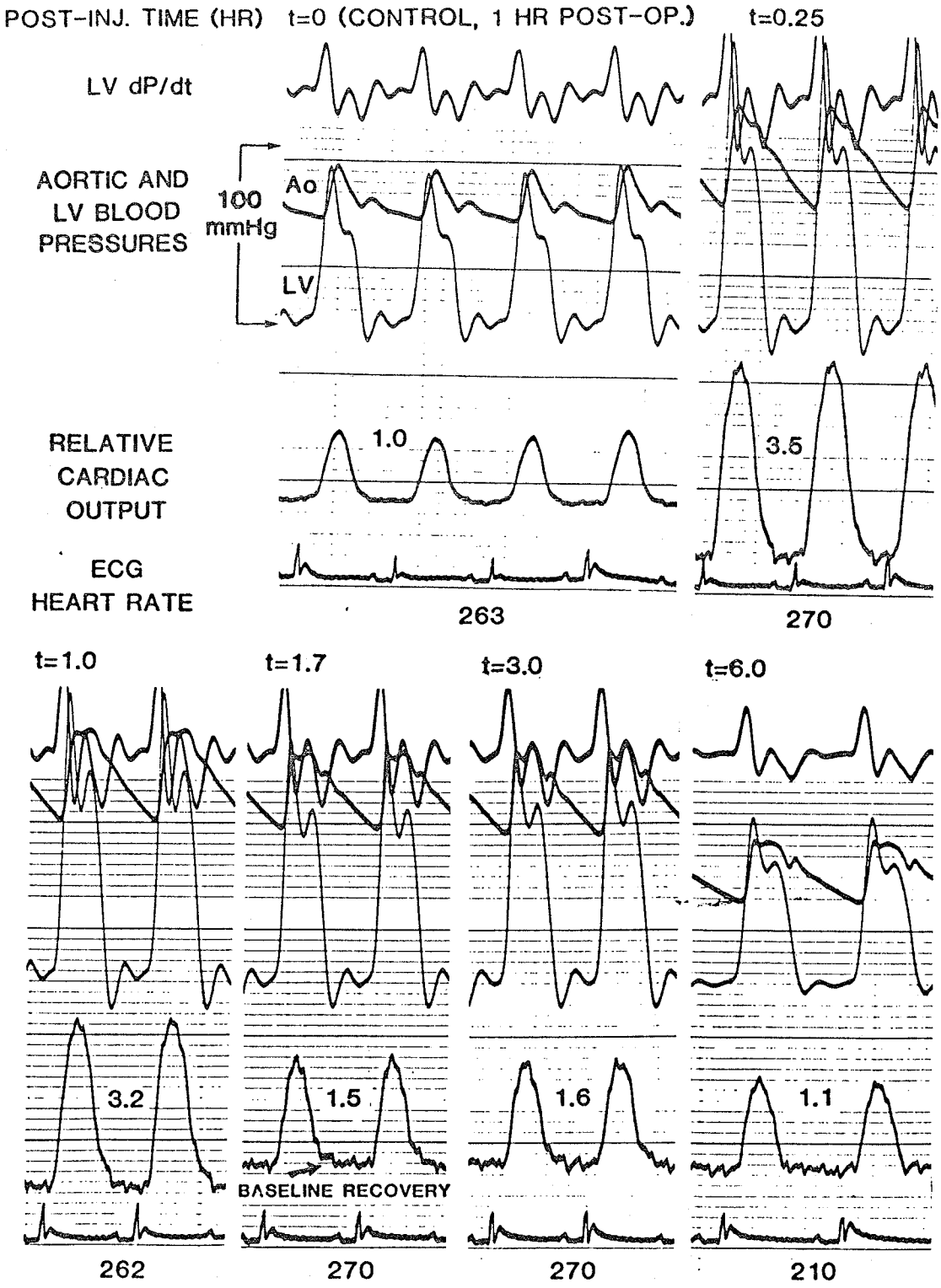

Figure 13. Effect of a 0.04% Separan AP-273 solution infused intravenously (6 ml/kg or 2.4 mg/kg, 0.25 ml/min) on aortic blood flow after a 1 h control period of constant (±10%) flow. Control blood flow was recorded just before the infusion commenced.

system. However, the hemodynamic effects were more commonly of shorter duration. By the sixth hour the pharmacological effects were markedly reduced, but several points are worth noting. Although peak and mean venticular blood pressures were both much lower than before, they were still somewhat higher than the control values despite the trauma of an open-chest, multi-probe experiment. Aortic end-diastolic pressure was now very low, indicating that the rate of arterial runoff was still higher and peripheral resistance--as confirmed by the Poiseuille relationship--remained below the control value. Whereas cardiac output had declined to only 10% above control, the stroke volume was still moderately elevated at nearly 40% over control.

With respect to the question concerning the significance of the baseline shift, it is noteworthy that the cardiac output was still substantially greater at t=1.7 h than during control although the baseline had recovered to its initial level. This was also evident at t=3.0 h. These two panels (t=1.7 and 3.0 h) indicate that the apparent increase of aortic flow following Separan infusion is not merely an artifact related to the baseline shift, although the possibility that flow values during the shift are exaggerated cannot be excluded. That is, probe calibration linearity might be lost during the shift.

Although the cause of baseline shift is not known, several facts suggest that the baseline must be readjusted to the original position to read flow off the meter. Firstly, at high polymer doses that usually cause a profound fall in the baseline, the meter sometimes read as low as zero flow without readjustment of the baseline, despite the

obvious vigor of cardiac activity and maintenance of blood pressure. Secondly, flow must be increased when the mean blood pressure is elevated, despite the steeper slope of the aortic diastolic pressure, which suggests that diastolic run-off is improved and thus peripheral resistance is reduced.

In the second experiment infusing Separan at a dose of 2.4 mg/kg, shown in Figure 14, a recording was made as early as one minute after infusion of the drug was complete. As in previous experiments the flow increased, but at this time the aortic pressure was lower than that of the control. In order for the flow to have increased during this brief period of hypotension, peripheral resistance must have had to decrease markedly. Such a drop in resistance is consistent with arterial vasodilation, hypoviscosity, drag reduction, or some combination of the three possible mechanisms.

This aspect of the study will be discussed in more detail in the Discussion section.

At t=15 minutes, the aortic and left ventricular pressures were well above those of control. How could these pressures have become elevated after the initial hypotension? There are at least three possible explanations. The first possibility is that there is a sympathetic response secondary to the initial hypotension, which provokes a positive inotropic effect and a vasoconstriction in both the arterial and venous vasculatures. Although this response commonly occurs when the baroreceptors detect a marked reduction in blood pressure, it should be noted that there is also an anomalous decrease

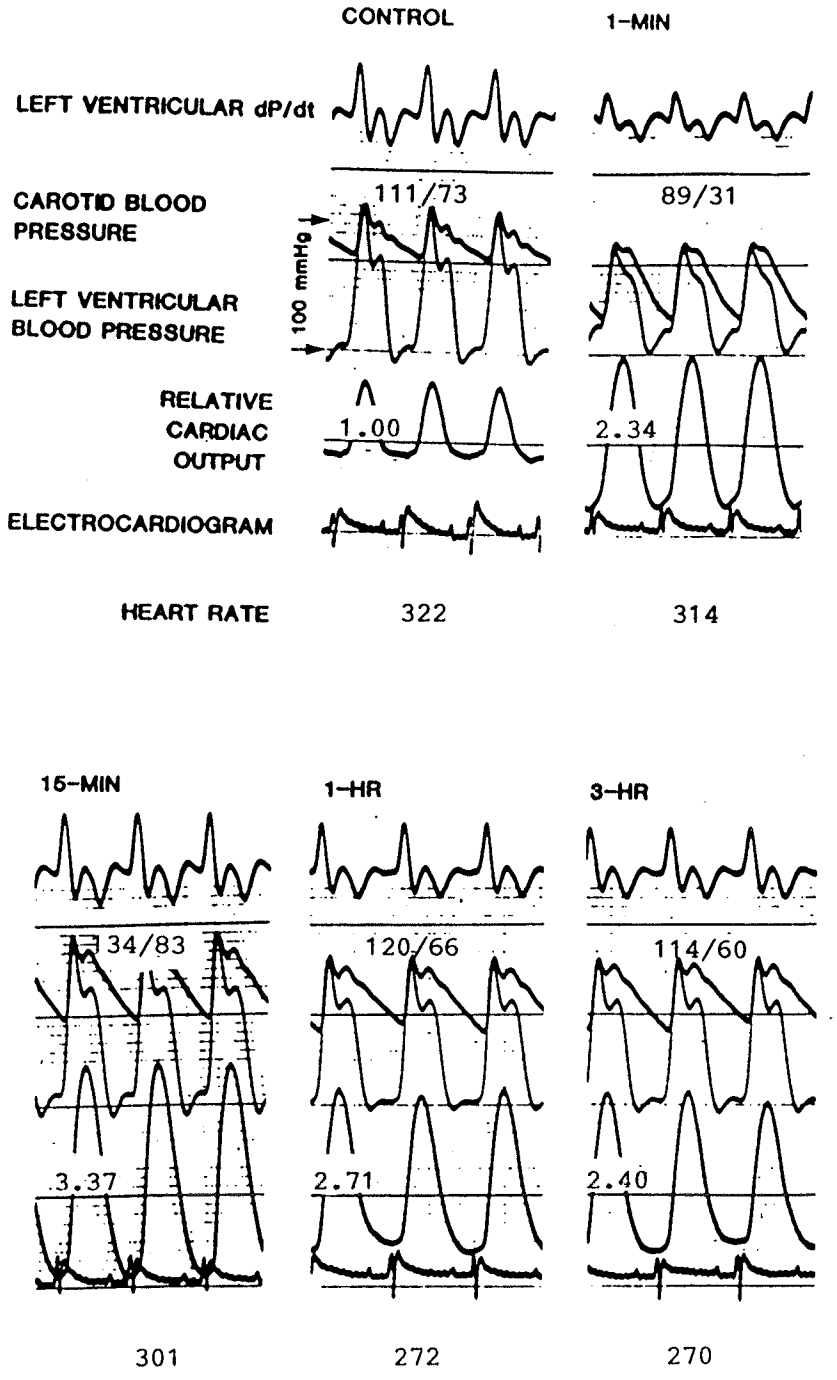

Figure 14. Hemodynamic effects of 2.4 mg/kg Separan AP-273 in the rat. Note marked decline in blood pressures 1 min post-infusion followed by an elevation of the pressures 15 min post-infusion. Changes in dP/dt were not readily interpretable in terms of a direct mechanism, but studies on isolated cardiac muscle exclude an inotropic effect (Bose et al., unpubl. obs.).

in heart rate which suggests that the situation is more complex than a simple baroreceptor reflex.

The Separan used in these experiments is an industrial product and it could have contained a number of impurities. Such substances, despite our efforts to dialyse any such contaminants out of the polymer solution, could have influenced myocardial function. Considerable variability was noted. Sometimes very large increases in flow were recorded, whereas occasionally the rat deteriorated and/or expired.

A possibility to be considered was that short fragments of the polymer itself caused the pressures to rise. Since the Separan used contained a wide range of polymer lengths, it was considered possible that the oligopolymer or even acrylamide itself might have an ionotropic effect. To further examine this point, experiments were conducted using an ultrafiltrate of Separan AP-273, described in Section E below.

D. Hemodynamic Effects of 2.0 mg/kg Dose of 0.04% Separan AP-273

Because a concentration of 2.4 mg/kg Separan AP-273 was found to be occasionally lethal as a single injection, a concentration of 2.0 mg/kg of Separan was tested more extensively.

Control values of cardiac output were recorded in 12 rats as before for one hour after stabilization ($\pm$10%). Separan solution at a concentration of 2.0 mg/kg was then injected at a rate of 0.12 ml/min, after which the hemodynamic variables were recorded again at 1 min, 15 min, 30 min, 1 h, 1.5 h and 2 h post-injection, with N=12 for all

points except at t=1 min (N=4) and 1.5 and 2 hours (N=11). As shown in Figure 15, the following hemodynamic variables are plotted in panels A-J:

1. Heart rate

The heart rate usually decreased immediately after infusion of the drug and tended to decline slowly compared to the initial fall (Fig. 15, panel A). The immediate fall in heart rate upon infusion of Separan is paradoxical when, as to be shown below (Panel D), arterial blood pressure is greatly reduced. A reflex sympathetic response would be expected during this acute hypotension, and thus an increase in heart rate would be predicted. The fact that cardiac output apparently is greater during this period suggests that venous return increases, which would be compatible with enhanced sympathetic activity. The paradox might be explained if there were a vagal reflex simultaneous with the sympathetic response, as occurs in the Cushing syndrome. Admittedly, such a dual response would be extraordinary, but then so is a reduction in heart rate during acute hypotension. The final heart rate showed an 8% decrease from the control.

2. Aortic Blood Flow Index.

The aortic blood flow index increased markedly immediately following infusion and by t=0.5 h the index was still 100% greater than control. At t=1 h, the index was elevated 50%, and at t=2 h, the index was 30% above control (Fig. 15, panel B).

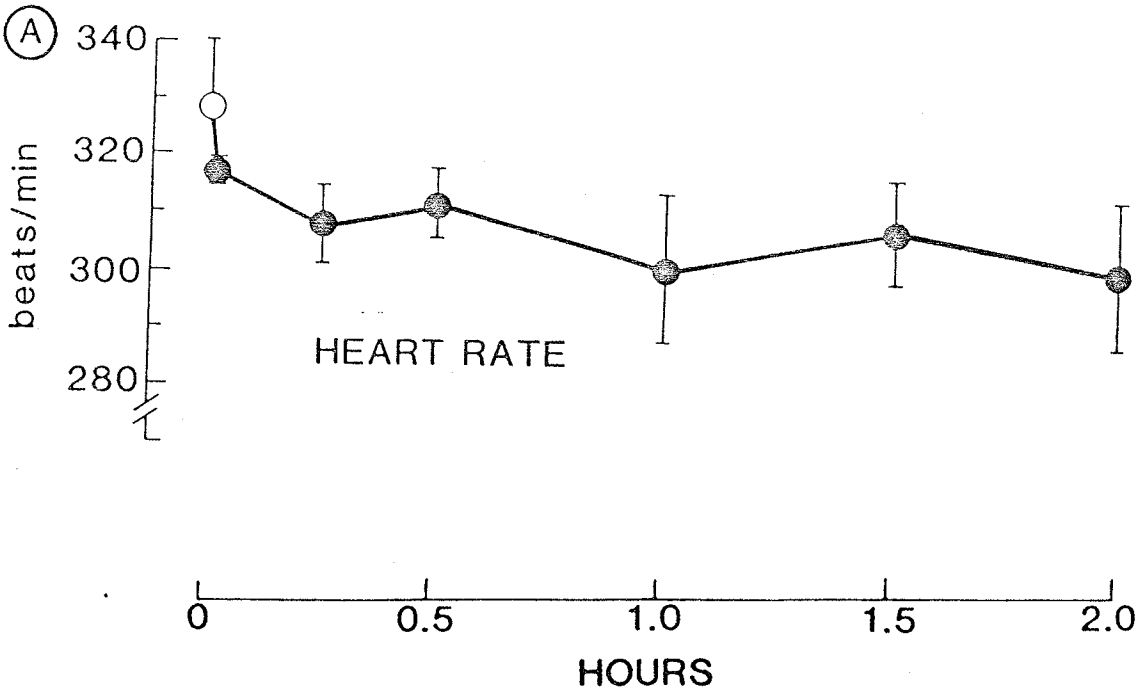

Figure 15, Panels A - J. Hemodynamic effects of 2.0 mg/kg 0.04% Separan AP-273 injected into the femoral vein. Control values (open circles) of hemodynamic variables were recorded in 12 rats after at least one hour of stable (±10%) cardiac output. Separan solution was then injected at a rate of 0.12 ml/min, after which the hemodynamic variables were recorded again 1 min, 15 min, 30 min, 1.0 h, 1.5 h, and 2.0 h after ending the injection (closed circles); N=12 for all points except at times 1 min (N=4) and 1.5 and 2.0 h (N=11), respectively. The following hemodynamic variables are plotted in panels A-J:

Panel A. Heart rate (beats/min).

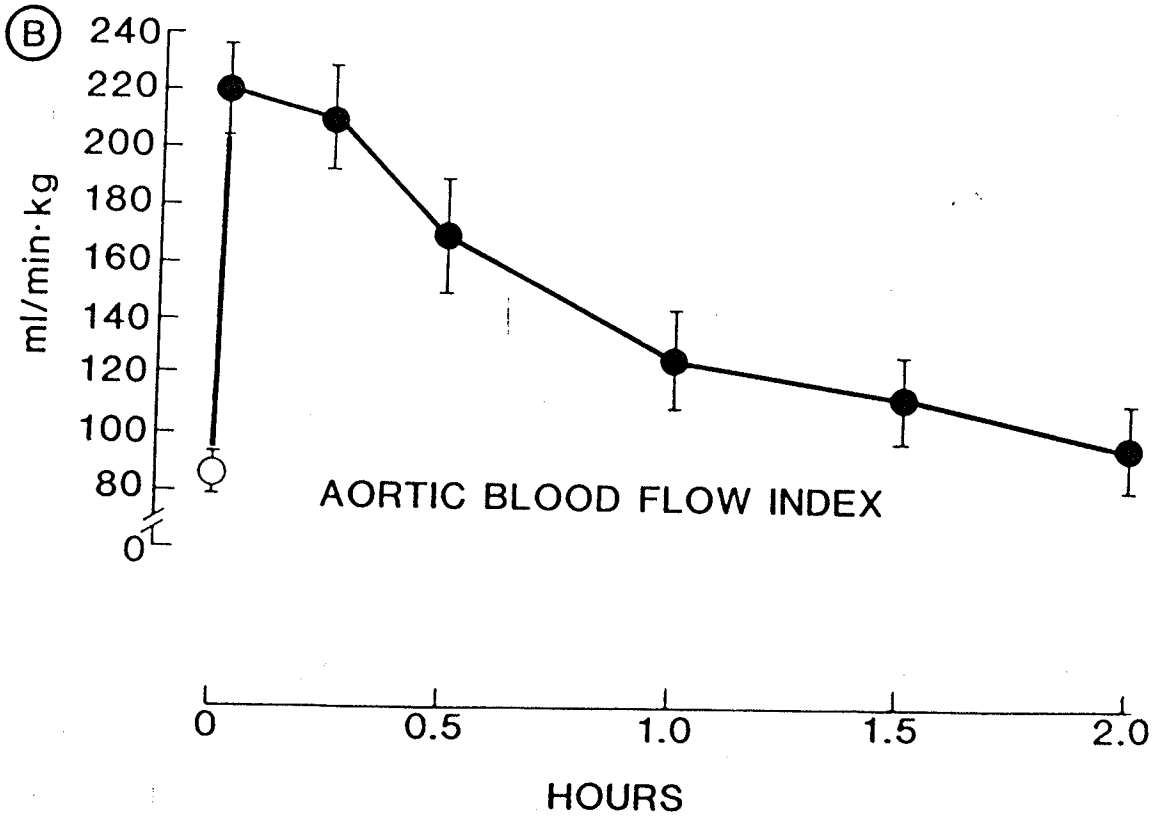

Figure 15 continued:

Panel B. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on aortic blood flow index (ml/min·kg). The index was statistically greater than control throughout the two hour post-infusion period.

3. Stroke volume index

A pattern similar to panel B is seen in the stroke volume index. The index rises immediately upon infusion and then makes a gradual decline towards the control value. But after two hours, the index is still somewhat above that of the control (Fig. 15, panel C).

4. Arterial blood pressure: peak, end-systolic, end-diastolic, and pulse pressures

Arterial (carotid) blood pressure quickly fell upon infusion of the Separan (Fig. 15, panel D). This was similar to what was found with RGGu, but unlike RGGu the Separan then increased 15-30 minutes later. One hour after infusion of the drug, peak pressure returned close to the control values even though aortic flow continued above control. The end-systolic pressure followed a pattern similar to that of the peak pressure, whereas the end-diastolic pressure fell precipitously immediately after infusion of the drug and quickly returned to control values. The initial decrease in end-diastolic pressure actually appeared during infusion of Separan; this was observed on the monitor, but not recorded. The fact that end-systolic pressure hardly ever fell, whereas end-diastolic pressure fell markedly at first, strongly suggests that peripheral resistance must have decreased at least initially. Moreover, this suggestion does not rely on the accuracy of the flow reading, but is independent of it. Finally, the pulse pressure rose after polymer infusion, then returned to control values within one hour.

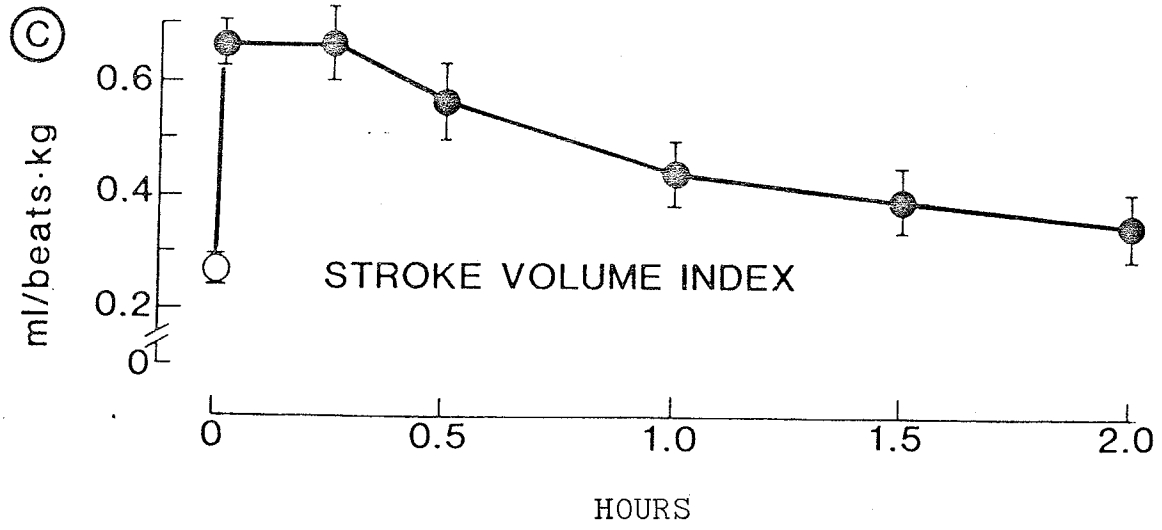

Figure 15 continued:

Panel C. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on stroke volume index (ml/beats·kg).

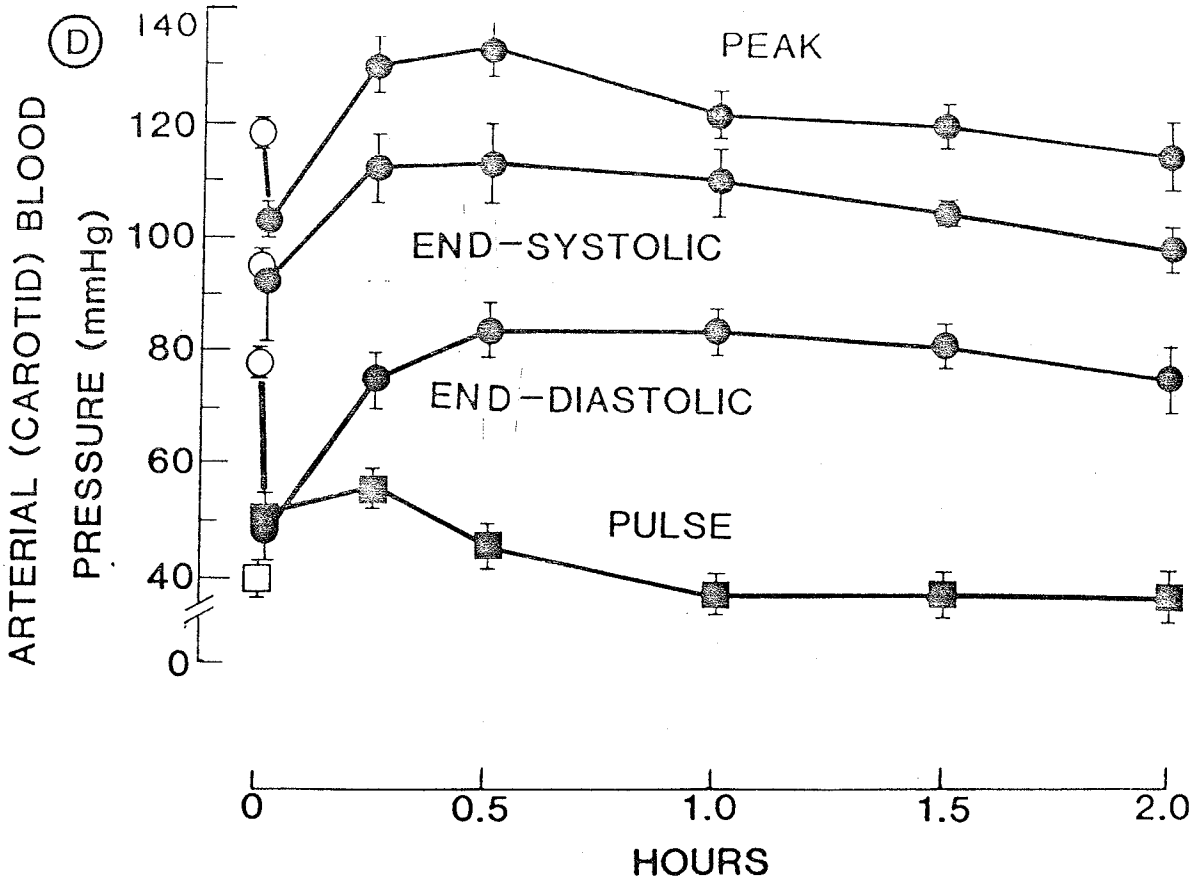

Figure 15 continued:

Panel D. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on arterial (carotid) peak, end-systolic, end-diastolic, and pulse pressures (mmHg).

5. Left ventricular blood pressure: peak and end-diastolic pressures

The peak systolic pressure of the left ventricle shows an expected similarity to the systolic arterial pressure (Fig. 15, panel E): a decrease in pressure in the first minute followed by an increase.

The diastolic left ventricular blood pressure initially increased upon infusion of the Separan. This increase, simultaneous with an increased aortic blood flow (Panel B), is surprising. It is paradoxical to have two pressure variables change in a manner suggesting that cardiac pump activity is compromised, while the aortic flow probe indicates that cardiac output is markedly enhanced. A possible explanation is that with the reduction of arterial resistance and subsequent reflex venoconstriction, venous return is greatly increased due to the reduction of venous capacitance combined with some reduction of venous flow resistance. In other words, although ventricular outflow is augmented, the increase in venous return is initially greater until outflow is increased further as left ventricular volume increases. The augmented arterial flow is not reflected in an expected elevation of arterial pressure, presumably because arterial flow resistance is greatly diminished. This initial diminuition of arterial pressure would also account for the dampening of arterial dP/dt.

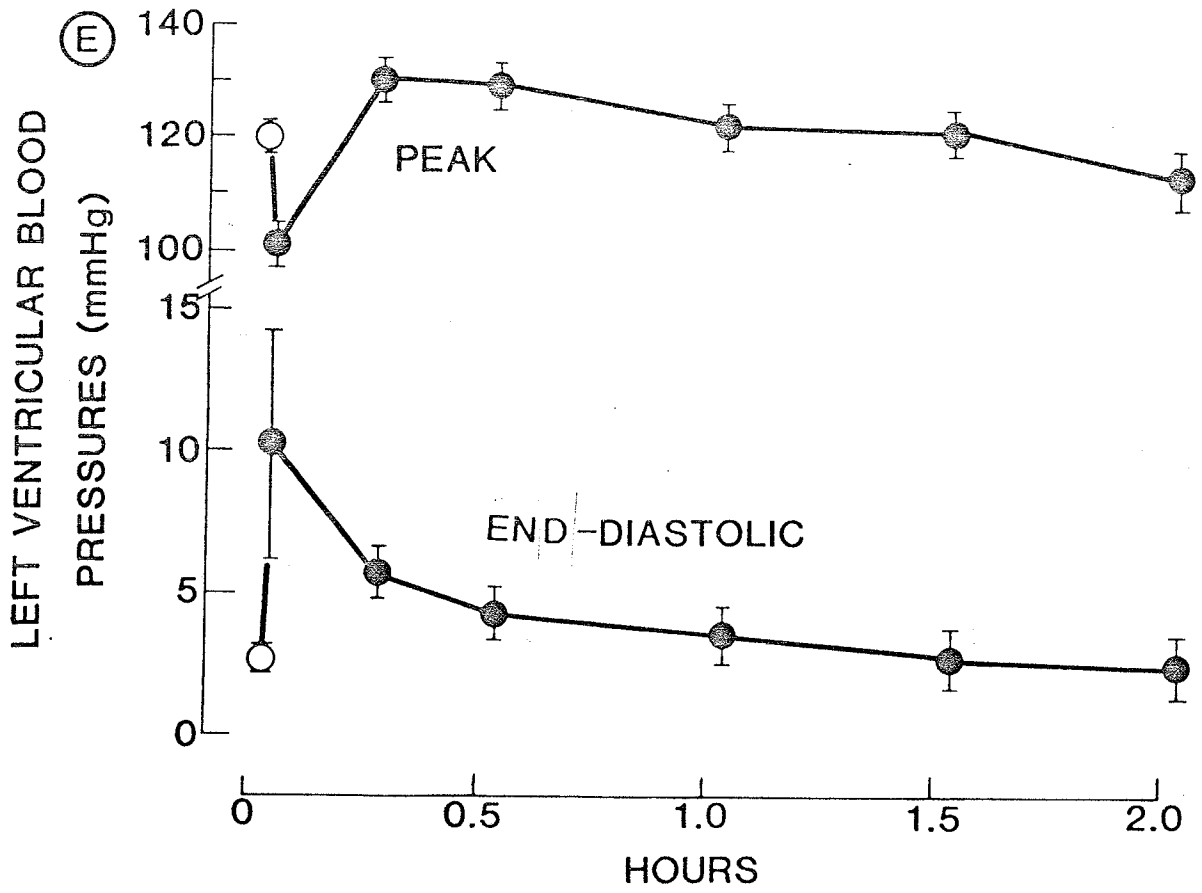

Figure 15 continued:

Panel E. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on peak and end-diastolic left ventricular pressures (mmHg).

6. **Effect of Separan on mean carotid and left ventricular blood pressures**

Mean carotid pressure initially dropped significantly, tended to overshoot the control value, and then returned to control pressure. The mean left ventricular pressure followed the same pattern, except that the initial drop was not as sharp as that of arterial pressure (Fig. 15, panel F).

7. **Effect of Separan on systolic ejection time**

After infusion, and throughout the duration of the experiments, systolic ejection time remained close to the control value (Fig. 15, panel G).

8. **Effect of Separan on the left ventricular ejection rate index**

Ventricular ejection rate index rose dramatically upon infusion of Separan (Fig. 15, panel H). The index remained above control during the two hours of experimentation, but decreased towards the control value with time. The striking increment in this variable in the first post-infusion minute was in marked contrast to reduced dP/dt amplitudes, but the two findings were qualitatively compatible if it is assumed that Separan causes a powerful reduction in peripheral resistance.

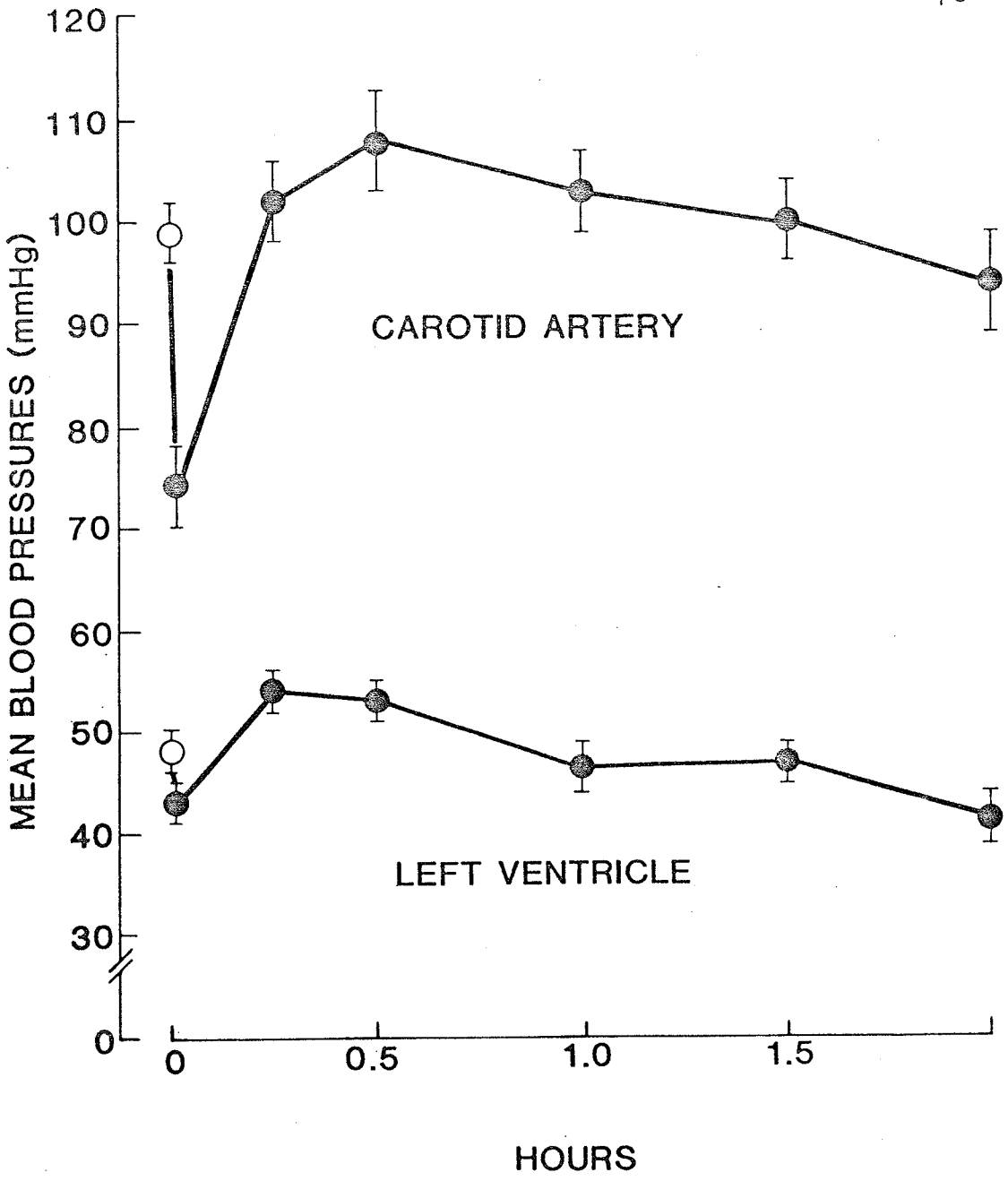

Figure 15 continued:

Panel F. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on mean carotid and left ventricular blood pressures (mmHg).

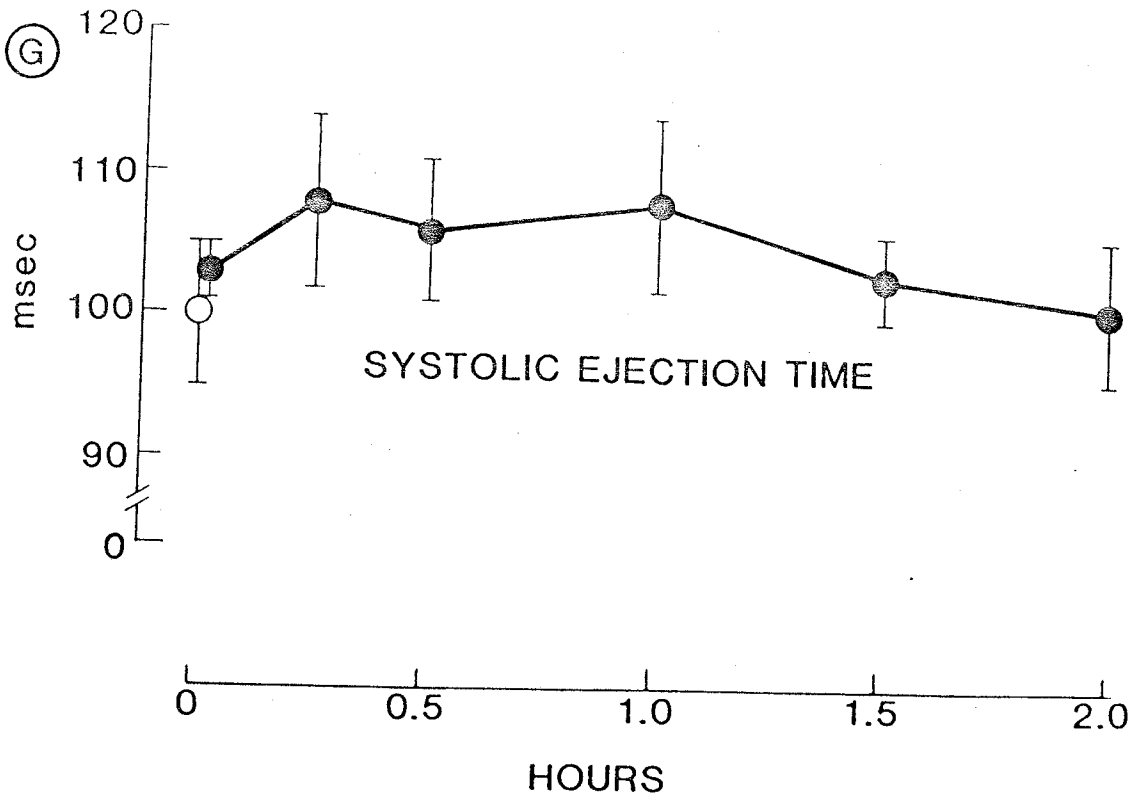

Figure 15 continued:

Panel G. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on systolic ejection time (msec).

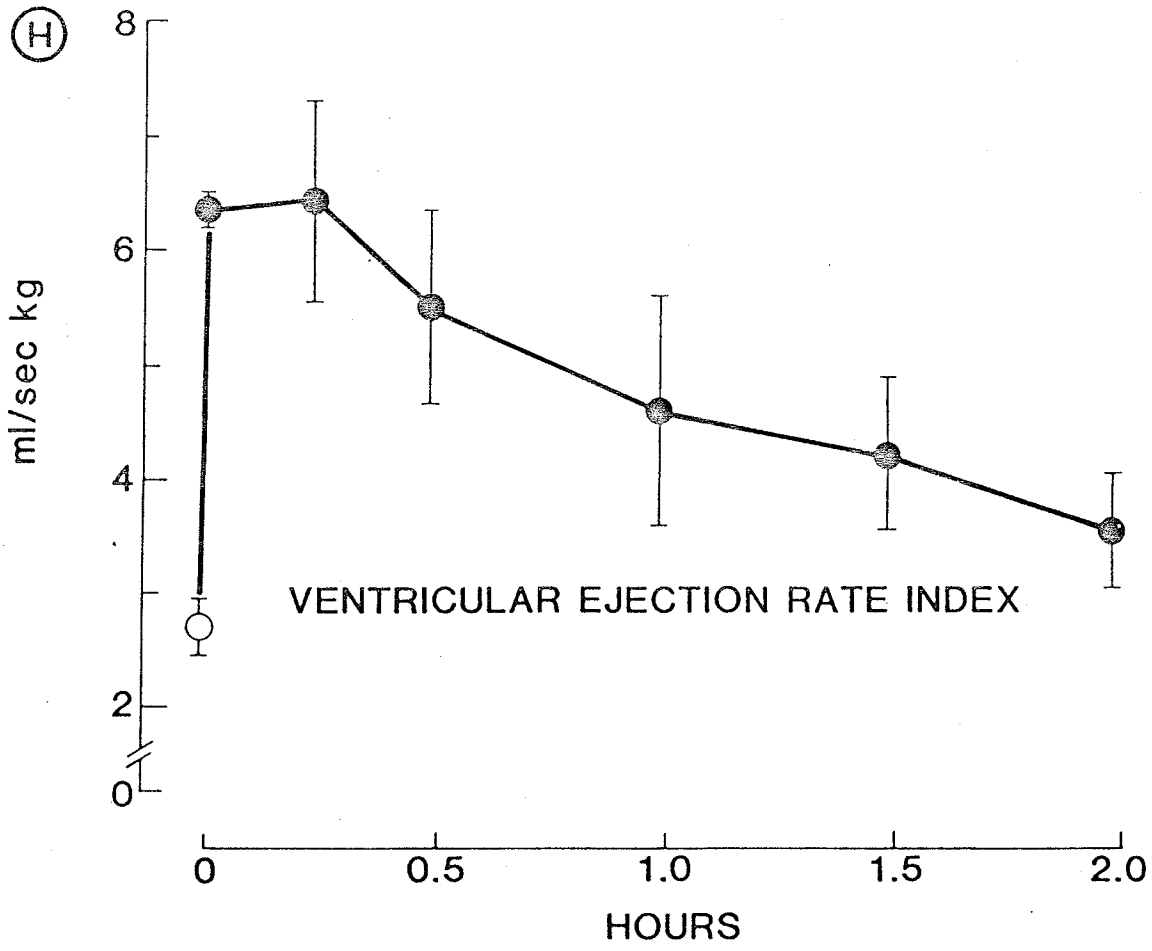

Figure 15 continued:

Panel H. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on left ventricular ejection rate index (ml/sec·kg).

9. Effect of Separan on cardiac work index

Since external work of the heart is defined by the product of mean pressure and volume outflow, it is not surprising that the cardiac work index increased given the improvement of outflow in the presence of Separan (Fig. 15, panel I). It is noteworthy that an increment in the work index was generated with hardly any change in mean left ventricular pressure, the component that requires by far the greatest increment in biochemical energy. That is, the biological work performed by the heart after infusion of Separan is probably much less than the external work achieved, which is another way of saying that Separan apparently enhances cardiac efficiency.

10. Effect of Separan on total peripheral resistance index

Aortic flow was seen to markedly increase (Panel B), whereas mean arterial blood pressure (Panel F) did not increase significantly after infusion of Separan and even fell initially. Thus, according to the Poiseuille relation, resistance must have dropped. As with other variables, the values returned towards control as the effects of the drug wore off (Fig. 15, panel J). This and other data obtained from the experiments illustrated in the Figure 15 panels are tabulated together with the electrocardiogram P-R interval (Tables 6A, B, and C).

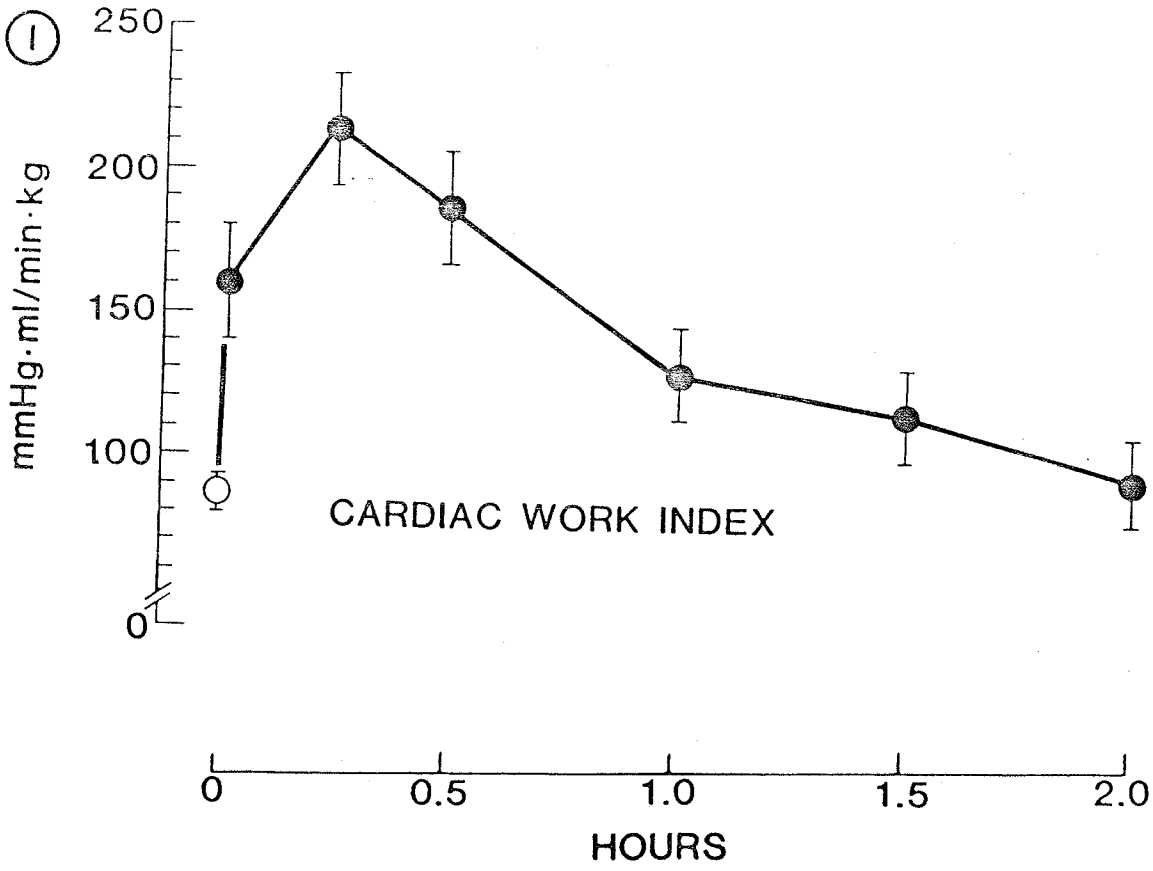

Figure 15 continued:

Panel I. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on cardiac work index (mmHg·ml/min·kg).

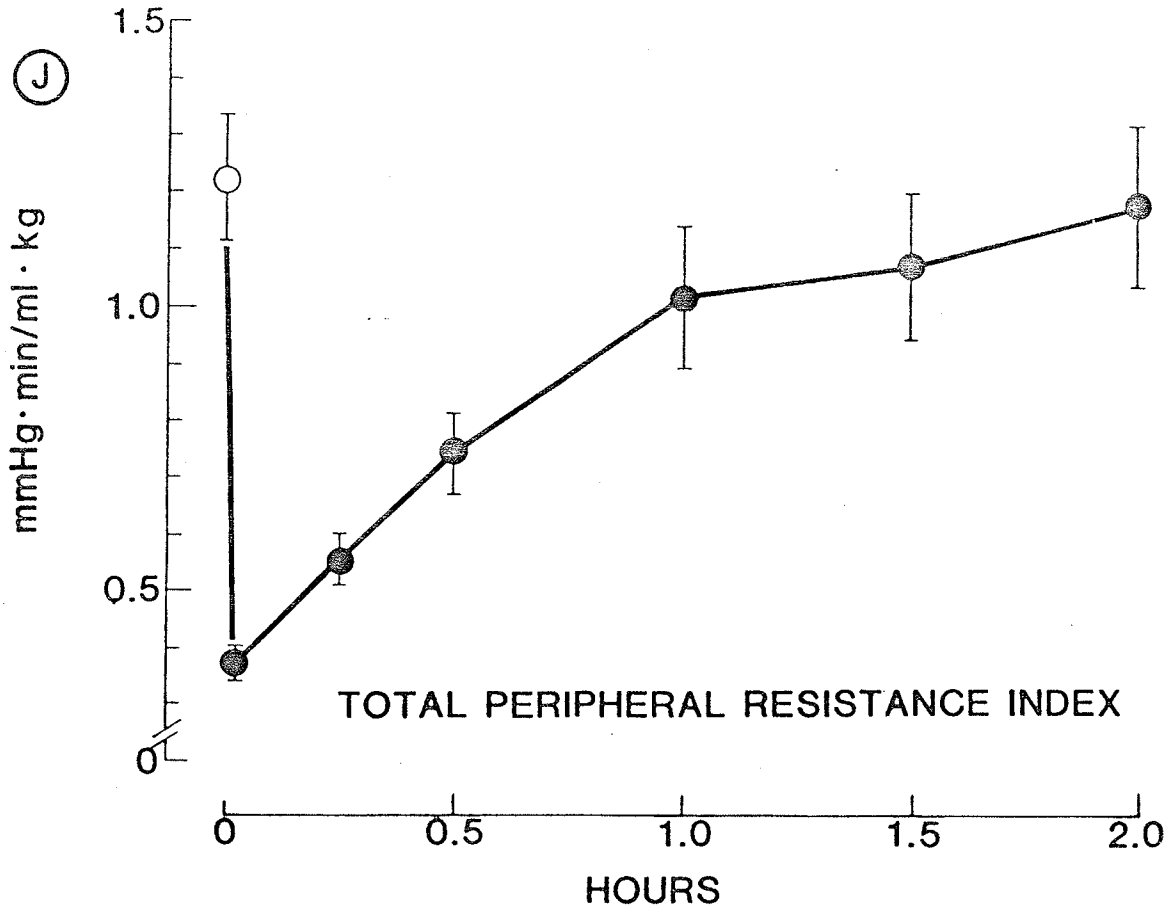

Figure 15 continued:

Panel J. Effect of 0.04% Separan AP-273 (2.0 mg/kg) on total peripheral resistance index (mmHg·min/ml·kg).

Post-Infusion Time	Heart Rate (bpm)	Cardiac Index (ml/min/kg)	Stroke Volume Index (ml/beat/kg)	Systolic Ejection Time (msec)	Ventricular Ejection Rate Index (ml/sec/kg)	P-R Interval (msec)
0	328 ± 12	87 ± 7	0.270 ± 0.024	100 ± 5	2.71 ± 0.23	55 ± 1
1 min	337 ± 2	189 ± 14	0.575 ± 0.030	95 ± 2	5.66 ± 0.32	58 ± 3
15 min	308 ± 7	211 ± 18	0.665 ± 0.063	108 ± 6	6.44 ± 0.87	59 ± 2
30 min	312 ± 6	170 ± 20	0.564 ± 0.066	106 ± 5	5.49 ± 0.84	57 ± 1
1.0 hour	301 ± 13	128 ± 17	0.439 ± 0.057	108 ± 6	4.60 ± 1.00	59 ± 2
1.5 hour	305 ± 9	119 ± 15	0.408 ± 0.060	106 ± 5	4.32 ± 0.73	57 ± 1
2.0 hour	297 ± 13	102 ± 16	0.362 ± 0.061	103 ± 5	3.63 ± 0.51	57 ± 2

Table 6. Summary of the hemodynamic effects of 2.0 mg/kg 0.04% Separan AP-273 solution over a two hour period (N=11 or 12, except at 5=1 min when N=3 or 4; ± SEM). The data were normalized against their respective control (t=0) values and recalculated on the basis of the mean of the corresponding control data. The tabulated data thus differs from some of the data plotted in Fig. 15, panels A-J, where the recalculations were based on a mean control value obtained with N=12. The differences in the data calculated by the two procedures were small (5%), except at t=1 min when the differences approached 14% for some variables. Panel A: heart rate, cardiac (aortic blood flow) index, stroke volume index, systolic ejection time, ventricular ejection rate index, and P-R interval.

Post-Infusion Time	Arterial (Carotid) Blood Pressure (mmHg)				
	Peak Systolic	End-Systolic	End-Diastolic	Pulse	Mean
0	118 ± 3	95 ± 3	78 ± 2	40 ± 3	99 ± 3
1 min	109 ± 4	92 ± 12	47 ± 5	64 ± 5	77 ± 4
15 min	130 ± 5	112 ± 6	75 ± 5	56 ± 3	102 ± 4
30 min	129 ± 5	113 ± 7	84 ± 5	45 ± 3	108 ± 5
1.0 hour	122 ± 4	110 ± 6	84 ± 4	38 ± 4	103 ± 4
1.5 hour	119 ± 4	105 ± 2	82 ± 4	37 ± 4	100 ± 4
2.0 hour	114 ± 5	100 ± 4	76 ± 6	37 ± 4	94 ± 5

Table 6. Panel B: Arterial (carotid) blood pressures, including peak, end-systolic, end-diastolic, pulse, and mean pressures.

Post-Infusion Time	Left Ventricular Blood Pressure (mmHg)			Cardiac Work Index (mmHg·ml/min/kg)	Total Peripheral Resistance (mmHg·min/ml/)
	Peak-Systolic	End-Systolic	Mean		
0	120 ± 3	2.7 ± 0.5	48 ± 2	86 ± 7	1.22 ± 0.11
1 min	108 ± 5	10.2 ± 4.2	47 ± 2	146 ± 17	0.43 ± 0.03
15 min	130 ± 4	5.7 ± 0.9	54 ± 2	214 ± 19	0.55 ± 0.05
30 min	129 ± 4	4.3 ± 0.9	53 ± 2	186 ± 20	0.74 ± 0.07
1.0 hour	122 ± 4	3.6 ± 1.0	47 ± 2	129 ± 16	1.01 ± 0.13
1.5 hour	120 ± 4	2.9 ± 1.0	46 ± 2	119 ± 16	1.01 ± 0.12
2.0 hour	112 ± 5	2.5 ± 1.1	41 ± 3	96 ± 16	1.11 ± 0.13

Table 6. Panel C: cardiac work index, total peripheral resistance, and left ventricular blood pressures, including peak, end-systolic, and mean pressures. Left ventricular and-systolic blood pressures are not normalized, because some control values are zero and thus the normalized values are indeterminate.

E. Hemodynamic Effects of Ultrafiltrated Separan AP-273

Up to this point, the Separan used for the infusions was a dialyzed, buffered solution of what had been furnished by Dow Chemical Co., that is, a polymer designed for industrial uses in bulk quantities. It was hoped that filtering the Separan solution through a molecular filtration system would eliminate impurities and Separan molecules less than 100,000 daltons. The infused solution would hopefully include only the longer, and hydrodynamically more effective, Separan molecules. Upon infusion of the Separan, peripheral resistance and systolic aortic pressure were expected to decrease initially, but soon after the pressure would return to its original level due to the baroreceptor reflex.

The Separan was ultrafiltrated (see Fig. 8 in Methods section), using a Minitan Ultrafiltration unit (Minitan, Mississauga, Ontario). The ultrafiltration procedure was designed to eliminate all impurities and Separan molecules less than about 100,000 daltons. Molecules lighter than 100,000 daltons make up about 1% of the total number of Separan molecules (see Fig. 7). It was noticed that the filtered Separan solution felt much less viscous when rubbed between the fingers, suggesting that a fraction of polymer much greater than 1% may have been lost during the filtration. This might be explained by the nature of the macropolymer and filter, which was designed to eliminate globular macromolecules of less than 100,000 daltons. Since Separan is linear, it is possible that the ends of the Separan molecules, even those much larger than 100,000 daltons, were drawn into the filter pore

head first once the heads were caught in the lateral current traversing the pore. Once caught in the current, the remainder of the molecule would be pulled through regardless of its length. Thus, Separan molecules greater than 100,000 daltons may have been eliminated, leaving a solution considerably more dilute than desired. It would have been easy to concentrate the remaining Separan, but at this point even an approximate polymer concentration was unknown.

As seen in Figure 16, the peak arterial pressure still was slightly higher than control after infusion of polymer, but the mean arterial pressure did not exceed that of the control during the first hour. The increase in aortic blood flow was extraordinary considering that perhaps as little as one-fifth of the original dose was infused. It should be borne in mind, however, that according to the hydrodynamic literature the Toms effect on flow is proportional to the longest polymer present and polymers of such length are present in both the filtered and unfiltered Separan solutions.

In one experiment a rat was infused intermittently with doses of the filtrated Separan solution to maintain the aortic blood flow at about 2.5-fold control flow over a six hour period (Fig. 17). The experiment using Separan was performed first. The polymer was infused when aortic blood flow began to decrease. Successive infusions maintained the flow at a fairly constant rate of 60 ml/min. There was one instance where flow decreased to 46 ml/min (control over flow was

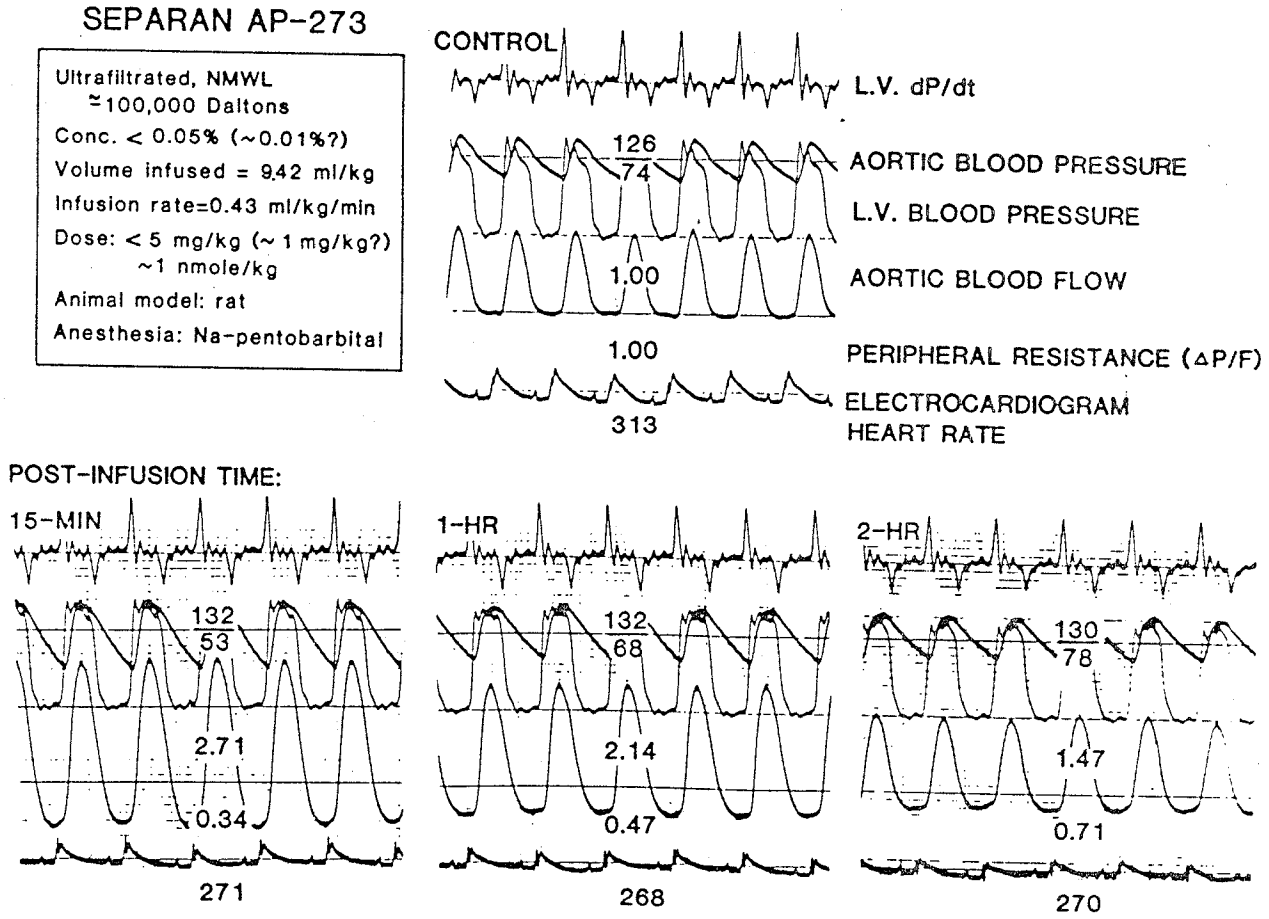

Figure 16. Hemodynamic effects of Separan AP-273 putatively free of substances of molecular weights below 100,000 daltons.

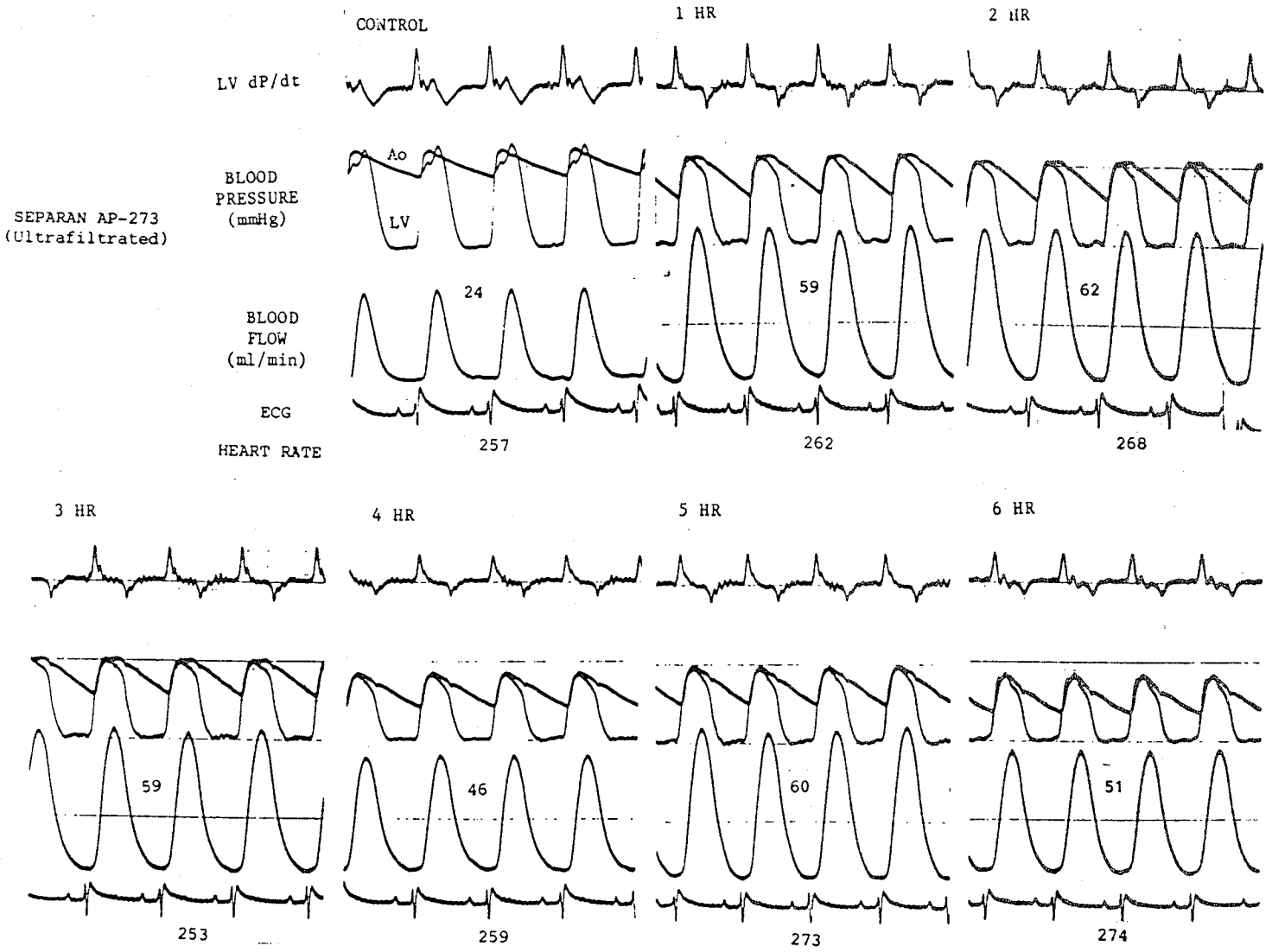

Figure 17. Hemodynamic effects of Separan AP-273 putatively free of low (<100,000 daltons) molecular weight substances. See text for details.

briefly lost in this instance). The experiment was then repeated with the same infusion protocol using the polymer vehicle alone.

The hemodynamic effects of the polymer solution and buffer solution are compared in Figure 18, panels A and B.

Panel A shows that aortic blood flow was much higher with Separan infusion compared to pre-infusion flow or flow following infusion with buffer solution alone. It is clear that infusion volume per se had no effect on flow. Peripheral resistance decreased in the animal receiving Separan and remained lower throughout the six hours with a constancy that contrasted sharply with the effect seen after a single injection. Heart rate seemed to be little affected by the drug vehicle or the drug itself.

In panel B, the mean aortic and aortic diastolic blood pressures show some elevation during the drug vehicle infusion, perhaps due to a volume effect, but both pressures fell during the Separan infusion. Mean left ventricular blood pressure followed similar patterns in both buffers and Separan experiments, except for a rise with buffer in the first hour.

F. Polymer Shear Degradation: Disappearance of Separan-induced Hemodynamic Effects

The hypothesis that the primary hemodynamic effect of Separan, like RGGu, is due to the long length of the polymer molecule was tested by comparing the hemodynamic effects of the intact polymer and the same

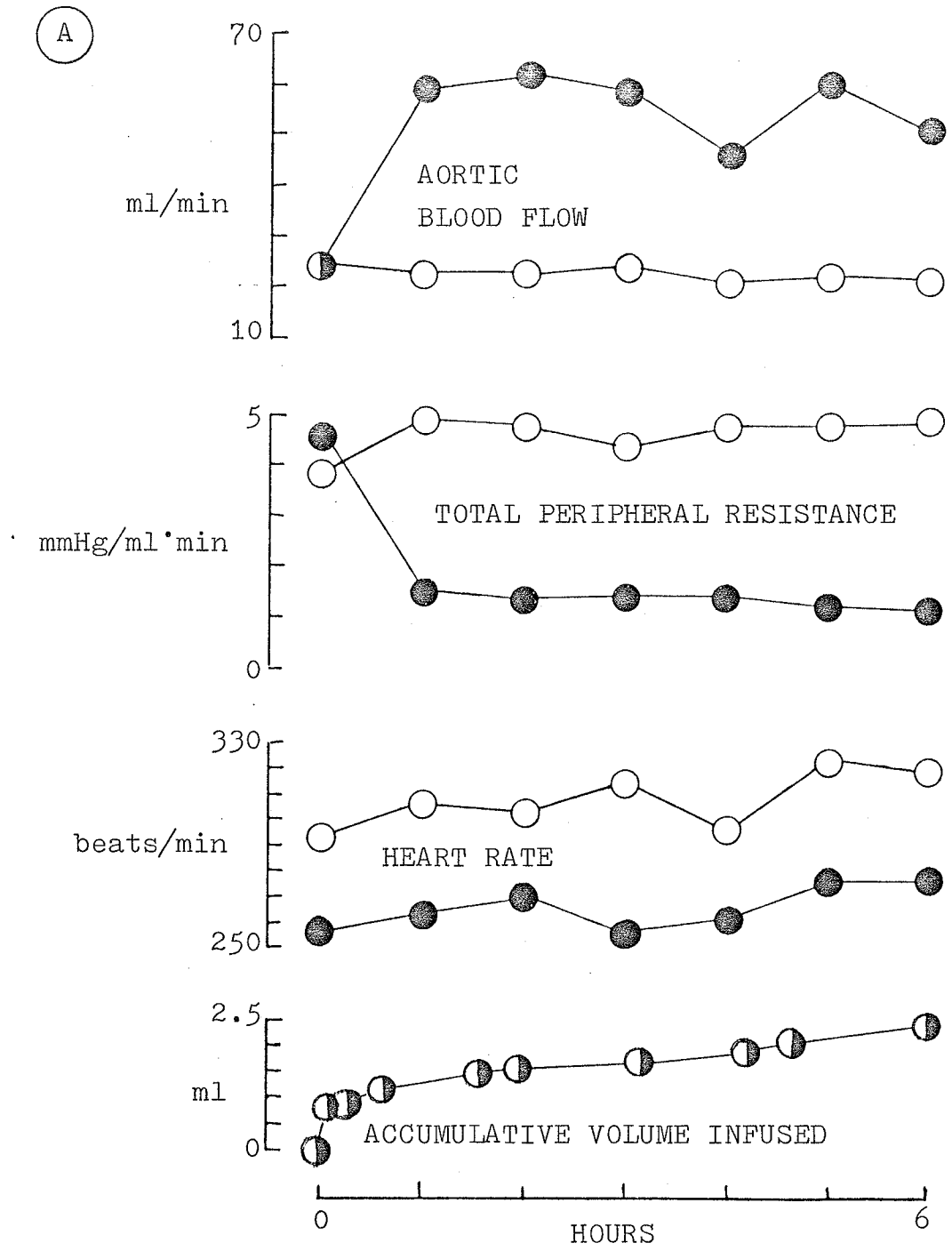

Figure 18. Hemodynamic effects of Separan AP-273 putatively free of low molecular weight substances (Separan, closed circles) compared with effects of drug vehicle alone (control, open circles). Panel A. Effects of drug or drug vehicle on aortic blood flow, total peripheral resistance, and heart rate.

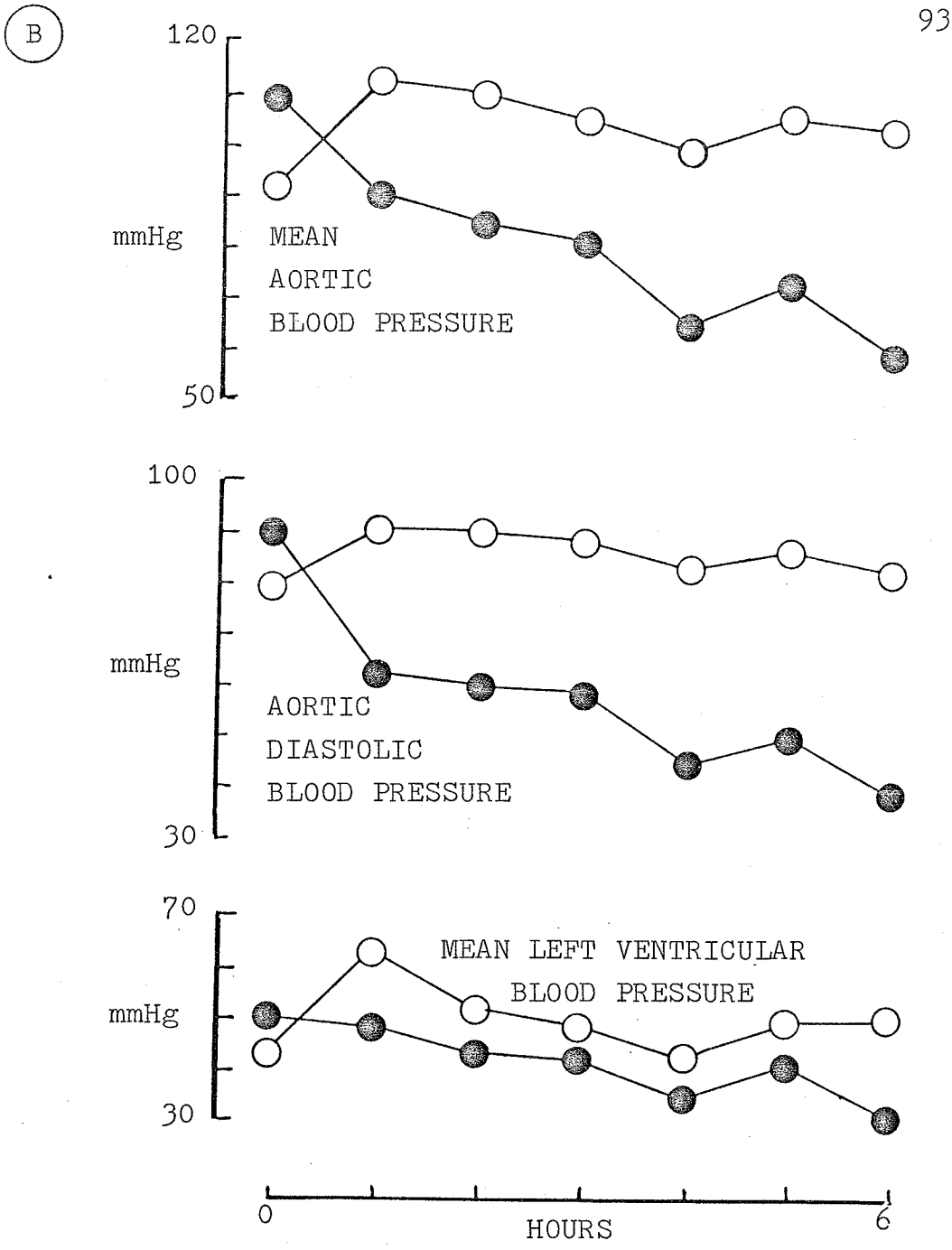

Figure 18 continued:

Panel B. Effects on mean aortic, aortic diastolic, and mean left ventricular blood pressures.

polymer subjected to sheer degradation. The experiments described in Section D (Figure 15 A-J) served as the control with all data normalized to the pre-infusion values. The experiments conducted with the sheared molecules used an identical protocol, except that these experiments were terminated one hour after infusion of the drug.

The polymer was stirred for 20 hours with a magnetic rod rotating at about 1000 rpm. The results of both sets of experiments is shown in Figure 19, A-F.

Panel A shows that shearing Separan molecules caused no large effect on heart rate, but the rate tended to be higher compared to the effect of the intact polymer. Mean aortic and aortic end-diastolic pressures seen in Panels B and C remained unchanged except for small elevations immediately after infusion, probably due to a transient volume effect, which contrast with the hypotension observed with the intact polymer.

Panels D and E show the cardiac index and the cardiac work index, respectively. Both show that the sheared Separan has little effect on these indices. This too is in sharp contrast to the unsheared Separan infusions. Again a small, transient volume effect is detectable.

Total peripheral resistance, shown in Panel F, remained basically unchanged the first 30 minutes after administration of the sheared polymer, and then tended to rise. As with Panels D and E, these results are quite different from what is seen with infusions of intact Separan molecules.

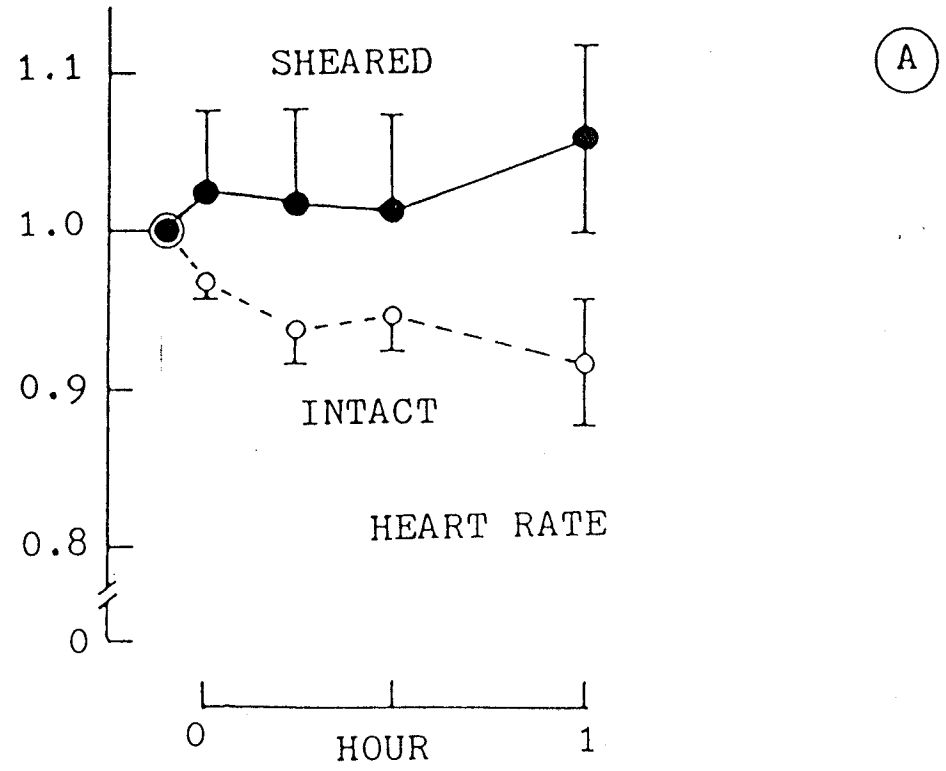

Figure 19 A - F. Hemodynamic effects of Separan AP-273 before (intact, open circles) and after (sheared, closed circles) mechanical shearing of the polymer. The control results are represented by the normalized data obtained with intact polyacrylamide molecules as shown in Fig. 14 A-J and Table 6 A-C. The same infusion protocol was used in the two studies, except that in the shear study (N=6) the polymer was subjected to 20 h shear with a magnetic stirrer rotating at about 1,000 rpm.

Panel A. Effect of sheared Separan AP-273 on heart rate.

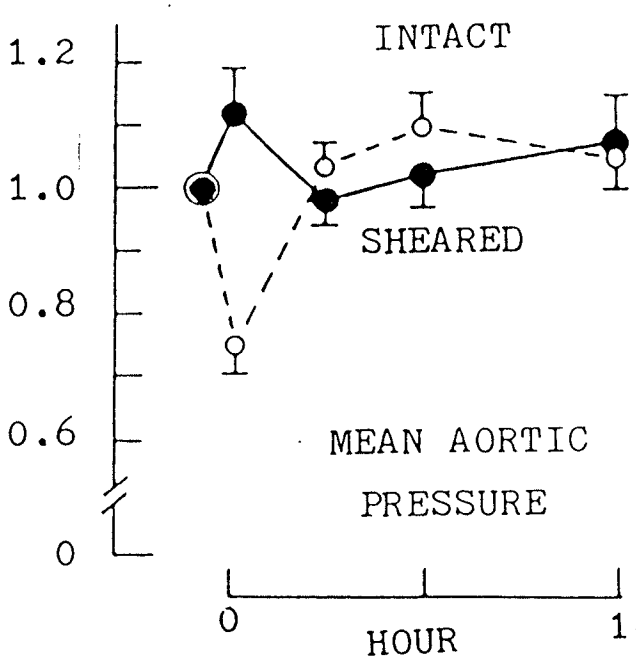

Figure 19 continued:

Panel B. Effect of sheared Separan AP-273 on mean aortic blood pressure.

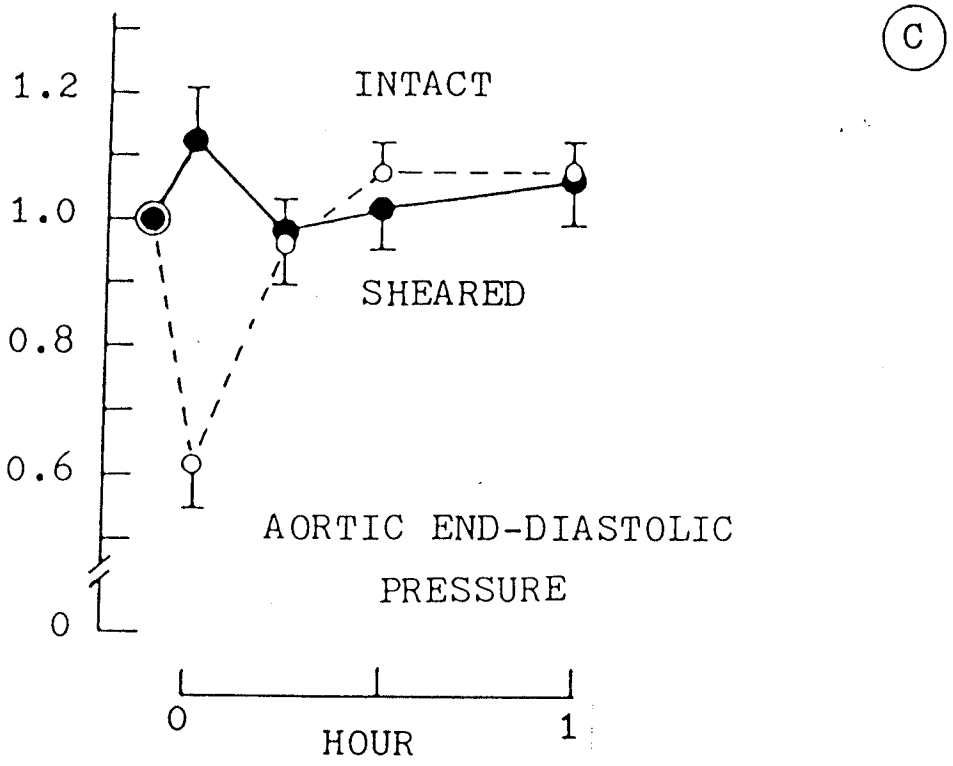

Figure 19 continued:

Panel C. Effect of sheared Separan AP-273 on aortic diastolic blood pressure.

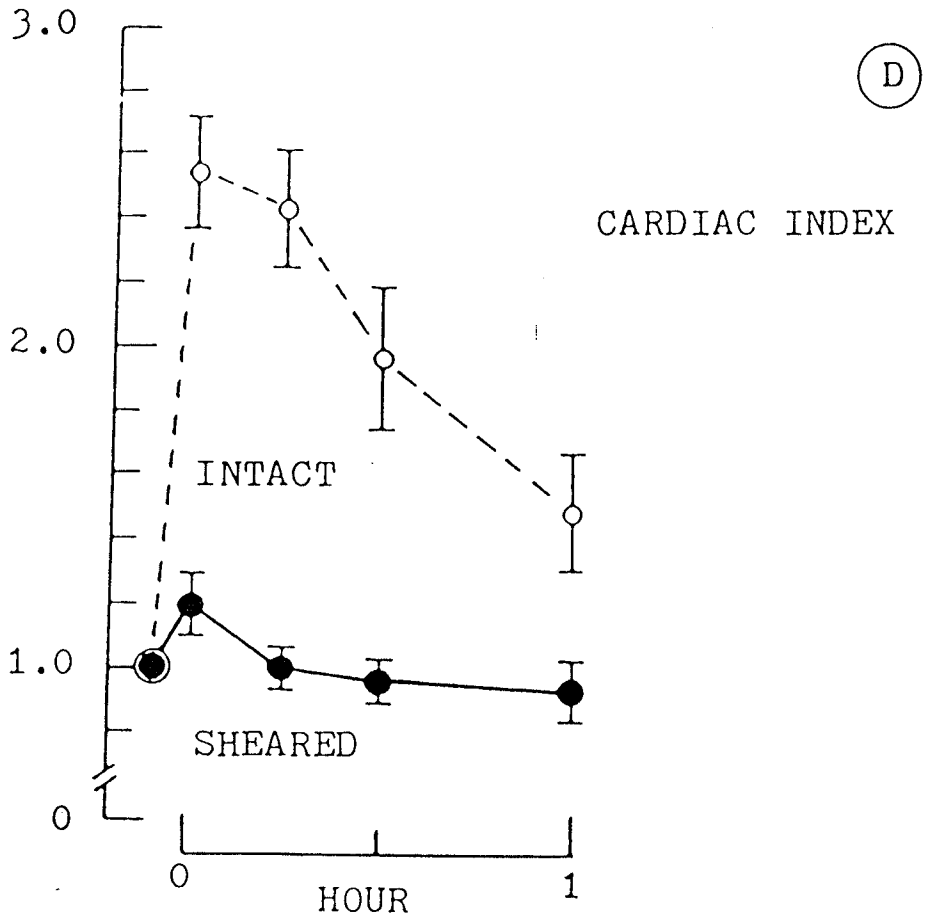

Figure 19 continued:

Panel D. Effect of sheared Separan AP-273 on cardiac index.

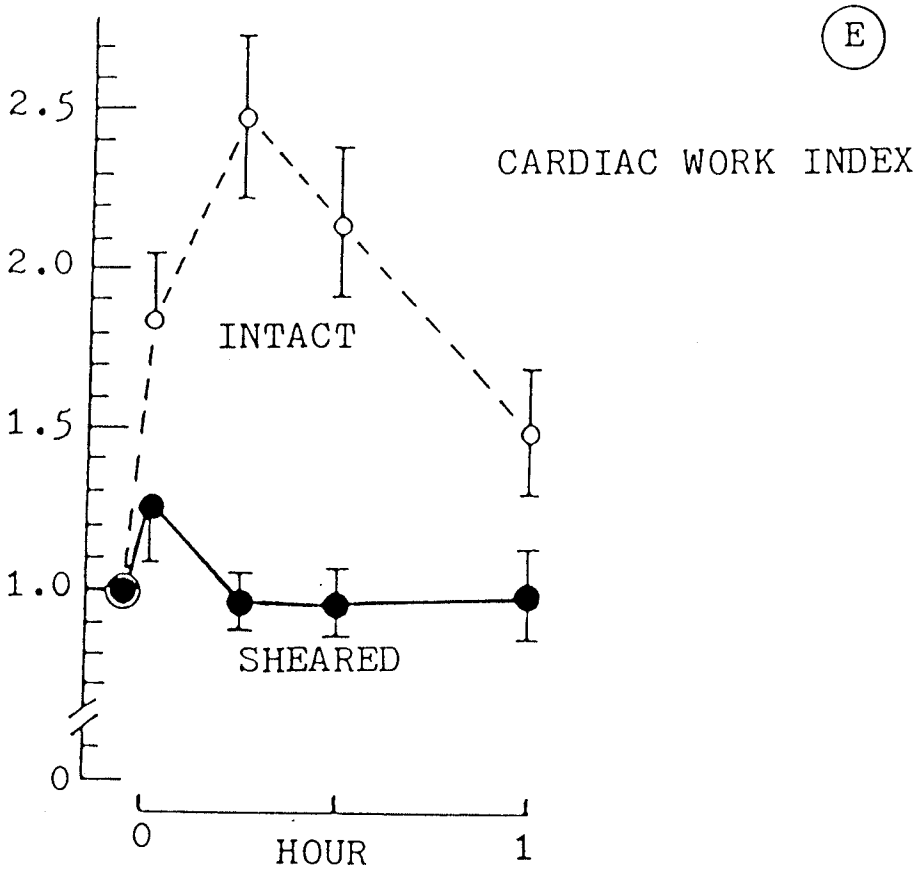

Figure 19 continued:

Panel E. Effect of sheared Separan AP-273 on cardiac work index.

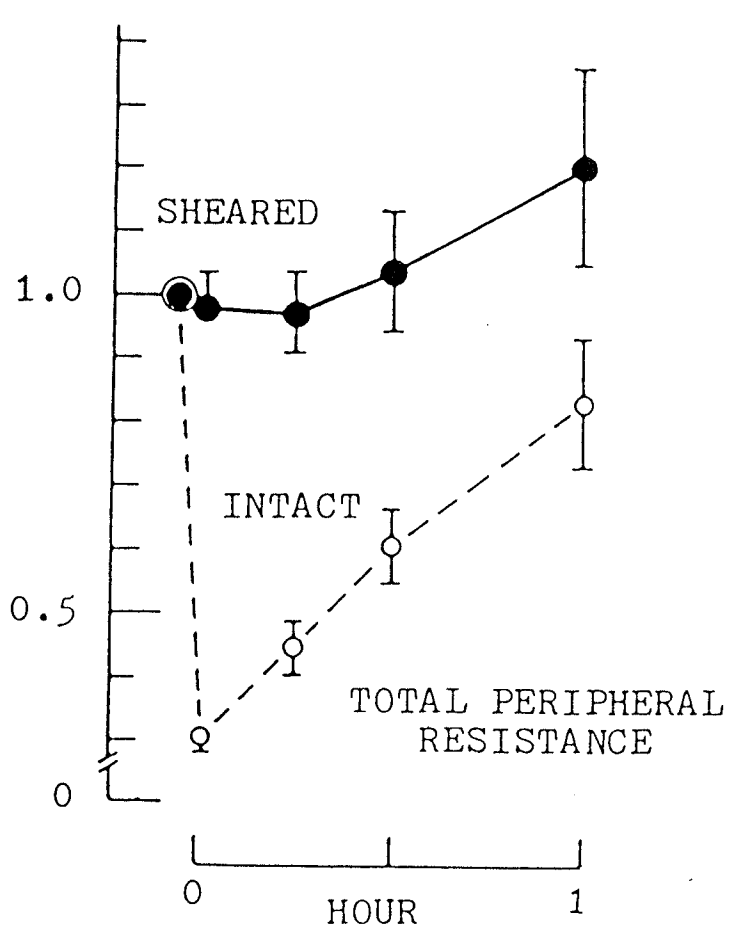

Figure 19 continued:

Panel F. Effect of sheared Separan AP-273 on total peripheral resistance.

The results from the sheared Separan experiments seen in Figure 19, panels A-E, are compatible with the hypothesis that the length of the Separan molecules is associated with the alterations in hemodynamic variables witnessed in the results with intact Separan (Fig. 15, panels A-J). Were the hemodynamic effects of Separan due to a conventional drug-receptor site interaction, we would expect that shearing a very long macropolymer -- with its repeating units -- would not usually inhibit a pharmacological response.

G. *In Vitro* Hemodynamic Study

Some of the hemodynamic data shown above is consistent with arterial vasodilation at least in a qualitative manner. The experiments with Separan were predicated on the assumption that polymer drag reduction, or some phenomenon related to it, occurs in the living circulation. It is well established that polymer drag reduction can occur in pipe blood flow, but only in the turbulent regime. The Toms phenomenon or something similar to it has been occasionally reported for flows at low Reynolds numbers, but this has never been reported for blood. It is the aim of this study to test the postulate that Separan can increase blood flow through a conduit system, described in the Methods section, at *low* Re. The demonstration of improved blood flow through such an *in vitro* system would establish that the *in vivo* hemodynamic effects of Separan are explicable by a physical mechanism novel to cardiology.

Two aliquots of human blood were well mixed with equal volumes of either 0.04% Separan solution, or the buffered saline solution alone, and the blood was then allowed to flow through a conduit system under a constant pressure gradient. The results are shown in Figure 20, Panel A and B. Each blood sample preparation was tested five times and the five results were averaged for the sample. The flow rates for the blood-saline and blood-Separan, relative to flow of blood without additive, were then calculated and averaged. A more thorough description of these experiments was described in the Methods section.

Flow remained unchanged when saline was added to blood, but when the Separan was added the flow increased by about 10%. This increment occurred despite the greater viscosity of the Separan solution compared to the saline solution or blood. Since the increases in blood flow in this system cannot be attributed to any in vivo mechanism, such as vasodilation, the possibility that Separan increased blood flow by drag reduction or a related mechanism must be considered to be plausible.

H. Toxic Effects of Separan AP-273

It quickly became clear in preliminary experiments that infusion of Separan into the rat could be deleterious or even fatal unless certain precautions were taken (Fig. 21). Many experiments were required to assess the minimal duration of mixing required for complete polymer solvation. This assessment had to be made by biological and particularly hemodynamic criteria. Outer limits had to be approximated for compatible polymer vehicle pH and storage temperatures. It soon

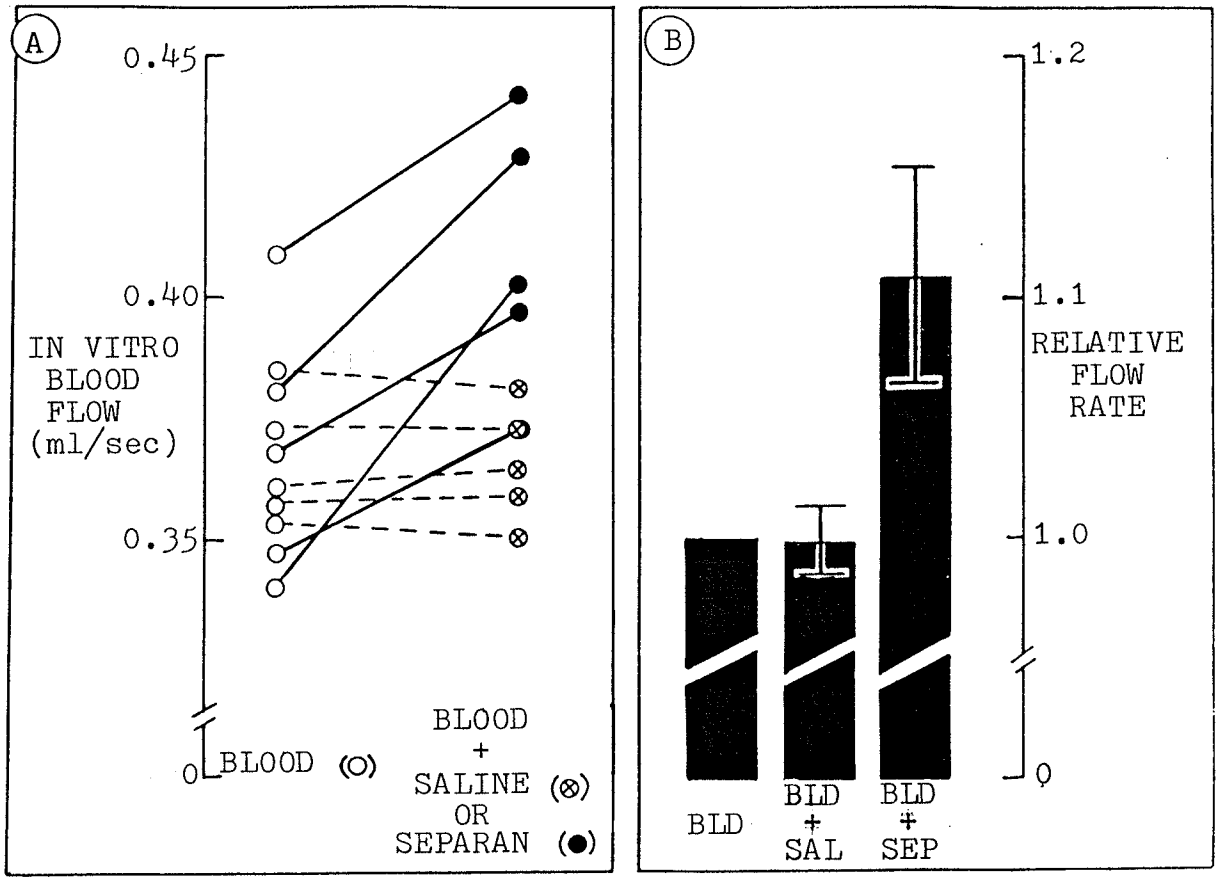

Figure 20. Effect of Separan AP-273 on <u>in vitro</u> blood flow at low Reynolds number. Panel A shows the flow rate of blood (open circles) under a constant pressure gradient through a non-linear conduit system. Two aliquots of human blood were well mixed with equal volumes of either 0.04% Separan solution (closed circles) or the solution medium alone (crossed circles) and the mixtures were then allowed to flow the conduit system. Each circle represents the mean of 5 measurements for each blood sample (N=5). The relative flow rates were then calculated and averaged in panel B. The Reynolds number of the blood flow through the apparatus was calculated to be about 40. Blood-saline and blood-Separan flows through the conduit system were statistically different (Student's t-test, $P < 0.05$).

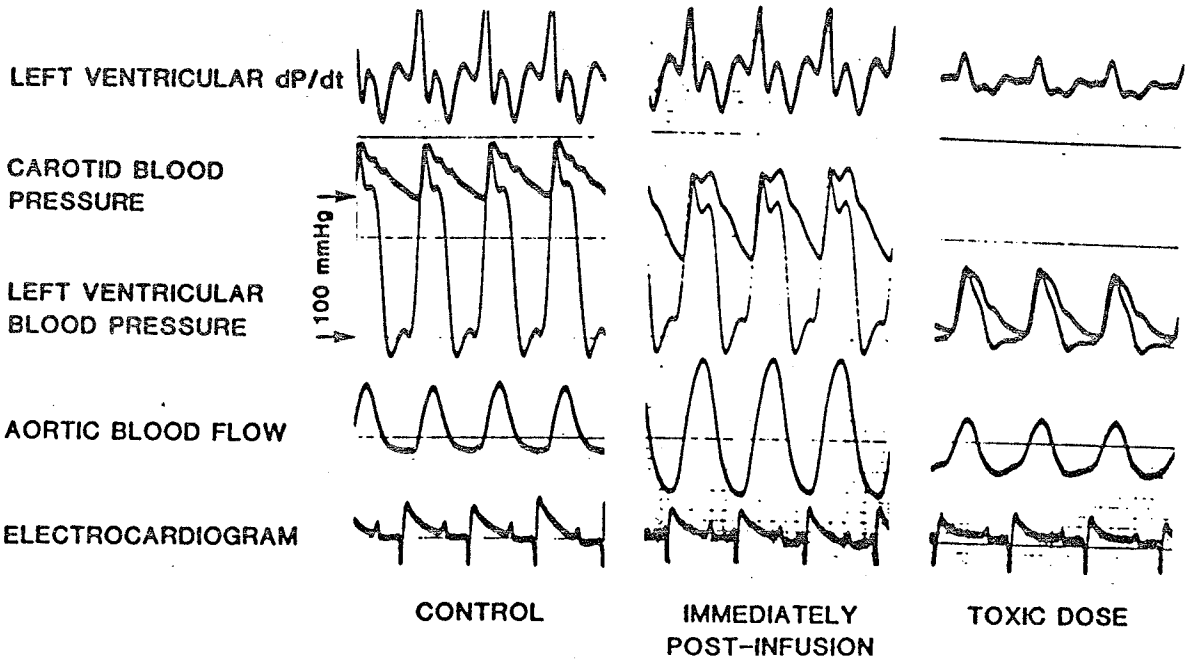

Figure 21. Effect of Separan AP-273 injection rate on hemodynamic variables. A 0.04% Separan solution was injected rapidly at low dose and caused a precipitous drop in aortic flow as well as in pressure. This result is a typical example of the deleterious effects of injecting the polymer too rapidly or at too high a concentration even at slower rates.

became apparent, however, that the major questions to be addressed concerned polymer concentration, dosage administered, and rate of polymer infusion. If any of these variables were too great, then the hemodynamic effects appeared to be the same. These effects can be divided into three stages. In the first stage the usual responses were exaggerated, particularly with regard to aortic end-diastolic pressure and blood flow. The second stage was characterized by bradycardia and a decline of cardiac output, all pressures, and dP/dt towards shock values; the heart now showed the dilatation characteristics of heart failure. In the third and final stage, the heart became increasingly arrhythmic and eventually went into ventricular fibrillation.

Although an extensive pathological or toxicological examination was not attempted, it soon became clear that even with doses of Separan eliciting the best hemodynamic response there was at least one deleterious effect, viz., the accumulation of a blood-red substance -- presumably hemoglobin -- in the urinary bladder. In experiments of long duration there appeared a yellowish bile-like material in the small intestine. At doses causing circulatory collapse the blood cells were crenated and the plasma took on a reddish hue suggestive of hemolysis. However, the hematocrit did not appear to be significantly altered.

Given the pituitous character of the polymer solution, it seems reasonable to speculate that ideally this solution should be introduced into the blood vessel with a consistency and at a rate conducive to the formation of a polymer thread that does not readily break apart.

Instead, blood flowing by the slowly moving thread should gently strip it molecule by molecule, and the anionic charges distributed along the lengths of these macro-ions ought to keep them extended parallel to the axis of flow and separate from each other. If the polymer thread were intermittently broken, however, the polymer bolus would clog the microvasculature at some point -- presumably in the lungs or heart.

IV. DISCUSSION

The main thrust of this thesis is that Separan AP-273, a linear macro-ion of extraordinary length, markedly augments cardiac output in the pentobarbital-anesthetized, open-chest rat by a novel physical mechanism (Coleman et al., 1985; Polimeni et al., 1985).

It is well established that linear macromolecules, particularly those with an extended and stiff axial structure (Sylvester & Tyler, 1970; Frommer et al., 1974), reduce resistance in many different types of non-laminar flow of aqueous or organic solutions and of blood. The chemical constitution of the macropolymer is irrelevant, providing that it is consistent with good solvation and minimal polymer-to-polymer interaction. Although a molecular weight in excess of 50,000 daltons is generally regarded as a necessary feature of the polymer to show a drag reducing effect, it is molecular length that is important rather than the weight per se. It would appear that a minimal length of 0.1 to 1 μm is necessary for drag reduction of homogeneous solutions, but the four macropolymers (Separan, Polyox, deoxyribonucleic acid, and the polysaccharide extract of okra) known to cause drag reduction in turbulent blood flow are among the longest molecules known. It appears that the Toms phenomenon occurs in blood only when the molecular length is at least 50 to 100 μm long. Although it is universally acknowledged that the mechanism(s) of polymer drag reduction is not fully understood, there is little doubt that the decreased flow resistance is

related to flow laminarization and the effect does not occur in the truly laminar regime. The alignment of the rod-like macromolecules parallel to the axis of flow apparently imposes an ordered dynamic structure similarly aligned with that axis and thereby dampens flow disturbances. The great majority of studies on the polymer drag reduction effect have been done in pipe flows above the critical Reynolds number of 1000, but a few reports indicate that the effect may be observed in oscillatory flows at low Re. The findings of Noselevich et al. (1979) are particularly interesting, for they suggest that drag reducing agents effectively reduce flow resistance through sand at head pressures comparable to those found in the vasculature. It is noteworthy that the tortuous flow pathways through the interstices of sand used in these experiments are of a magnitude comparable to the microcirculation.

A. Effects of Polymer Drag-Reducing Agents on the Cardiovascular System

In the mid-1970's two laboratories were simultaneously and independently testing two drag reducing agents in experimental animals. Mostardi and his colleagues at the University of Akron in Ohio successfully tested first the hypothesis that Separan AP-30 would reduce aortic post-stenotic flow turbulence in dogs (Mostardi et al., 1976) and later that this polymer would protect rabbits on high-cholesterol diets from developing atherosclerosis (Mostardi et al., 1978). Polimeni and his colleagues at the University of

Chicago initially tested an extract of okra similar to that shown by Castro (1972) to reduce drag in blood pipe flow. Eventually the hemodynamically active substance in the okra was purified and shown to be a polysaccharide -- consisting of rhamnose, galactose, and galacturonic acid -- of extraordinary length. Although this polysaccharide markedly enhanced cardiac output in the rat (Polimeni et al., 1977, 1978, 1979), only milligram amounts of the substance could be extracted and purified over a two week period -- an amount greatly hampering progress. Other drag reducing polymers were tested for their hemodynamic effects, but none outside the four polymers known to reduce drag in turbulent blood flow through pipes showed an increased cardiac output. Excluding Separan AP-273, the following is known about the in vivo effects of these polymers, all characterized by extraordinary molecular length.

1. Rhamnogalactogalacturonan (RGGu)

The hemodynamic effects of RGGu are described elsewhere (Polimeni et al., 1979) and alluded to in the Introduction. The most striking feature of this substance is the large increase in cardiac output (Fig. 22, panel A) concommitant with a profound diminuition of peripheral resistance. There is little effect on left ventricular dP/dt and electrical activity, but there is a noteable fall in end-diastolic blood pressure. Benjamin et al. (1951) apparently first infused a mixture of okra polysaccharides, including RGGu, into dogs in hemorrhagic shock, using the substance as a plasma expander. These

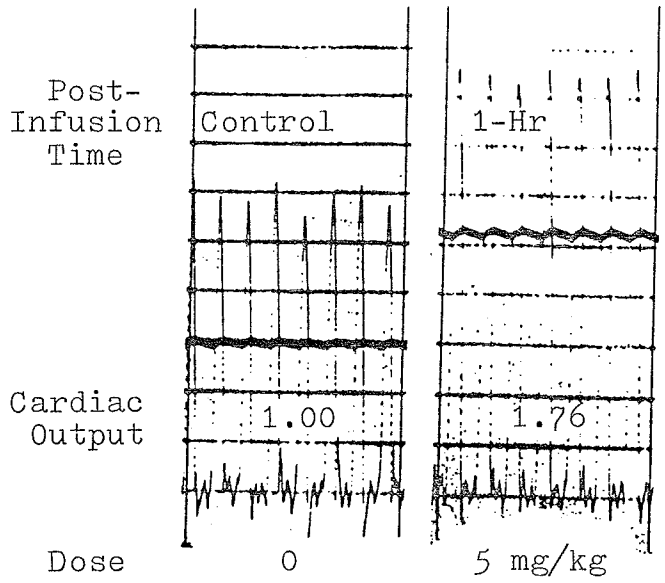

Figure 22. Drag reducing polymers enhancing cardiac output in the rat. Panel A. Rhamnogalactogalacturonan, a vegetable polysaccharide (Polimeni et al., unpubl. obs.)

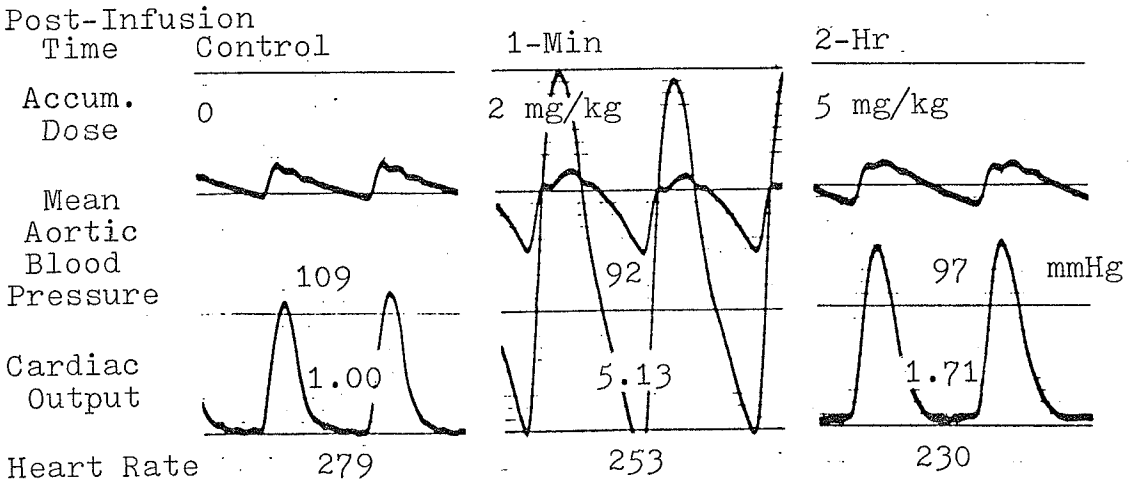

Figure 22, Panel B. Polyox, a poly(ethylene oxide) (Polimeni & Ottenbreit, unpubl. obs.).

investigators were clearly impressed with the therapeutic effects of the polymer, which appeared to go beyond improvements associated with other plasma expanders. They also reported that no toxic effects could be detected with this material.

2. Poly(ethylene oxide)

It has recently been found that Polyox WSRN-60K, a polymer with an average molecular weight of about $2 \cdot 10^6$ daltons, produced by Union Carbide Corp., enhances aortic blood flow in the rat (Polimeni and Ottenbreit, unpubl. obs.). Figure 22, panel B, shows the first recording of this flow enhancement *in vivo*. Polyox is the most effective polymer drag reducing agent known for a given molecular length, presumably because a given weight of this compound is longer than any other organic compound due to its simple chemical composition. Although the polymer is not ionic, the molecule tends to stiffen and become extended when its oxygen atoms complex with metal salts -- particular sodium -- in an ion-dipole interaction (Sylvester & Tyler, 1970).

3. Separan AP-30

Hot film anemometric recordings gave direct proof that aortic post-stenotic turbulent blood flow *in vivo* is laminarized after the injection of Separan AP-30 by Mostardi *et al.* (1976). Based on this finding Mostardi *et al.* (1978) postulated and showed that the polymer protects rabbits on high-cholesterol diets against the formation of

atherosclerotic plaques. This finding has recently been confirmed by morphometric analysis of the entire length of the aorta in our laboratory (Faruqui et al., 1985). In several exploratory studies with Separan AP-30, a polymer similar to but shorter than Separan AP-273, Polimeni and Ottenbreit (unpubl. obs.) have found that this substance also markedly augments cardiac output in the rat. The average molecular weight (and presumably also length) of Separan AP-30 is about two-thirds that of Separan AP-273.

4. Deoxyribonucleic acid

DNA obtained from calf thymus has been reported to reduce drag in pipe flow (Hoyt, 1966), but only a DNA obtained from roe has been tested in vivo. This substance had no discernible effect on rat hemodynamics (Polimeni et al., unpubl. obs.), perhaps because it lacked sufficient length or was too flexible. Clearly these experiments should be repeated with the same DNA that effectively reduced drag in blood flow in vitro.

5. Renatured xanthan

Although this substance does not properly belong in this group because the renatured polymer has never been tested for its drag reducing properties in vitro, it is another example that might support the suggestion that polymer drag reduction or some related physical phenomenon occurs in vivo. Xanthan is a natural linear polymer shown to be a drag reducing agent in water and aqueous solutions, but there

is no indication in the literature that it improves *in vitro* blood flow. Like other drag reducing polymers of moderate molecular lengths, xanthan was without effect *in vivo* (Polimeni *et al.*, unpubl. obs.). Several years after these experiments were performed Holzwarth and Prestridge (1977) published an electron micrograph of a linear xanthan constructed from several lengths of denatured xanthan joined end-to-end. A sample of this substance, which Holzwarth called "renatured" xanthan, was made available for *in vivo* tests in 1982. Unfortunately the renatured polymer could not be well solubilized and the solution was opaque and heterogeneous in its diffusion of light. This solution was injected into the canine femoral artery to utilize the microcirculation of the leg as a filter. Although the results of four experiments were quite variable, aortic blood flow measured with an intravascular electromagnetic velocity probe indicated that the polymer increased cardiac output. In one experiment this effect was particularly striking (see Fig. 6A). While it is acknowledged that these results are problematic, because the results were quite variable and it was not possible to exclude some reflex mechanisms related to ischemia in the limb acting as a filter. Nonetheless, the fact that short-chained xanthan had no hemodynamic effect, whereas the renatured xanthan increased aortic flow, is compatable with the hypothesis that relatively stiff polymers of very long lengths reduce peripheral resistance by a physical mechanism.

B. No Evidence of Direct Effects by Separan on Myocardial Inotropy or Vasoactivity

The hemodynamic data suggest that upon the injection of Separan there is a tremendous fall in total peripheral resistance, with left ventricular and aortic blood pressures falling concommitantly with a marked increase in blood flow. The hypotension is transient and the pressures quickly reach or exceed control values. Evidence of reduced peripheral resistance is suggested, independently of the validity of the flow probe recordings, by the clear indication that arterial runoff has improved, viz., the fall in arterial pressure during diastole is much steeper than before administration of the drug. This diastolic slope is steeper even during the brief period of transient hypotension, during which time peripheral resistance must have fallen. This interpretation of the hemodynamic data does not depend on the flow recording. Therefore the possibility that Separan is a powerful vasodilator must be considered, even if the marked augmentation of cardiac output is not generally associated with vasodilation. The possibility that Separan is initially a negative inotropic agent and/or later becomes a positive inotropic agent also cannot be excluded from the in vivo hemodynamic data.

In a recent study by Bose et al. (1985) isolated smooth muscle preparations obtained from several rat blood vessels were incubated with Separan solutions containing concentrations of the polymer comparable to those applicable in vivo. The vessels included the aorta, renal, mesenteric, and carotid arteries, and portal and jugular

veins. Separan did not provoke any changes in resting isometric tension, nor did it alter the effects on vascular tone by submaximal concentrations of noradrenaline.

The results of Bose *et al.* complement earlier preliminary observations in the rat *in vivo* preparation, where RGGu had no effect on the diameters of small mesentery arteries and arterioles as measured electronically (Polimeni, personal communication). Mesenteric venules did expand slightly in the *in vivo* study, but this response probably represented a passive dilatation of a highly compliant tube receiving a large increment of flow. Although flow was not quantified in these exploratory studies, individual red blood cells -- observable as distinct entities before injection of RGGu -- became a fast-moving blur after administration of the polysaccharide. It would be difficult to interpret the venodilation -- apparently limited to the immediate post-capillary region -- as an active phenomenon, because such a response would be incompatible with a large increase in cardiac output.

The published results of Vetterlein *et al.* (1979) tend to support the view that arterial vasodilation is not likely to explain the effects of Separan on blood flow. In their study of the effects of six vasodilators on cardiac output in the urethan-anaesthetized rat only modest increases in flow could be elicited at best (Table 7). Isoproterenol and pentoxyfylle both increased cardiac output by nearly one-fourth, but four other vasodilators had no significant effect (bencylane, acetylcholine, dihydroergotoxine) or diminished it

Drug	Normalized cardiac output		
	Control		Test
Isoproterenol	1.00±0.06	*	1.23±0.08
Pentoxifylle	1.00±0.11	*	1.24±0.11
Bencylane	1.00±0.06		1.13±0.07
Acetylcholine	1.00±0.06		1.04±0.10
Dihydroergotoxine	1.00±0.05		1.02±0.05
Nitroprusside	1.00±0.05		0.83±0.08

*P < 0.05 (Wilcoxon matched-pairs signed-rank test)
Anesthesia : Urethan

Table 7. Effect of vasodilators on cardiac output in the rat. The effects of six vasodilators on the normalized cardiac output of urethan-anesthetized, open-chest rats (N = 6) are compared. Only isoproterenol and pentoxifylle showed a statistically significant increase in cardiac output. Modified from Vetterlein et al., 1979.

(nitroprusside). Thus, the large increase in blood flow obtained in the rat with linear polymers is not characteristic of vasodilators.

The direct effect of Separan on myocardial inotropy was tested in isolated trabeculae from the right ventricle of the rat and dog stimulated at 1 Hz at optimum resting length (Bose et al., 1985). No inotropic effects were observed at Separan concentrations comparable to those used in vivo without deleterious results. But at slightly higher polymer concentrations a small negative inotropic effect was demonstrated, i.e., isometric contractile tension declined. Therefore, it is concluded that any direct effect that Separan might have on the myocardium would not explain the improvement in cardiac output in terms of inotropy.

C. Does Separan Increase Flow By an Effect on Blood?

In the last decade several drugs have been demonstrated to improve blood flow by their hemorheologic effects (Dormandy & Matrai, 1983) i.e., by deceasing blood viscosity. The two best known mechanisms of reducing blood viscosity, apart from hemodilution and mechanical defibrinogenation, involve the lysis of fibrinogen and rendering erythrocytes more flexible. The snake venom extract, Arwin (Ehrly, 1973), and pentoxifylline (Aviado & Porter, 1984), respectively, are examples of these types of hemorheologic agents. Is Separan such an agent?

No rheological studies have been made on blood containing Separan and the question of a possible direct effect of the polymer on blood

remains open. However, the little information there is relating to the subject suggests that the answer is no. Firstly, the Separan solution is itself somewhat more viscous than blood over a range of shear rates (Polimeni, unpubl. obs.) and the volume injected is too small to significantly alter blood viscosity in any case. Secondly, the rheological properties of blood containing RGGu solution, which was extensively studied, were found to be slightly higher than blood containing an equal volume of saline solution over the entire range of shear rates known to exist in vivo (Polimeni and Al-Sadir, unpubl. obs.). This observation conforms to similar findings in many engineering and hydrodynamic studies of both aqueous and organic solvents. Thirdly, it is unlikely that the central characteristics -- molecular linearity, stiffness, and great extension -- of at least three polymers showing powerful hemodynamic effects have anything in common that would be expected to alter blood rheology, except to make the blood more rather than less viscous.

Another possibility that must be considered is that the polymer draws interstitial fluid into the vascular compartment, thereby reducing blood viscosity by hemodilution and increasing blood volume -- both results being capable of increasing cardiac output. Such a possibility is plausible, because even though the blood concentration of Separan is only about 5 nanomoles (based on polymer average molecular weight) there can be a marked Donnan effect. However, this possible fluid shift does not appear to occur in vivo. Polimeni and Otten (unpubl. obs.) have found that during the dialysis of a

polymer-saline buffer solution against the saline buffer alone there is an anomalous shift of fluid (~10%) from the polymer retentate solution into the dialysate. This shift is in the opposite direction from that normally expected and from what in fact does happen when the vehicle solution is water instead of saline buffer. This phenomenon is qualitatively explicable (Polimeni and Otten, paper in prep.) by a consideration of the second virial coefficient in the van't Hoff equation for the osmotic pressure of charged macromolecules in the presence of two monovalent ions (Tombs & Peacocke, 1974; Eisenberg, 1976). However, the Separan solution is presumably balanced osmotically after dialysis and there is no basis for assuming that the solution would draw interstitial fluid into the vasculature. The fact that hemotocrit is found to be entirely normal even after long duration experiments with Separan supports this view.

D. Does Separan Increase Cardiac Output By Drag Reduction?

The overwhelming majority of reports on polymer drag reduction concern flows at high Reynolds numbers, generally well into the turbulent regime. Some studies have already been alluded to of a similar phenomenon in various types of disturbed flow, i.e., flow that is neither laminar nor frankly turbulent (Barnes et al., 1969; Voitkounsky et al, 1972; Driels & Ayyash, 1976; Noselevich et al., 1979). These disturbances may be found even at very low Re in pulsatile flow or flow through conduits of complex geometry, particularly elastic conduits.

The in vitro experiments conducted with blood containing Separan in the present study confirms that Separan can indeed reduce drag in blood flowing at a Re that would be considered to be laminar were the flow through a straight pipe. It has long been known that the critical Re is much lower not only in oscillating flow (Meisner & Rushmer, 1963), but even in steady flow (Stehbens, 1960; Roach et al., 1972) when the pipe is not straight. Despite considerable progress in the understanding of fluid mechanics in the last quarter-century, it is still true that the generally accepted definitions of turbulence apply to steady flow and the situation in pulsatile flow remains obscure (McDonald, 1960). There can be little doubt that the pulsatile flow of a non-Newtonian fluid through an elastic system of tubes -- curving, bifurcating, converging, tapering, and dilating -- must have a considerable component of frictional resistance apart from the viscous component, particularly when tumbling and obligue (to the axis of flow) movements of blood cells are considered. What is questionable is whether or not a laminarization of such disturbed flow would be quantitatively significant in reducing flow friction. In principle, one way that in vivo polymer drag reduction could be established would be by comparing the pressure gradients between the femoral artery and vein, before and after addition of Separan or polymer vehicle to blood perfusing a maximally dilated limb vasculature at a constant rate of flow.

Analysis of the hemodynamic data obtained in the present experiments suggests that the results are compatible with the

hypothesis of an *in vivo* Separan-induced drag reduction, but it does not prove it. However, several considerations give support to this hypothesis. Firstly, the data are not readily explicable by the usual cardiovascular mechanisms. Secondly, the *in vitro* flow study demonstrates that polymer drag reduction can occur at low Re when the pipe system is even minimally complex. Thirdly, it is extremely unlikely that three, possibly four, chemically different polymers -- having only linearity, stiffness, and extension in common -- would fortuitously have fundamentally similar hemodynamic effects both *in vitro* and *in vivo*. As difficult as it might be to accept the drag reduction hypothesis for blood flow in the living organism, a unifying hypothesis explaining the hemodynamic effects of the drag reducing polymers is more difficult still. This is particularly true when it is remembered that when the RGGu and Separan macro-ions were shortened the hemodynamic effects seen with the intact macropolymers disappeared. Were the polymer effect not based on a physical mechanism, but on some receptor interaction, shortening of polymers with repeating units even to the order of a single micrometer would not be expected to significantly alter a receptor response.

E. Comparison of Hemodynamic Data in Present Experiments With Those Obtained in Conscious Rats by Iriuchijuna and Teranishi

Comparison of the present hemodynamic results with those of Iriuchijuna and Teranishi (1982) obtained in the conscious rats (Table 8) is instructive. It is clear that the cardiac index is

HEMODYNAMIC VARIABLES	CONSCIOUS RAT (Iriuchijuna & Teranishi, 1982)	PENTOBARBITAL-ANESTHETIZED OPEN-CHEST RAT		
		CONTROL		SEPARAN
HEART RATE (beats/min)	405 ± 46	328 ± 40	*	307 ± 42
CARDIAC INDEX (ml/min/100g)	23.9 ± 5.8	8.7 ± 2.2	**	20.7 ± 6.1
MEAN ARTERIAL PRESSURE (mmHg)	111 ± 12	99 ± 11		102 ± 16
CARDIAC WORK INDEX (mmHg·ml/min/100g)	2650 ± 660	856 ± 237	**	2086 ± 603
T.P.R. INDEX (mmHg·min/ml/100g)	4.91 ± 1.14	12.2 ± 3.8	**	5.44 ± 2.16
Number of Experiments:	25	12		

Table 8. Comparison of several hemodynamic variables in conscious rats (Iriuchijuna and Teranishi, 1982) and pentobarbital-anesthetized, open-chest rats before (control) and after (Separan) intravenous injection of 5ml/kg of 0.04% Separan AP-273. The asterisks indicate statistical significance (Students paired t-test) between control and Separan data: *$P < 0.02$ and **$P < 0.001$. Assuming a molecular weight of 6,000,000, a polymer dose of 2 mg/kg is equivalent to an *in vivo* molal concentration less than 5 nmol./liter blood.

markedly lower in the Separan control rats. Much of the difference in the cardiac indices can be attributed to the cardiodepressant effects of pentobarbital, which perhaps accounts for one-fourth to half of the difference (Popovic & Kent, 1964; Salgado & Krieger, 1976; Smith & Hutchins, 1980). Part of the difference is undoubtedly due to the reduction of venous return subsequent to increased venous resistance associated with elevated intrathoracic pressure after thoracotomy. Some of the difference may have been due to inexperience with the open-chest model, because more recent values of cardiac index tend to be at least 50% greater. Smith and Hutchins (1980) did not observe an increase in peripheral resistance in the pentobarbital-anesthetized intact rat. Because of technical differences a detailed comparison of the two sets of data probably is not warranted. However, the comparison suggests that Separan does not improve the cardiovascular variables beyond normal, but causes an amelioration bringing the variables to approach normal values. This conclusion is in line with our subjective impression that hemodynamic improvements appear to be greatest in those hearts that initially appear to be functioning poorly -- i.e., relatively low cardiac index and blood pressures. There was a similar impression also with RGGu (Polimeni, personal communication).

F. Separan's *In vivo* Mechanism of Action: a Hypothesis

For reasons discussed above, the primary mechanism of hemodynamic action by Separan appears to involve a powerful fall in peripheral resistance, putatively due to a physical phenomenon related to polymer

drag reduction. That peripheral resistance falls initially is clear and not dependent on the accuracy of the aortic flow probe, because diastolic runoff is facilitated even when the pressure head has fallen. This facilitation is evidenced by the steeper slope of aortic pressure during diastole.

If the effect of Separan involves some form of drag reduction, then the primary effect is not necessarily limited to the arterial side of the vasculature. It would be effective wherever there are flow disturbances. Although postcapillary resistance has relatively little direct effect on cardiac output, changes in venous resistance significantly influence the output by altering the preload (Greenway, 1982). Given the profound hypotension observed immediately after injection of Separan, it can reasonably be assumed that a powerful sympathetic response will ensue via the baroreceptor reflex. This reflex can be expected to cause both arterial and venous constrictions, thereby directly increasing arterial pressure and, more importantly, increase venous return due to reduced venous capacitance. Thus the initial increase in cardiac output is probably due to reduced afterload, but the situation is quickly altered. With the reduction of venous, and to a lesser extent arterial, capacitance there is a shift of blood volume from the vasculature to the heart. It is difficult to be certain by visualization that ventricular volume has expanded, although that is our impression. However, the atria can readily be observed to be distended and left ventricular end-diastolic blood pressure rises despite the marked increase in ventricular outflow.

It is well established that distension of the right atrium, left ventricle, and the baroreceptors, all apparently occurring after administration of Separan, can cause reflex bradycardia and systemic hypotension. These reflexes, if elicited by Separan, would usually be mediated by increased vagal activity to the heart and inhibition of sympathetic vasoconstriction. Heart rate is indeed slightly (~6%) diminished and the likelihood of vagal involvement is suggested by the observation that the P-R interval is increased. The paradox is that not only is there no evidence that sympathetic activity is inhibited, but the hemodynamic data are consistent with a continuation of sympathetic activity even after mean arterial blood pressure has risen by about 10%. The main reason for this suggestion is that the increased venous return, visual distention of the heart, and left ventricular hypertension are inconsistent with venodilation. While there is no obvious explanation why Separan should maintain sympathetic activity above normal after the initial hypotension is reversed, simultaneous stimulation of vagal and sympathetic activities is known to be possible (e.g., in the Cushing reflex). A negative chronotropic response is consistent with such a dual neural activity, because vagal activity dominates over sympathetic activity with respect to heart rate but not contractile force.

Examination of the heart, particularly the atria, after injection of Separan resembles cardiac congestion. Aside from the flow recording, which indicates a voluminous cardiac output, the associated hypertension is consistent with increased contractile force and not

myocardial failure. If our suggestion of enhanced sympathetic activity is correct then hypertension and improved flow would partly be due to a catecholamine-induced positive inotropy. However, what is less doubtful is that part of enhanced vigor with which the heart contracts is related to the myocardial distention. That is, the volume shift from vasculature to heart moves the length-tension relationship up the ascending limb of the Frank-Starling curve.

Whatever the actual cardiovascular mechanism might be in the augmenting of cardiac output by Separan, it is clear that changes in two of the major determinants of myocardial work, blood pressure and heart rate, tend to offset each other. Ventricular tension development is likely to increase disproportionately to the afterload increment, due to the Laplace relationship between circumferential tension and ventricular radius. It is therefore probable that myocardial oxygen consumption increases with Separan administration, but cardiac efficiency might still be improved if the increment in flow exceeds that of oxygen consumption.

G. Toxic Effects of Separan

A formal toxicological examination of Separan has not been attempted in our laboratory, but some information on the subject is available from the industrial literature and from general observation. The lethal oral dose of Separan exceeds the rat's body weight, but about 6 mg Separan per kg body weight appears to be a lethal intravenous dose for the rat. The optimal dose for enhancing cardiac

output, 4 mg/kg, is dangerously close to a fatal dose (Polimeni and Ottenbreit, unpubl. obs.).

A peculiarity of Separan is that the pentobarbital-anesthetized, open-chest rat seems to be more susceptible to the toxic effects of the polymer than does the intact rat, which shows no obvious acute or long-term effects by visual inspection and autopsy after single doses of Separan. Rabbits on high-cholesterol diets receiving tri-weekly injections of Separan for up to 5 months appear far more healthy than rabbits on the same diet not receiving the drug, although not as healthy as rabbits on a regular diet. The body weights parallel this subjective assessment (Mostardi et al., 1978; Faruqui et al., unpubl. obs.). The situation is different in the open-chest rat, where a substantial amount of hemoglobin accumulates in the urinary bladder. After several hours of experimentation with high doses of Separan the erythrocytes are sometimes crenated but the hematocrit is normal. Under these conditions a yellowish fluid, probably bile, appears in the small intestine.

An acutely fatal dose usually provokes cardiac arrythmia followed by profound hypotension and eventually reduced cardiac output associated with cardiac congestion. Clearly an in depth toxicological study would be necessary before the drag reducing agents could be considered for therapeutic application given the tendency of macromolecules to occasionally cause hemostatic alterations, renal dysfunction, and anaphyllactoid reactions (Laxenaire et al., 1976).

H. Some Practical Considerations

Whether or not Separan is therapeutically useful and what the precise mechanism turns out to be is of secondary importance. The value of this thesis depends on whether or not the postulated principle -- that stiff macromolecules of extraordinary linear dimensions cause increased flow by a novel physical mechanism -- is or is not valid. The number of suitable polymers is likely to become large and the polymers more homogeneous with recent advances in polymer synthesis (Teyssie, 1984). Thus those polymers with serious side-effects should be easily replaceable, providing that great linear size *per se* is not problematic.

Polymer drag reduction has several features that are relatively advantageous, particularly when it is considered that this mechanism is likely to be applicable in conjunction with other categories of drugs.

A physical mechanism that reduces flow friction is likely to offer an uniquely novel approach to a wide range of cardiac and vascular diseases.

V. CONCLUSION

Separan AP-273, a polydisperse polyacrylamide of linear conformation, markedly enhances cardiac output in the pentobarbital-anaesthetized, open-chest rat. Although the mechanism appears to be complex, it is concluded that the primary hemodynamic effect of the macro-ion is based on a physical phenomenon probably related to polymer drag reduction.

REFERENCES

Aviado, D.M., and Porter, J.M. Pentoxifylline: a new drug for the treatment of intermittent claudication. Pharmacotherapy 4: 297-307, 1984.

Bark, F.H., Hinch, E.J., and Landahl, M.T. Drag reduction in turbulent flow due to additives: a report on Euromech 52. J. Fluid Mech. 68: 129-138, 1975.

Barnes, H.A., Townsend, P., and Walters, K. Flow of non-Newtonian liquids under a varying pressure gradient. Nature 244: 585-587, 1969.

Benjamin, H.G., Ihrig, H.K., and Roth, D.A. The use of okra as a plasma replacement. Rev. Can. Biol. 10: 215-221, 1951.

Block, E.H. High speed cinephotography of the microvascular system. In: Hemorheology, edited by Copley, pp. 655-667, Pergamon, Oxford, 1968.

Bose, R., Bose, D., and Polimeni, P.I. Do polymer drag-reducing agents enhance cardiac output by flow laminarization? Can. Fed. Biol. Soc., abstract in press.

Bove, A.A., Gimenez, J.L., and Stauffer, H.M. Addition of polymer to radiopaque contrast material to decrease catheter flow resistance. Radiology 93: 381-383, 1969.

Brandt, H., McDonald, A.T., and Boyle, F.W. Turbulent skin friction of dilute polymer solutions in rough pipes. In: Viscous Drag Reduction, edited by C.S. Wells, pp. 159-171, Plenum, New York, 1969.

Burger, E.D., Chorn, L.G., and Perkins, T.K. Studies of drag reduction conducted over a broad range of pipeline conditions when flowing Prudhoe Bay crude oil. J. Rheol. 24: 603-626, 1980.

Caro, C.G., Pedley, T.J., Schroter, R.C., and Seed, W.A. The Mechanics of the Circulation. Oxford Univ. Press, New York, 1978.

Castro, W.E. Reduction of flow friction with polymer additives. Water Resources Research Institute, Clemson Univ., Report #24, 56 pp., 1972.

Castro, W.E., and Neuwirth, J.G. Reducing fluid friction with okra. Chem. Technol. 1: 697-701, 1971.

Coleman, P., Ottenbreit, B., and Polimeni, P.I. Increasing cardiac output with a polymer drag-reducing agent, Separan AP-273. Can. Fed. Biol. Soc., abstract in press.

Dinner, U. Cardiovascular Fluid Dynamics. CRC Press, Boca Raton, 1981.

Dormandy, J.A. Clinical significance of blood viscosity. Ann. R. Coll. Surg., Engl. 47: 211-228, 1970.

Dormandy, J.A., and Matrai, A. Assessment of pharmacological agents with a hemorheological action. Ann. N.Y. Acad. Sci. 416: 599-610, 1983.

Driels, M.R., and Ayyash, S. Drag reduction in laminar flow. Nature 259: 389-390, 1976.

Dunlop, E.H., and Cox, L.R. Influence of molecular aggregates on drag reduction. Phys. Fluids 20: S203-213, 1977.

Ehrly, A.M. Influence of Arwin on the flow properties of human blood. Biorheology 10: 543-549, 1973.

Eisenberg, M. Biological Macromolecules and Polyelectrolytes in Solution. Clarendon Press, London, 1976.

Elata, C., Lehrer, J., and Kahanovitz, A. Turbulent shear flow of polymer solutions. Israel J. Technol. 4: 87-95, 1966.

Elata, C., and Tirosh, J. Frictional drag reduction. Israel J. Technol. 3: 1-6, 1965.

Fabula, A.G. The Toms phenomenon in the turbulent flow of very dilute polymer solutions. 4th Int. Congr. Rheol. Providence, R.I., 1963.

Fabula, A.G., Lumley, J.L., and Taylor, W.D. Some interpretations of the Toms effect. In: Modern Development in the Mechanics of Continua, Academic Press, New York, 1966.

Faruqui, F., Otten, M.D., and Polimeni, P.I. Protection against atherogenesis using a polymer drag-reducing agent, Separan AP-30. Can. Fed. Biol. Soc., abstract in press.

Fowler, N.O., and Holmes, J.C. Blood viscosity and cardiac output in acute experimental anemia. J. Appl. Physiol. 39: 453-456, 1975.

Frommer, M.A., Feder-Lavy, A., and Kraus, M.A. Dependence of the drag reduction efficiency of polyelectrolytes on their conformation in aqueous solutions. J. Colloid. Interface Sci. 48: 165-169, 1974.

Gadd, G.E. Turbulence damping and drag reduction produced by certain additives in water. Nature 206: 463-467, 1965.

Gadd, G.E. Reduction of turbulent drag in liquids. Nature 230: 29-31, 1971.

Gampert, B., and Wagner, P. Turbulent flow with polymer additives. Arch. Mech. 34: 493-502, 1982.

Gilbert, C.G., and Ripken, J.F. Drag reduction on a rotating disk using polymer additive. In: Viscous Drag Reduction, edited by C.S. Wells, pp. 252-263, Plenum, New York, 1969.

Giles, W.B. Orifice flow of polyethylene oxide solutions. Nature 224: 584-585, 1969.

Good, N.E. Hydrogen Ion Buffers for Biological Research. Anal. Biochem 104: 300-310, 1980.

Granville, P.S. The frictional resistance and velocity similarity laws of drag-reducing dilute polymer solutions. NSRDC Report 2502, 1967. Cited by Hoyt, 1972.

Green, J.H. Effect of polymer additives on nozzle stream coherence: A preliminary study. Naval Undersea R&D Center TN 504, 1971. Cited by Hoyt, 1972.

Greene, W.H., Farrel, G.A., and Schneck, D.V. In vivo demonstration of junctional blood flow disturbance by hot wire anemometry. Atherosclerosis 11: 485-496, 1970a.

Greene, H.L., Nokes, R.F., and Thomas, L.C. Drag reduction in pulsed flow of blood. Med. Res. Engng. 9: 19-21, 1970b.

Greene, H.S., Sebok, D.A., and Jenkins, D.L. Characterization of disturbed flows of blood and sucrose solutions during extracorporeal pumping. A.I.Ch.E. J. 74 (Symp. Ser. #182): 22-26, 1978.

Greenway, C.V. Mechanisms and quantitative assessment of drug effects on cardiac output with a new model of the circulation. Pharmacol. Rev. 33: 213-251, 1982.

Grigoryan, S.S., et al. (11 co-authors). On the mechanism of the Toms effect. Sov. Phys. Dokl. 24: 829-832, 1979.

Gutstein, W.H., Farrell, G.A., and Schneck, D.J. In vivo demonstration of junctional blood flow disturbance by hot wire anemometry. Atherosclerosis 11: 485-496, 1970.

Hansen, R.J., The reduced drag of polymer solutions in turbulent and transient laminar shear flows. Trans. A.S.M.E., J. Fluids Engng. 95: 23-28, 1973.

Hansen, R.J., and Little, R.C. Pipe diameter, molecular weight, and concentration effects on the onset of drag reduction. Chem Engng. Progr. Symp. Ser. 111, 93-97, 1971.

Hershey, H.C., and Zakin, J.L. Existence of two types of drag reduction in pipe flow of dilute polymer solutions. I&EC Fundamentals 6: 381-388, 1967.

Holt, J.B. Drag reducers boost crude-line throughput. Oil and Gas J. 79: 272-276, 1981.

Holzwarth, G., and Prestridge, E.B. Multistranded helix in xanthan polysaccharide. Science 197: 757-759, 1977.

Hoyt, J.W. Turbulent-flow properties of deoxyribonucleic acid solutions. Nature 211: 170-171, 1966.

Hoyt, J.W. Drag-reduction effectiveness of polymer solutions in the turbulent-flow rheometer: a catalogue. Polymer Lett. 9: 851-862, 1971.

Hoyt, J.W. The effect of additives on fluid friction. J. Basic Engng., Trans. A.S.M.E. D94: 258-263, 1972.

Hoyt, J.W. Recent progress in polymer drag reduction. Int. Conf. Polymers & Lubrication, Brest, 1974.

Hoyt, J.W., and Soli, G. Algal cultures: ability to reduce turbulent friction in flow. Science 149: 1509-1511, 1965.

Hoyt, J.W., and Taylor, J.J. Turbulence structure in a water jet discharging in air. Phys. Fluids. 20: S253-257, 1977a.

Hoyt, J.W., and Taylor, J.J. Waves on water jets. J. Fluid Mech. 83: 119-127, 1977b.

Hunston, D.L., and Reischman, M.M. The role of polydispersity in the mechanism of drag reduction. Phys. Fluids 18: 1626-1629, 1975.

Iriuchijuna, J., and Teranishi, Y. Cardiac output in conscious and almost unrestrained spontaneously hypertensive rats. Hiroshima J. Med. Sci. 31: 259-265, 1982.

James, D.F., and Saringer, J.H. Extensional flow of dilute polymer solutions. J. Fluid Mech. 97: 655-671, 1980.

Keller, A., Kiss, G., and Mackley, M.R. Polymer drag reduction in Taylor vortices. Nature 257: 304-305, 1975.

Kenis, P.R. Drag reduction by bacterial metabolites. Nature 217: 940-942, 1968.

Kohn, M.C. Energy storage in drag-reducing polymer solutions. J. Polymer Sci. 11: 2339-2356, 1973.

Ku, D.N., and Giddens, D.P. Pulsatile flow in a model carotid bifurcation. Arteriosclerosis 3: 31-39, 1983.

Kuo, J.T., and Kovasznay, L.S.G. Drag reducing polymer in helicoidal flow. J. Fluids Engng. 103: 491-496, 1981.

Laxenaire, M.C., Moneret-Vautrin, D., Sigiel, M., Jacob, F., and Picard, J.M. Incidents et accidents observés lors de l'utilisation des macromolécules. Anesth. Anat. Réan, 33: 691-702, 1976.

Lee, W.K., Vaseleski, R.C., and Metzner, A.B. Turbulent drag reduction in polymeric solutions containing suspended fibers. A.I.Ch.E. J. 20: 128-133, 1974.

Lefort, M., Stoltz, J.F., and Larcan, A. Singularités vasculaires et mécaniques des écoulements. Aggressologie 17: 189-194, 1976.

Little, R.C. Effective salt concentration on the drag reduction efficiency of polyethylene oxide. Nature 242: 79-80, 1973.

Lumley, J.L. Drag reduction by additives. Am. Rev. Fluid Mech. 1: 367-384, 1969.

MacGregor, E.A., and Greenwood, C.T. Polymers in Nature. Wiley, New York, 1980.

McComb, W.D., and Rabie, L.H. Drag-reducing polymers and turbulent bursts. Nature 273: 653-654, 1978.

McDonald, D.A. The velocity profiles of pulsatile blood flow. In: Flow Properties of Blood, edited by A.L. Compey and G. Stainsby, pp. 84-95, Pergamon, New York, 1960.

Meisner, J.E., and Rushmer, R.F. Eddy formation and turbulence in flowing liquids. Circ. Res. 12: 455-463, 1963.

Metzner, A.B., and Park, M.G. Turbulent flow characteristics of viscoelastic fluids. J. Fluid Mech. 20: 291-303, 1964.

Molyneux, P. Water-Soluble Synthetic Polymers: Properties and Behavior, 2 volumes. CRC Press, Boca Raton, Florida, 1983.

Mostardi, R.A., Greene, H.L., Nokes, R.F., Thomas, L.C., and Lue, T. The effect of drag reducing agents on stenotic flow disturbances in dogs. Biorheology 13: 137-142, 1976.

Mostardi, R.A., Thomas, L.C., Green, H.L., Van Esson, F., and Nokes, R.F. Supression of atherosclerosis in rabbits using drag reducing polymers. Biorheology 15: 1-14, 1978.

Mysels, K.J. Flow of thickened fluids. U.S. Patent 2,492,173, Dec. 27, 1949.

Narkis, N., and Rebhun, M. Aging effects in measurements of polyacylamide solution viscosities. Polymer (London) 7:507-512, 1966.

Nerem, R.M., and Seed, W.A. An in vivo study of aortic flow disturbance. Cardiovasc. Res. 6: 1-4, 1972.

Noselevich, V.A., Kalinichenko, N.M., and Pilipenko, V.N. Resistance during filtration of dilute polymer solutions. Sov. Phys. Dokl. 24: 830-832, 1979.

Ohlsson, N.M. Left heart and aortic blood flow in the dog. Acta Radiol. Suppl. 213: 1-80, 1962.

Patterson, R.W., and Abernathy, F.H. Turbulent flow reduction and degradation with dilute polymer solutions. J. Fluid Mech. 43: 689-710, 1970.

Peyser, P., and Little, R.C. The drag reduction of dilute polymer solutions as a function of solvent power, viscosity, and temperature. J. Appl. Polymer Sci. 15: 2623-2637, 1971.

Polimeni, P.I., Al-Sadir, J., and Cutilletta, A.F. Rhamnogalacto-galacturonan (RGGu): a drug increasing cardiac output in rat, perhaps by a novel mechanism. Physiologist 20: 75, 1977 (Abstract).

Polimeni, P.I., Al-Sadir, J., and Cutilletta, A.F. Polysaccharide for enhancement of cardiac output. U.S. Patent 4,154,922, May 15, 1979.

Polimeni, P.I., Cutilletta, A.F., and Al-Sadir, J. Increased cardiac output by a drag reducing agent extracted from okra. J. Molec. Cell. Cardiol. 10: 78, 1978 (Abstract).

Polimeni, P.I., Ottenbreit, B., and Coleman, P. Enhancement of aortic blood flow with a linear anionic macropolymer of extraordinary molecular length. J. Molec. Cell. Cardiol., in press.

Popovic, V.P., and Kent, K.M. 120-day study of cardiac output in unanesthetized rats. Am. J. Physiol. 207: 767-770, 1964.

Ram, A., Finklestein, E., and Elata, C. Reduction of friction in oil pipelines by polymer additives. Ind. Engng., Chem. Proc. Design Dev. 6: 309-314, 1967.

Roach, M.R., Scott, S., and Ferguson, G.G. The hemodynamic importance of the geometry of bifurcations in the circle of Willis (glass model studies). Stroke 3: 255-267, 1972.

Rodriguez, J.M., Zakin, J.L., and Patterson, G.K. Correlation of drag reduction with modified Deborah number. Soc. Petrol. Engng. J. 7: 325-331, 1967.

Ruckerstein, E. On the mechanism of drag reduction in turbulent flow of viscoelastic liquids. Chem. Engng. Sci. 26: 1075-1079, 1971.

Rudd, M.J. Velocity measurements made with a laser dopplermeter on the turbulent pipe flow of a dilute polymer solution. J. Fluid Mech. 51: 763-685, 1972.

Salgado, M.C.O., and Krieger, E.M. Cardiac output in unrestrained rats. Exp. Pharmacol. Physiol. 4 (Suppl. 3): 165-167, 1976.

Sarnoff, S.J., Braunwald, E., Welch, Jr., G.H., Case, R.B., Stainsby, W.N., and Macruz, M. Hemodynamic determinants of oxygen consumption of the heart with special reference to the tension-time index. Am. J. Physiol. 192: 148-156, 1958.

Savins, J.G. Drag reduction characteristics of solutions of macromolecules in turbulent pipe flow. Soc. Petrol. Engng. J. 4: 203-214, 1964.

Savins, J.G. Contrasts in the solution drag reduction characteristics of polymeric solutions and micellar systems. In: Viscous Drag Reduction, edited by C.S. Wells, pp. 193-212, Plenum, New York, 1969.

Sellin, R.H.J., and Ollis, M. Effect of pipe diameter on polymer drag reduction. Ind. Engng., Chem. Prod. Res. Dev. 22: 445-452, 1983.

Sharma, R.S., Seshadri, V., and Malhotra, R.C. Parameters affecting the onset of drag reduction in polymeric solution. Polymer 20: 257-259, 1979a.

Sharma, R.S., Seshadri, V., and Malhotra, R.C. Drag reduction by centre-line injection of fibres in a polymeric solution. Chem. Engng. J. 18: 73-79, 1979b.

Shipman, C. Evaluation of 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) as a tissue culture buffer. Proc. Soc. Exp. Biol. Med. 130: 305-326, 1969.

Shyluk, W.P., and Stow, F.S. Aging and loss of flocculation activity of aqueous polyacrylamide solutions. J. Appl. Polym. Sci. 13: 1025-1033, 1969.

Silberberg, A. Conditions of flow at interfaces with flexible walls. Ann. N.Y. Acad. Sci. 275: 2-9, 1976.

Smith, T.L., and Hutchins, P.M. Anesthetic effects on hemodynamics of spontaneously hypertensive and Wistar-Kyoto rats. Am. J. Physiol. 238: H539-H544, 1980.

Stehbens, W.E. Turbulence of blood flow in the vascular system of man. In: Flow Properties of Blood, edited by A.L. Copley and G. Stainsby, pp. 137-140, Pergamon, New York, 1960.

Stein, P.D., Parsons, E.D., and Blick, E.F. Modification of dynamic flow properties of turbulently flowing human blood by long chain polymers. Med. Res. Engng. 11: 6-10, 1972.

Stenberg, L.G., Lagerstedt, T., and Lindgren, E.R. Polymer additive and turbulent drag reduction. Phys. Fluids 20: S276-279, 1977.

Streeter, V.L., Keitzer, W.F., and Bohr, D.F. Pulsatile pressure and flow through distensible vessels. Circ. Res. 12: 3-20, 1963.

Sylvester, N.D., and Tyler, J.S. Dilute solution properties of drag-reducing polymers. Ind. Eng. Chem. Res. Develop. 9: 548-553, 1970.

Taylor, A.R., and Middleman, S. Turbulent dispersion in drag-reducing fluids. A.I.Ch.E. J. 20: 454-461, 1974.

Teyssie, D. Polymeres sur mesure? La reponse de Du Pont. Recherche 15: 84-87, 1984.

Tiu, C., and Chee, N.O. Turbulent flow behavior of dilute polymer solutions in an annulus. Can. J. Chem. Engng. 57: 527-577, 1979.

Tombs, M.P., and Peacocke, A.R. The Osmotic Pressure of Biological Macromolecules. Clarendon Press, Oxford, 1974.

Toms, B.A. Some observations of the flow of linear polymer solutions through straight tubes at large Reynolds numbers. Proc. 1st Int. Cong. Rheol., vol. 2, pp. 135-141, North Holland Publ. Co., Amsterdam, 1949.

Van der Meulen, J.H.J. Friction reduction and degradation in turbulent flow of dilute polymer solutions. Appl. Sci. Res. 29: 161-174, 1974.

Vetterlein, F., Halfter, R., and Schmidt, G. Regional blood flow determination in rats by the microsphere method during i.v. infusion of vasodilating agents. Arzneim.-Forsch. 29: 747-751, 1979.

Virk, P.S., Merrill, E.W., Mickley, H.S., Smith, K.A., and Mollo-Christensen, E.L. The Toms phenomenon: turbulent pipe flow of dilute polymer solutions. J. Fluid Mech. 30: 305-328, 1967.

Voitkounsky, Y.I., Amfilokhiev, W.R., and Pavlofsky, V.A. On the theoretical description of the flows of dilute polymer solutions. Paper distributed on the occasion of the O.N.R. 9th Symp. on Naval Hydrodynamics, Paris, 1972, cited by W.J. Hoyt, 1974.

Whistler, R.L., and Conrad, H.E. 2-O-(D-galactopyranosyluronic acid)-L-rhamnose from okra mucilage. J. Am. Chem. Soc. 76: 3544-3546, 1954.

White, W.D. Drag-reduction measurements for three polymers at 4°C. In: Viscous Drag Reduction, edited by C.S. Wells, pp. 173-182, Plenum, New York, 1969.

White, W.D., and Hoyt, J.W. The effect of linear-high molecular weight polymers on the turbulent flow properties of human blood. Report, Hemodynamics Session II, p. 11, 8th I.C.M.B.E., Palmer House, Chicago, Ill., 1969.

Whitmore, R.L. Rheology of the Circulation. Pergamon, Oxford, 1968.

Wu, J. Drag reduction in external flows of additive solutions. In: Viscous Drag Reduction, edited by C.S. Wells, pp. 331-350, Plenum, New York, 1969.

Yamashita, F., Nakamura, K., and Nakagawa, T. Study of drag reduction due to aqueous sodium polyacrylate by the rolling ball method. Polymer J. 15: 563-567, 1983.

Zalosh, R.G., and Nelson W.G. Pulsating flow in a curved tube. J. Fluid Mech. 59: 693-705, 1973.