

Isolated Organ Perfusion: Physiology and Application*

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History

The perfusion of isolated organs is certainly not a new idea (Table 1). Le Gallois, although probably not the first to perfuse isolated organs, predicted in 1812 that life could be maintained in organs by ex vivo perfusion (Norman, Covelli and Sise, 1968).

Elaborate mechanical perfusion systems were developed by Ludwig and Schmidt in 1868 and Lindbergh and Carrel in the early 1900's (Carrel, 1912; Perry et al., 1968). Our current techniques have added significantly to knowledge in many fields, including physiology, transplantation, immunology, toxicology, pathology and pharmacology. I will attempt to cover some of the

more interesting and important recent developments in perfusion and show its application to understanding fundamental physiology.

Systems

Basically, there are three types of isolated perfusion systems. An *isolated in vivo system* has an intact animal in which a specific organ is isolated by cannulation. The importance here is that the neural system remains intact while the "isolated" organ and its responses are being studied. An *isolated ex vivo system* has an organ removed from an animal, and that animal (isologous) or another animal (homologous or heterologous) has its blood circulated through the organ,

TABLE 1

History of Organ Perfusion*

1812	Le Gallois	"If one could substitute for the heart a kind of injection...of arterial blood, either natural, or artificial... , one would succeed easily in maintaining alive indefinitely any part of the body whatsoever."
1828	Kay	first artificial circulation to restore muscle
1849	Löbell	kidney perfusion
1858	Brown-Séguard	brain perfusion
1866	de Cyon	heart and liver perfusion
1869	Ludwig and Schmidt	perfusion apparatus
1875	Luchsinger von Fry and Gruber Brodie, Hamel and Jacobj	liver perfusion pulsatile pump aeration and perfusion
1913	Carrel	culture of organs
1931	Lindbergh	coil apparatus
1935	Carrel and Lindbergh	thyroid perfusion lasting 18 days

* (Carrel and Lindbergh, 1938.)

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which is usually contained in a box or bag. In this system, the neural factors are severed where as, the hormonal factors remain intact. Finally, a complete *isolated in vitro perfusion system* utilizes an artificial pump and various perfusate mixtures or anticoagulated blood. Neither hormonal nor neural response mechanisms are intact in this system, and the circulation is regulated by the pump and the intrinsic neural mechanisms.

Problems in Perfusion

With these systems in mind, let us look at some of the problems in organ perfusion. In the intact *in vivo* system, problems of cell death are seldom encountered unless the drugs administered to the organ or the perfusion apparatus directly damage the cells. *In vitro* systems inevitably result in cell death no matter how elaborate the system or how nutritive the perfusate. It is imperative to resolve the etiology of cell death in *ex vivo* and *in vitro* systems in order to establish better maintenance of organs and thereby prolong survival for the purposes of study and, perhaps more importantly, transplantation.

Whether or not cell or organ death is due to vasospastic phenomena, lack of endothelial integrity, or plugging by embolic fatty products is not yet clear. One or several of these phenomena may be causative, depending upon such factors as the mechanisms of perfusion, the choice of perfusate, the oxygenation and the organ. It has been suggested (Belzer et al., 1968) that one cause of organ death in some isolated systems is emboli of lipid aggregates. According to Belzer, these emboli could cause tissue hypoxia with resultant cellular swelling, anaerobic metabolism, acidosis and death. The common denominator in cell and organ death is hypoxia due to inadequate perfusion. It has been recently demonstrated (Cohen and Folkman, 1968) that the pathogenesis in-

involved in hypoxic cell death is inadequate perfusion or inadequate oxygenation. Hypoxemia leads to anaerobic glycolysis within the cell and produces an intracellular lactic acidosis. As a result, there is a lack of energy because of the anaerobic metabolism. Without energy the sodium pump mechanism fails, and water is imbibed into the cell, producing edema. The lysosomes swell and the resultant release of enzymes caused by acidity, temperature rise, or decreasing osmotic pressure produces self-destruction of the cell.

Assessing Viability

For transplantation purposes, it is imperative that organ viability be assessed with some degree of assurance prior to reimplantation.

How can one, then, detect cellular death early in the perfusion of isolated organs so that something can be done? The answer to this question is far from being simple depending, as it does upon many factors. It is dependent upon the organ under study. If one is studying the kidney, for example, it is difficult to correlate specific enzyme changes with cell death. Other factors, including urine output, blood flow and appearance, do not necessarily correlate with ultimate viability of the organ. A group of investigators (Belzer et al., 1968) have recently demonstrated that there may be some correlation between LDH isozymes and the prediction of viability of perfused kidneys. Rising perfusion pressure has also been shown to correlate with total renal function in isolated organs

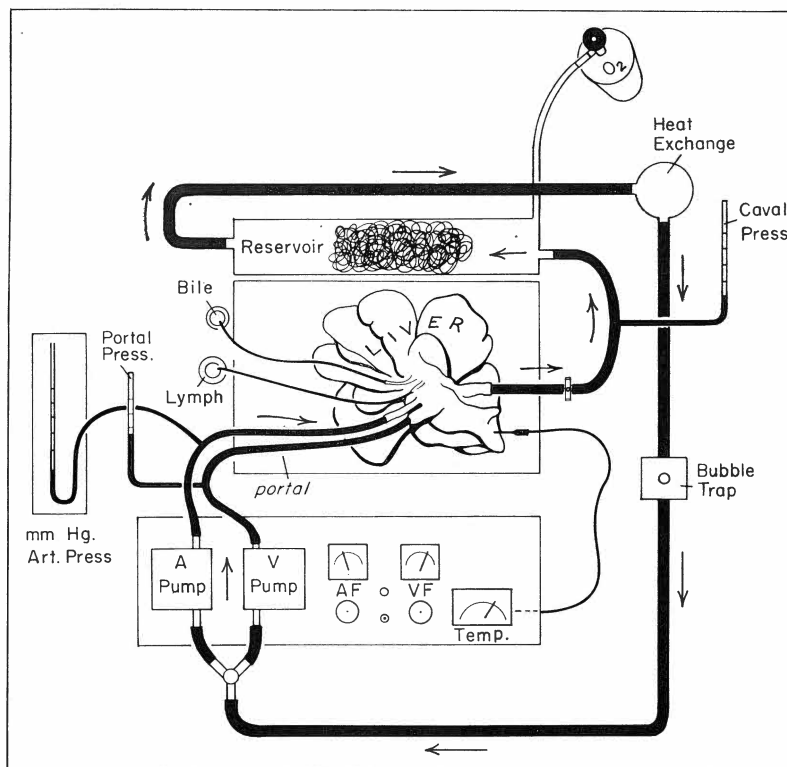


Fig. 1—The pump regulates arterial flow (A pump) and venous flow (V pump) separately through the liver. A bubble oxygenator is used with a heat exchanger. Pressures and flows (AF and VF) are continuously monitored. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 807.)

and probably indicates renal death (Belzer et al., 1968). The pathologic mechanism is obstruction to the renal vasculature, and the perfusion pressure rises because of inadequate perfusion of these small vessels. In an organ which has an active enzyme system, such as the liver, there are more readily detectable enzymatic changes which correlate with cell death. The major question here is: To what degree enzyme change indicates whether the liver will or will not survive and immediately support life after reimplantation. This is dependent upon the amount of anoxia it suffers during extirpation, perfusion, preservation, and reimplantation. There are many parameters for studying acute changes of liver damage, the more common being the measurement of SGOT (serum glutamic oxaloacetic transaminase), SGPT (serum glutamic pyruvic transaminase), potassium and glucose changes and lactate-pyruvate ratios.

Regarding the applicability of isolated organ perfusion to transplantation, one should closely examine the various ways of assaying viability of different organs prior to reimplantation. Appearance of the organ is often helpful in detecting the presence of edema, poor venous return, or the presence of inadequate oxygenation, as, with these conditions, there may be blotchiness or poor color in general. Excretory output can also be measured, although this does not necessarily correlate with function.

Chemical assays, including those of enzyme changes in the kidney, liver, and heart, are important, as changes in perfusate pH, temperature, oxygen concentration, and carbon dioxide content. Pressure changes in the chambers of the heart as a measure of contractility may be beneficial in assaying the viability of the heart. Tetrazolium bromide dye is a rapid method for attempting to assay cardiac viability (Maginn and Hadjimichalis, 1968). In studies by Idezuki et al.

(1968), pancreatic viability was determined by giving an isolated pancreatic graft a glucose stimulation test which, if the graft was viable, brought about the immediate production of insulin. Even with minimal output of insulin after 48 hours of perfusion, this test indicated a viable graft that functioned on reimplantation.

As mentioned previously, in an *ex vitro* perfusion system, the excretory functions of organs, such as the kidney and liver, were not totally reliable as adequate assays of viability. The kidney can put out large amounts of urine which is isosmolar with the perfusate and represents simply an ultrafiltrate of a dying kidney. Similarly, the liver can put out bilious-appearing material which, if assayed, proves to be a poor quality of true bile and probably represents an ultrafiltrate type of material. Flow rates through organs depend upon several factors: whether the perfusion is normothermic or hypothermic; the method of perfusion; the type of pump used; whether or not the pressure is constant; and the amount of vascular resistance within the organ system. One elaborate system (Norman et al., 1967) provides the ability to monitor the intake and output via a computer-type setup and, thereby balance and control the precise amount of flow. In our laboratories we utilize a pressure-sensitive Edwards-Bosher† pump, which detects resistance and, as the resistance increases, automatically and proportionately diminishes the flow (Fig. 1). Diminishing flow rate provides us with immediate evidence of intravascular resistance secondary to vasospasm, occlusive disease, or other problems which raise resistance and diminish inflow. We feel that this is a highly physiologic system in that, essentially, it allows the organ to control its own flow by intrinsic autoregulation.

† Edwards-Bosher Coronary Perfusion Unit. J. F. Edwards Company, Harrisonburg, Virginia.

Clearance studies, such as excretion of dyes in the isolated kidney preparation or excretion of bromsulphalein or ammonia in the isolated liver preparation, are also utilized for detection of viability. These are probably somewhat more reliable than the simple measurement of excretory rate alone, because excretion of the dye demands cellular function.

Another method of assaying viability is pathology, including light and electron microscopy. Because it takes time for the preparation of sections, these methods prevent obtaining conclusive evidence of viability immediately prior to implantation.

The *sine qua non* of assaying viability, of course, is normal function after reimplantation. This is the only reliable indicator of adequate viability of the organ. The obvious problem here is that, where human transplantation is concerned, this implies surgical manipulation of the patient (recipient) in order to detect viability of the organ involved. The immediate need, of course, is for some quick, reliable, and accessible method of assaying viability of isolated organs being perfused for transplantation. Various aspects of such a method, including chemical changes, excretory clearance functions and vital dye studies, are presently being pursued with the greatest interest at the present time.

Mechanics of Perfusion

Returning to more basic physiology, let us consider some of the actual mechanics of perfusion of isolated organs. There are many variables in an isolated system. The first of these is *temperature*. Perfusion at normothermia (37 C), at room temperature (25 C), or at considerable hypothermia (4-10 C), for the purposes of prolonged preservation, depends upon the desires of the investigator. Almost all physiologic, pharmacologic, toxicologic, and immunologic studies are per-

formed at body temperature, but, for the purposes of studying preservation techniques, hypothermia is usually utilized, since the metabolic rate is markedly diminished. However, it produces problems, such as associated vasospasm and sludging of blood when cellular products are present, inactivation of heparin at low temperatures, and inactivation of certain drugs.

Another basic consideration in the mechanics of perfusion is the *presence or absence of hyperbaria*. Belzer (Belzer, Ashby and Dunphy, 1967) has adequately demonstrated that hyperbaria is not necessary for the preservation of kidneys for a period of up to 24 and, perhaps, even 72 hours with the use of hypothermia and a plasma solution. Other investigators (Brettschneider et al., 1968) have demonstrated that, for their purposes, hyperbaria is necessary to maintain viability during preservation of human and canine livers. Recently it has been demonstrated that hyperbaria sufficiently suppresses cellular swelling in the liver for a period of 24 hours (Huntley et al., 1968). However, after 24 hours the enzymes are markedly altered, and the liver is not sufficiently functional to support life. The perfused liver gains weight at a rapid rate after the first 24 hours, whether or not it is in a hyperbaric atmosphere. Hyperbaria may be necessary for prolonged preservation of the pancreas and, perhaps, the heart and the lung. However, there are certain inherent disadvantages to hyperbaria: 1) Compression and decompression take several extra hours; 2) There is potential danger of an accident with the high pressure system; and 3) Excess oxygen will damage in vitro tissue culture cells, although it is difficult to assess its effect on whole organs. It has been shown that hyperbaria (using nitrogen or helium) will accomplish effects similar to those accomplished by hyperbaric oxygen, and it is speculated that pressure alone may be the important

factor (Lyons, Dietzman and Lillehei, 1966).

A third consideration in perfusion mechanics is the *type of pump and oxygenator* utilized. As previously mentioned, we use the Edwards-Bosher pump, because we think it is physiologically sound. Belzer and Folkman both utilize different types of pumps and obtain very satisfactory results. These pumps are basically pulsatile. It has been demonstrated by work in our own laboratory (Robertshaw and Gayle, unpublished data) and by others (Tait and Eisenman, 1966) that pulsatile perfusion allows lymphatics to empty during the diastolic phase and, therefore, diminishes edema formed in the parenchyma and around the capsule of the organs. The type of oxygenator is also important. It has recently been demonstrated that disc oxygenators, which have a large surface contact with the actual perfusate, have increased screen filtration pressure (Ashmore, Svitek and Ambrose, 1968) and as previously mentioned (Belzer et al., 1968), may cause problems with embolization of fatty materials. Membrane oxygenators have been utilized for bypass over periods of 12 to 24 hours and have a very minimal increase in screen filtration pressure. We have recently switched to a membrane oxygenator for our preservation system but have, however, been able to successfully preserve some kidneys for 24 hours with a disc oxygenator.

A fourth area of importance in perfusion is the *choice of perfusate*. Again, there is much controversy as to the exact type of perfusate to use. This is dependent upon the type of study desired by the individual investigator. If the system is to be as physiologic as possible, a whole blood or dilute whole blood solution is utilized. Problems involved, however, are sludging and outflow block—especially in the liver—if a whole blood solution is utilized. Regardless of the perfusate used, perfusion of the lungs, without ventilation, is problematical for maintenance of viability (Stevens et al., 1968). In preservation (Table 2), dilute whole blood solutions have worked satisfactorily with lung and liver. With kidney preservation, the use of a plasma solution has been shown by Belzer to be the most satisfactory (Belzer et al., 1967). The use of crystalloid solutions has been shown by some investigators to be nearly as effective as that of plasma. However, one does run into the problem of edema after occurring long preservation. Depending upon the study, many additives have been utilized in perfusates, including magnesium, steroids, antibiotics, adenosine (Stewart and Wilbrandt, 1968), chlorpromazine, buffering agents, glucose and insulin.

The question of inclusion of cells in the perfusate has been a very controversial one. Up until recently it was thought that platelets might be harmful; however, it appears that

TABLE 2

Perfusates for Preservation

Kidney—	homologous undiluted ACD plasma, room air, pulsatile perfusion, moderate hypothermia
Liver—	dilute heparinized whole blood, hyperbaric oxygen, hypothermia
Lung—	ventilation, ? perfusion (dextran-ACD-blood), ? hyperbaria (2A)
Pancreas—	non-perfusion relatively successful to 22 hours
Thymus—	Eagle's MEM, fetal calf serum

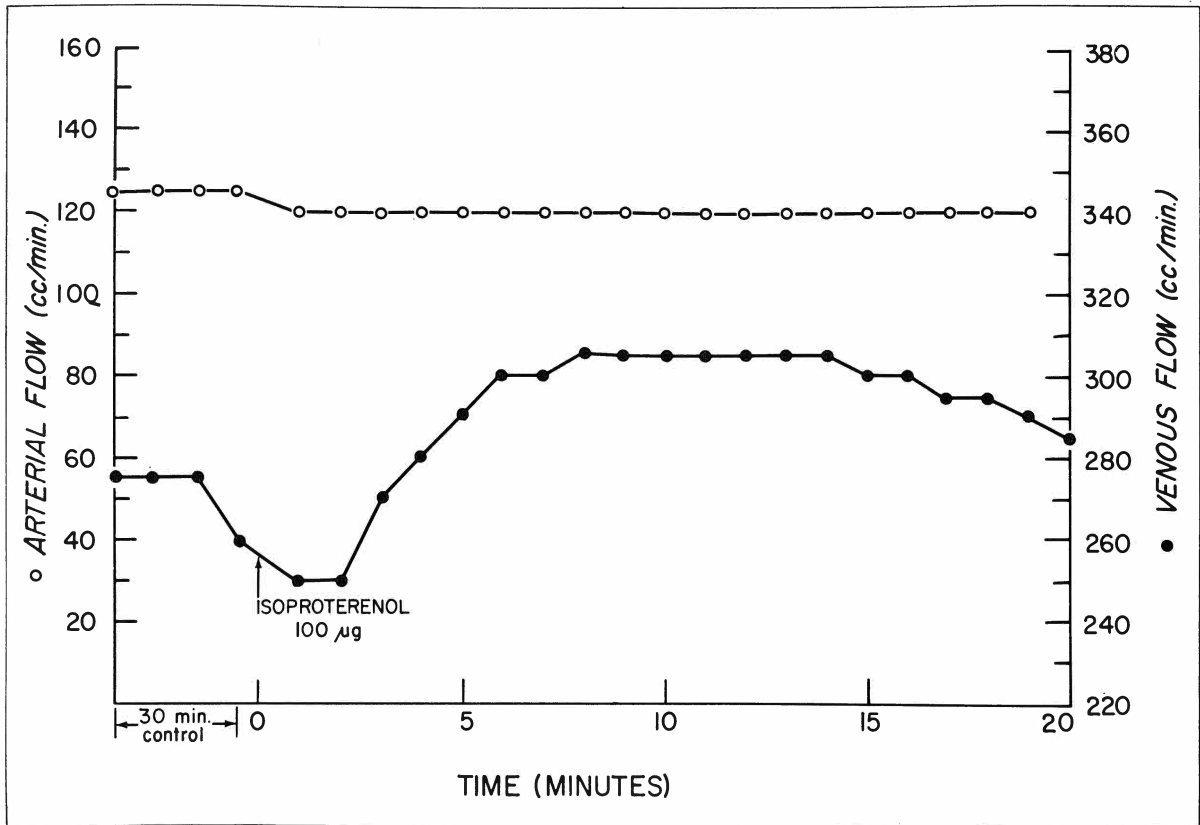


Fig. 2—Isoproterenol added to an isolated perfused liver with a falling venous flow produced immediate increase in flow.

Folkman has renewed the desire to have platelets present to insure physiologic preservation of the endothelium (Gimbrone et al., 1969). He and his colleagues have demonstrated that the platelet, which is thought by some to contribute to problems in isolated organ perfusion, may be an important factor in maintaining the integrity of the vascular endothelium. They have recently demonstrated this by perfusing dog thyroid glands with platelet-rich plasma and comparing them to thyroids perfused simultaneously with platelet-poor plasma under identical conditions. The endothelium seems to be maintained with adequate function after re-anastomosis in organs perfused with platelet-rich plasma for 24 hours. Platelet-poor perfused thyroids, however, after a period of three to four hours, swell and have petechial and purpuric hemorrhages

in their subcapsular area on reimplantation. This is perhaps the newest and most exciting concept in organ perfusion and preservation and goes against the theory of many workers in the past (Humphries, 1967).

One final perfusate which has been used for maintenance of perfusion in isolated organs (Paton et al., 1968) and in the total rat model (Geyer, Monroe and Taylor, 1968) is the fluorocarbon perfusate (Pluronic F-68). Geyer et al. have described a highly intriguing application in which rats were completely exchanged with a fluorocarbon-serum emulsion without cells, maintained at 100% oxygen atmosphere, and kept alive for six to eight hours. If washed erythrocytes were added to fluorocarbon emulsion, the animal survived indefinitely after being perfused for up to 140 minutes.

MCV Data

At this point I would like to relate some of the work recently done in our laboratory to the aspects of organ perfusion and transplantation. Doctor William Harlan (O'Brien, Harlan and White, unpublished data) has recently shown that palmitic acid, which is probably the major source of energy for the renal cortex, is markedly diminished in canine renal allografts as compared to isografts and normals. It is hoped that we can apply this work and its implications to the study of rejection.

Other studies relevant to transplantation are being performed by one of our medical students, Mr. Harold Levinson, who is using an isolated rat perfusion system modeled after that of Doctor Judah Folkman to study in vitro aspects of rejection. Utilizing PA and Lewis

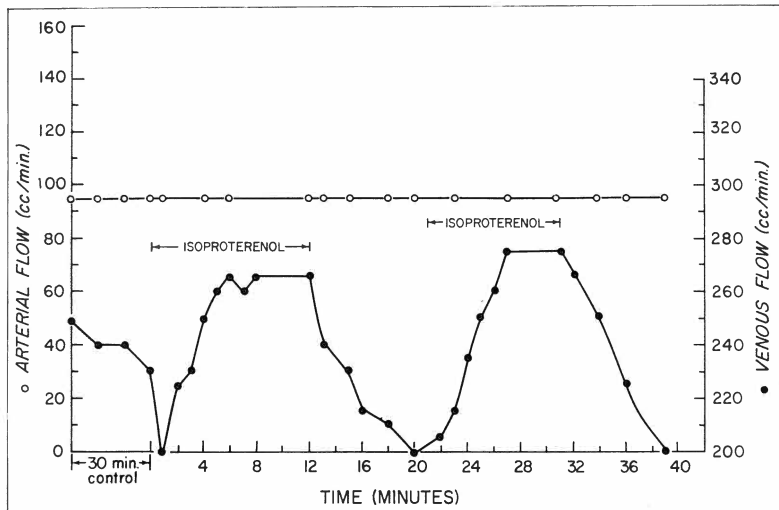


Fig. 3—Intermittent intravenous isoproterenol drip produced an immediate rise in venous flow. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 812.)

rat strains, lymphocytes can be obtained from the thoracic duct of one rat strain. Either a culture medium which is isologous to one rat strain or a medium, such as Medium 199, which is indifferent to both strains, is used to suspend the cells. Thoracic duct lymphocytes, tagged with chromium 153, are utilized to perfuse syngeneic and allogeneic rat strains. Counts of the venous effluent as well as counts of the total kidney are monitored after a standard perfusion time. These data appear to demonstrate that the allogeneic kidney took up more sensitized lymphocytes than did the syngeneic kidney. Preliminary data on the venous effluents, however, have not proved consistent in their results. Utilizing tagged red cells to reveal whether the kidneys were being

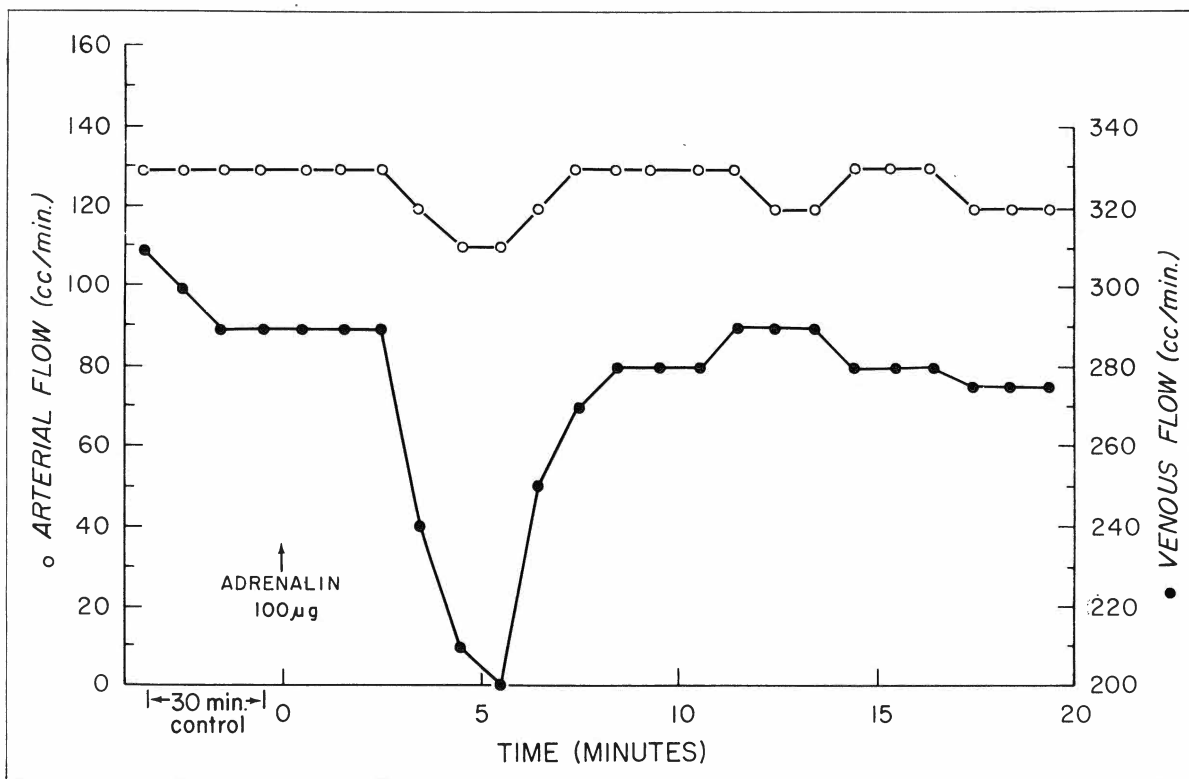


Fig. 4—Vasospasm with arterial and venous flow diminution is produced with epinephrine. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 810.)

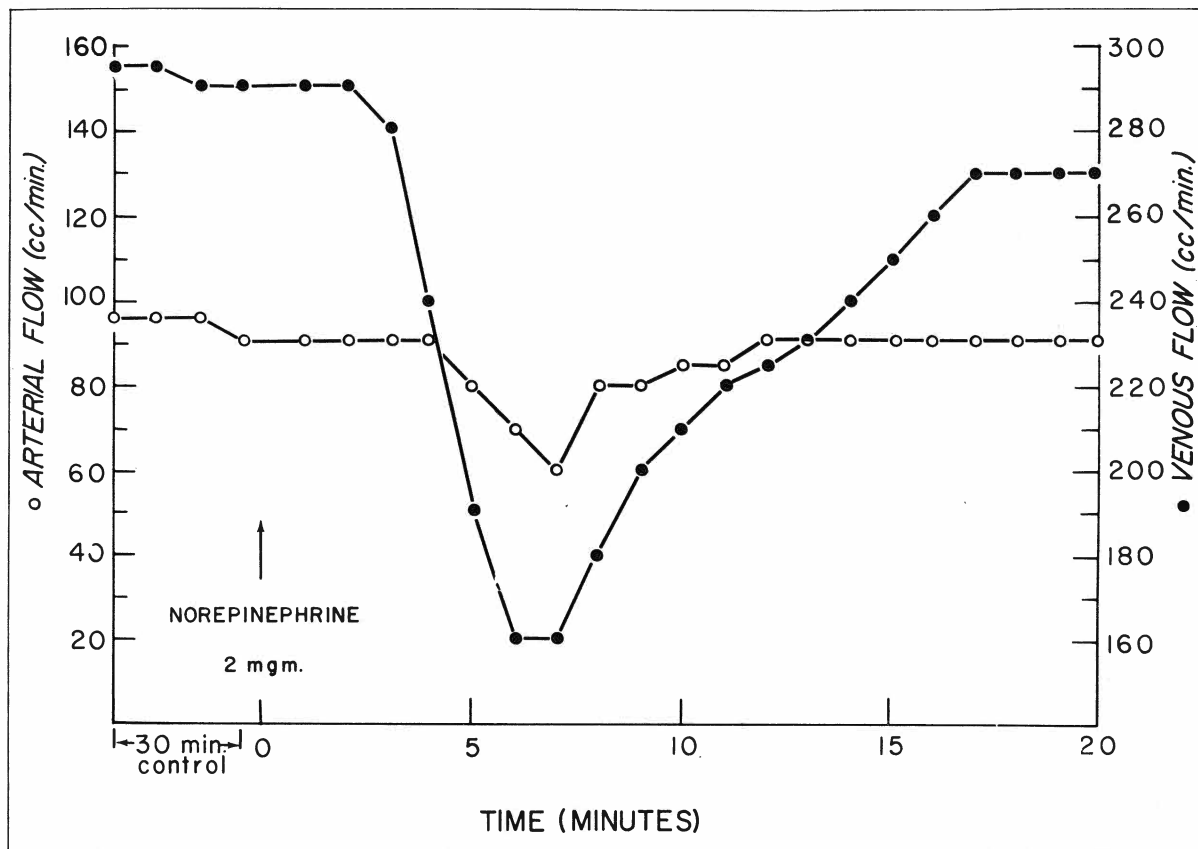


Fig. 5—Norepinephrine produced similar results.

adequately perfused, Levinson demonstrated that the kidneys are not always being perfused equally in the cortical and medullary areas.

Doctor Gilbert Robertshaw has published an excellent report on the presence of serum antibody which is not cell-bound (Robertshaw et al., 1967). One of a set of unrelated mongrel dogs was subjected to a first-set transplant that used a kidney which was allowed to reject and was then removed. After a short rest period, a second-set graft was performed utilizing either skin or spleen. Skin grafts were subsequently performed until a "white-graft" reaction was obtained, implying hyperacute rejection. The kidneys of the original donor were placed in a perfusion circuit, and the serum of the hypersensitized

recipient dog was added to the perfusion system after a baseline was obtained. It was immediately demonstrated that the donor kidney swelled, and the urine output diminished rapidly. A recipient kidney was used as a control. Upon analyzing these data, Robertshaw found that there was a decrease in renal arterial flow, oxygen uptake across the kidney, urine output and urine osmolality in the donor kidney. Pathologic examination revealed proteinuria and increased edema.

In a more recent study (Robertshaw et al., unpublished data) the donor kidney, after being perfused, was replaced in the donor animal and allowed to perfuse for 30 minutes to 2 hours. Urine output remained low. On pathologic exami-

nation, polymorphonuclear leukocytes were shown to adhere to the endothelial lining of the kidneys, demonstrating continuance of the hyperacute rejection phenomenon.

Work done with isolated liver perfusions in our laboratory has demonstrated that there are probably beta-adrenergic receptors within the vasculature of the liver which are affected by isoproterenol (Figs. 2, 3) and tend to cause vasodilatation of the postsinusoidal sphincter mechanism (Gayle, Williams and Hume, 1968). This causes relaxation and allows increased flow, especially if the system has been previously subjected to vasospasticity with either norepinephrine or epinephrine, both of which produce marked vasoconstriction and diminution of flow through the liver

(Figs. 4, 5). With the administration of isoproterenol, increased flow is more marked in the venous phase than in the arterial phase (Figs. 6, 7). Lymphatic output, as measured by direct cannulation of perihilar lymphatics, is increased with the administration of vasoconstrictive agents. With the relaxation of the sphincter accomplished by isoproterenol, the lymphatic output diminishes. It is our feeling that if post-hepatic transplantation support to the cardiovascular system were needed, isoproterenol would probably be the drug to choose, since vasopressor agents, such as norepinephrine or epinephrine, would harm the liver by causing vasospasm. However, not enough work has been done with human livers to definitely corroborate this in the human system. We are also, at present, working on effects of certain anesthetic agents on hepatic flows and pathologic changes.

Conclusions

Isolated organ perfusion provides us with an excellent tool for investigating the physiology and the function of a variety of individual organs. It enables us to explore these organs, their pharmacologic reactions, their ability to withstand various stresses, their pathology after stress and their suitability for preservation and transplantation. Isolated perfusion has allowed us to better understand hemodynamics, effects of various perfusates, and toxicity of drugs on individual organs. We have recently been enlightened about the vital role of platelets and endothelial integrity. We now have a model for assessing individual hormonal systems and qualitative and quantitative endocrine and exocrine functions. Organ perfusion has provided us with a method for preservation and a means for studying organ viability prior to transplantation. We now have an invaluable isolated system in which we can study certain

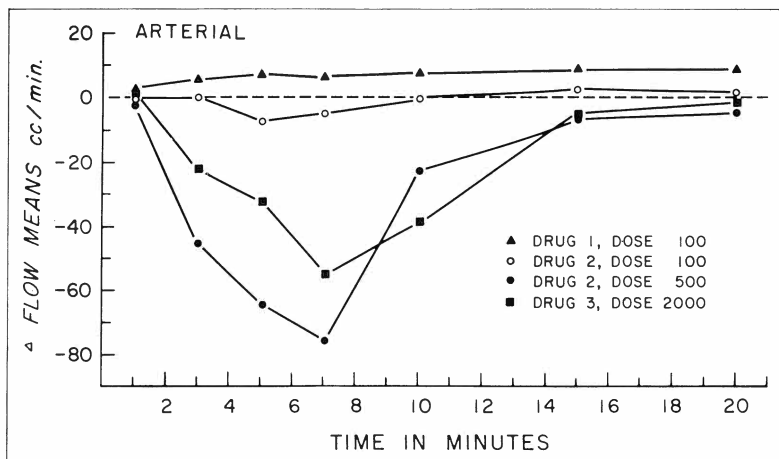


Fig. 6—Mean arterial flow was slightly but consistently increased only by isoproterenol (Drug 1) when the mean changes in flow were recorded for 15 dog livers after injection. Drug 2 is epinephrine and Drug 3 is norepinephrine. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 811.)

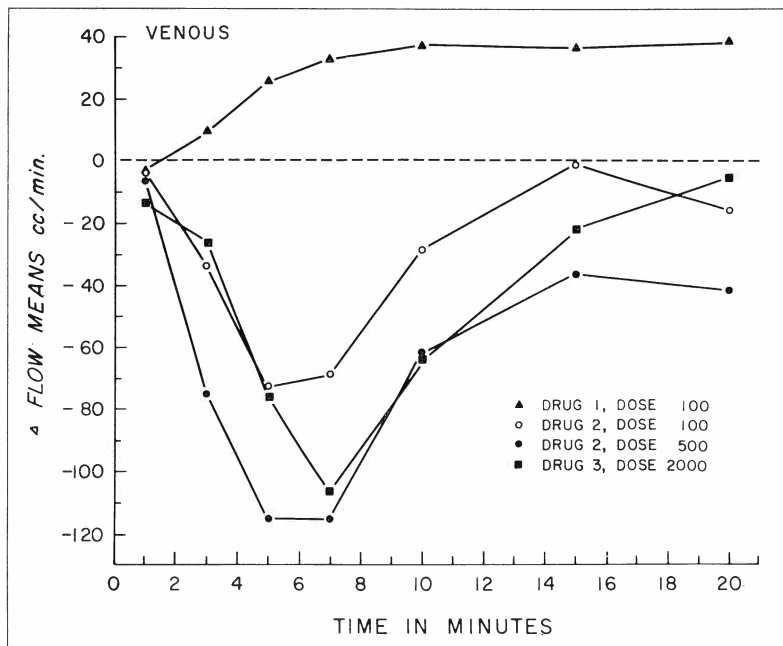


Fig. 7—Mean venous flows were increased only by isoproterenol (Drug 1). Norepinephrine and epinephrine diminished flows consistently after injection. (Reprinted with permission from W. E. Gayle, Jr., G. M. Williams and D. M. Hume in *Organ Perfusion and Preservation*, Appleton-Century-Crofts, 1968, p. 811.)

aspects of immunology, including the rejection mechanism, sensitization, and the evaluation of serum and cell-bound phenomena. Our immediate goals include detection of cell or organ death prior to re-implantation by some rapid and accurate method and prolongation of preservation time for in vitro organ systems.

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