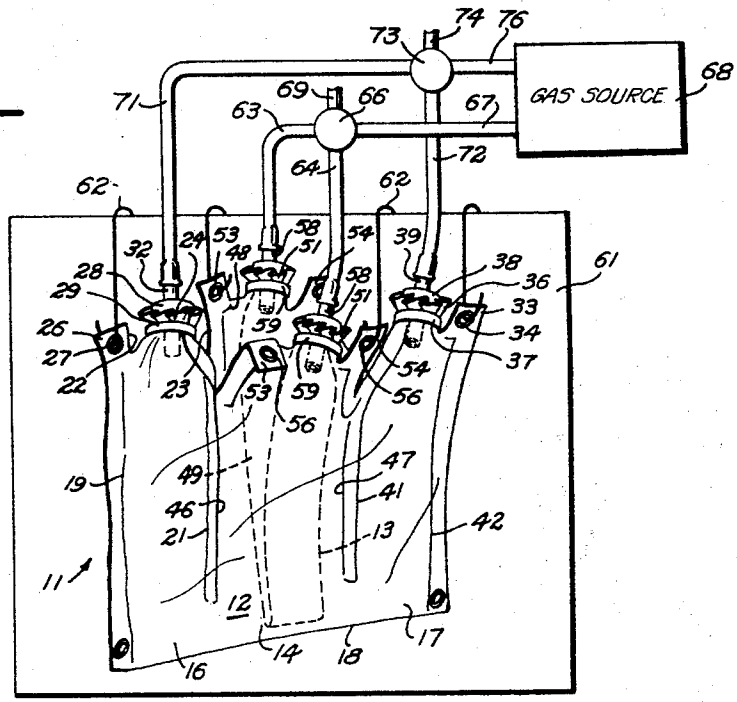
April 29, 1969 L. JANKAY 3,441,479

METHOD AND APPARATUS FOR IN-VIVO-LIKE MAINTENANCE OF BLOOD IN VITRO

Filed March 19, 1964

FIG. 1



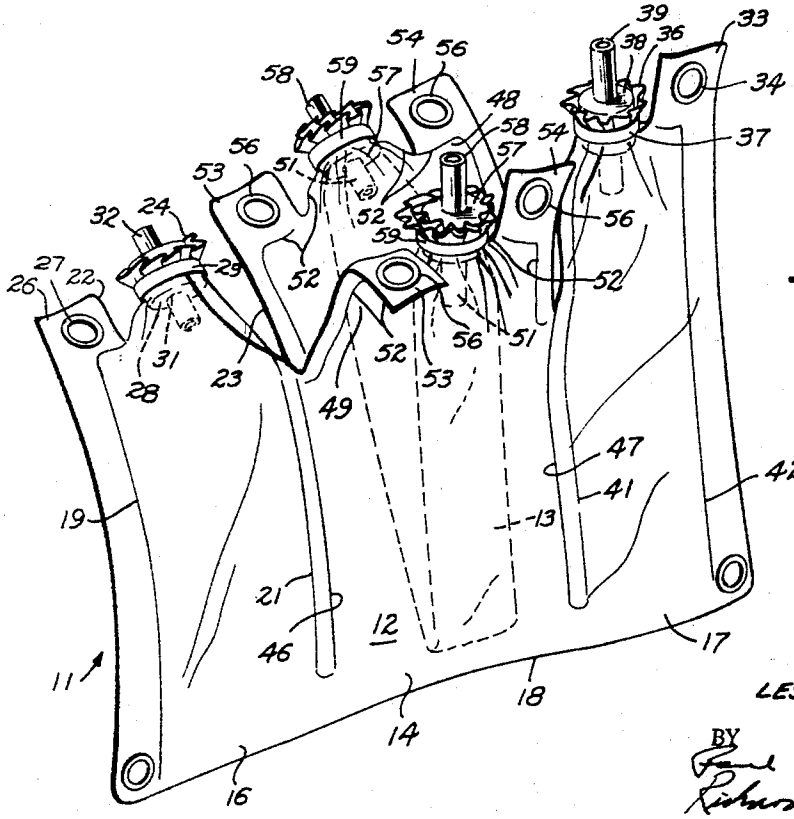


FIG. 2

INVENTOR.
LESTER (NMI) JANKAY

BY

ATTORNEYS

United States Patent Office 3,441,479
Patented Apr. 29, 1969

3,441,479

METHOD AND APPARATUS FOR IN VIVO-LIKE MAINTENANCE OF BLOOD IN VITRO

Lester Jankay, 518 E. Rustic Road,
Santa Monica, Calif. 90402

Continuation-in-part of application Ser. No. 160,376, Dec. 18, 1961. This application Mar. 19, 1964, Ser. No. 353,304

Int. Cl. C12k 9/00

U.S. Cl. 195—1.8 13 Claims

ABSTRACT OF THE DISCLOSURE

The method employs a U-shaped, tubular sac member formed of a permeable material to hold the blood culture to be treated. A dialysing bag formed of a vinyl contains the dialysing fluid, such as blood plasma, and also contains the tubular sac. Preferably, both of these members are coupled to a source of a gas (oxygen and carbon dioxide) under pressure which is applied in a timed pulsating manner to cause the fluids contained in the members to oscillate relative one to the other. The oscillation renders dialysis more effective, while the addition of CO_2 controls the pH level during dialysis. Further, blood cultures normally are heparinized and the oscillation permits minimum heparin concentration. Excess heparin is toxic to blood. The method further contemplates a preheating of the blood culture to a temperature of about 57° C. before commencing the dialysing process and during the processing, electrolytic equilibrium is maintained by adding distilled water or the like. Also the sugar content of the blood is replenished.

The invention described herein may be manufactured and used by or for the Government of the United States of America for governmental purposes without the payment of any royalties thereon or therefor.

This application is a continuation-in-part of application Ser. No. 160,376, filed Dec. 18, 1961, issued Oct. 4, 1966, as U.S. Patent No. 3,276,589 and entitled "Method and Apparatus for Maintenance and Treatment of Blood in Vitro."

The present invention relates to a method and apparatus for the maintenance and treatment of blood "in vitro" and, more specifically, it relates to producing an in vitro environment for blood which substantially approximates the "in vivo" environment of the blood involved.

The realization of an in vivo-like in vitro environment for extracorporeal blood is a highly desirable objective from a twofold standpoint. On the one hand, it enables a satisfactory extracorporeal maintenance of blood which is important, per se, as it relates to the abilities to conduct in vitro blood studies, to recondition human blood affected by disease and to extracorporeally maintain blood as an aid to surgical procedures. And, on the other hand, it enables the realization of an in vitro tissue culture medium which is vastly superior to previous such in vitro media.

During the last few decades a great variety of methods and different culture media have been employed to cultivate cancer and other kinds of tissues in vitro. These prior methods and culture media suffer the disadvantage that, during cultivation and particularly during long-term cultivation, the cells under cultivation undergo changes and at times develop what is referred to as a "cell line." Involved is the question of how much the original morphology and biological characteristics of the cultivated cells are lost or changed during short or long-term cultivation. There is also the question as to whether the results of the experimental studies done on the "cell lines" or on other cultured tissues can be applied to the tissues from

which they originate. All of the prior conventional tissue culture media constitute a foreign environment for the cultured explants and this fact, no doubt, is one of the primary reasons for the undesirable changes which take place to the tissue explant during cultivation. The present invention substantially avoids the undesirable morphological and biological changes which typify prior in vitro cultivation of tissue explants by creating an in vitro environment wherein blood can be satisfactorily extracorporeally maintained. In this particular in vitro environment autologous heparinized whole blood is employed as the tissue culture medium and through the use of a "circulating-and-dialysing" chamber peculiar to the invention in conjunction with various procedural steps, also peculiar to the invention herein, this whole blood culture medium is maintained in such a condition that it provides, for tissue explants cultured therein, an in vitro environment which substantially approximates their in vivo milieu.

In such an environment, as produced by the subject invention, cancer cells can be introduced into blood taken from a cancer patient and in another like environment the same cancer cells can be introduced into blood taken from a healthy person and the two different specimens can then be observed in vitro for any differences in behavior. Thus can an effective comparison be drawn between the reaction to cancer cells by normal healthy blood, on the one hand, and by cancer-diseased blood, on the other. In like manner, such an in vivo-like in vitro environment can provide the ability to study the effect of various anti-cancer substances on a cancer patient's blood. Such an environment also will permit the study of "anti-bodies" as a possible defense to cancer. For instance, the combination of normal healthy blood, normal lymph node tissue and a small quantity of homogenated cancer cells as an antigen can be observed in such an in-vivo-like in vitro environment to determine how anti-body-producing lymph nodes in a healthy person react to the challenge of the presence of cancer cells.

The in vitro environment of the invention can also be employed to produce a whole-blood culture medium for effective cultivation of bone marrow, skin fragments, lymph nodes, etc. It can render feasible the study of radiation effects on biologically active blood (at approximately 37° C.) without the necessity of exposing the human body to such radiation. The whole-blood culture medium produced herein can also be used for "homografting" purposes to adapt a "donor's" tissue (such as skin, etc.) to a "recipient's" blood.

The above-noted examples are but a few of the possibilities presented by the current invention which is particularly applicable to the performance of tasks and studies which are impossible of performance in an in-vivo environment because of the risk involved to the patient and which previously have been rendered impossible in vitro because of failure of previous in vitro environments sufficiently to achieve the in-vivo-like character required.

Among objects of importance of the present invention are the following:

To produce an in vitro environment which successfully approximates the natural in-vivo environment of the being involved.

To produce an in vitro environment in which cell explant maintain their original in-vivo status.

To produce a culture medium in vitro wherein explants maintain their original mophological and biological characteristics.

To maintain blood in vitro in such a way as to maintain its in-vivo status whereby such in vitro blood can adequately serve as a tissue culture medium or lend itself to other biological purposes.

To adequately maintain blood in vitro circa 37° C.

To provide an apparatus for performing the combined functions of circulating, oxygenating, dialysing, and properly buffering a blood culture in vitro as well as maintaining said blood culture in osmotic equilibrium.

To provide a blood circulating-and-dialysing chamber.

To provide a cheap, simple, readily-portable blood circulating-and-dialysing chamber.

Other objects and many of the attendant advantages of this invention will be readly appreciated as the same becomes better understood by reference to the following detailed description when considered in conjunction with the accompanying drawing wherein:

FIG. 1 portrays an illustrative embodiment of the invention wherein the blood circulating-and-dialysing chamber is shown in perspective and the balance of the apparatus which is employed in conjunction with the chamber, per se, is generally shown schematically; and

FIG. 2 is a somewhat enlarged showing of the circulating-and-dialysing chamber, per se.

Blood circulating-and-dialysing chamber **11** consists primarily of a wash fluid or dialysing bag **12** which is adapted to hold the wash fluid which herein is heated blood plasma and a sac member **13** which is adapted to hold the blood culture and to serve as the dialysing membrane.

Wash fluid or dialysing bag **12** is formed of vinyl or some like appropriate inert material, and comprises essentially three separate compartments: a medial compartment **14** and respective outer compartments **16** and **17**, one of which is located on each side of medial compartment **14**. Bag **12** is formed of unitary construction largely from a single sheet of vinyl which has been folded back upon itself to form two juxtaposed layers which are then appropriately cut and fused together at various positions to form the three compartments noted above. The description below of the various compartments of wash fluid or dialysing bag **12** will make reference to various locations where these adjacent vinyl layers are fused together.

The inner (wash-fluid-containing) portion of left-hand outer compartment **16** is defined at its bottom by the line **18** where the single vinyl sheet was folded, at one side by the fusion-line **19** and at the other side by the fusion-line **21**. It will be noted that fusion-line **21** does not run to the bottom fold line **18**, but stops short thereof by some distance. This is to permit passage of wash fluid (i.e. blood plasma) between this compartment **16** and the medial compartment **14**. The respective fusion lines **19** and **21** run upwardly to the upper edges of the two adjacent vinyl layers. These layers have been cut along the respective lines **22** and **23** (located respectively outboard of fusion lines **19** and **21**) to form an open-ended throat portion **24**. The cut along line **22** has also yielded an upstanding flap appendage **26** at whose upper end there is fixedly positioned a metallic eye **27**. Disposed within throat portion **24** is a centrally-bored cylindrically-shaped access fitting **28**. By means of a resilient annular fastening member **29**, which is disposed outwardly of throat portion **24** and which resiliently bears against the outer portion of access fitting **28** and throat portion **24**, which is sandwiched therebetween, throat portion **24** is sealed off to fluid except for the bore **31** longitudinally running through access fitting **28**. Located in an interference fit in the bore of access fitting **28** is an access tube **32** which projects above and below the access fitting **28** in which it is disposed.

Right-hand outer compartment **17** is, in effect, a mirror image of left-hand outer compartment **16** and is similarly constructed, having an appendage flap **33** with a metallic eye **34** located therein, a throat portion **36**, with enclosing resilient annular fastening member **37** and enclosed access fitting **38** and access tube **39**. The inner wash-fluid-carrying portion of this right-hand outer compartment **17** is defined at its respective left and right sides by the fusion-lines **41** and **42**, at the bottom by fold line **18** and is limited at its upper portion by throat-enclosed access fitting **38** with its access tube **39**. Similarly to left-hand outer compartment **16**, fusion-line **41** begins some distance removed from the bottom fold line **18** so as to permit passage of wash fluid back and forth between this right-hand outer compartment **17** and medial compartment **14**.

Medial compartment **14** of wash fluid or dialysing bag **12** is meant to accommodate sac member **13** and correspondingly differs in construction from the outer compartments **16** and **17**. The wash-fluid-containing interior portion thereof is defined at the bottom by fold line **18** and at its vertically-lower portion by the respective fusion-lines **46** and **47** whose lowermost limits are located at the same distance from the bottom fold line **18** as are the lowermost limits of fusion-lines **21** and **41** of the respective outer compartments **16** and **17**. At its vertically-upper portion medial compartment **14** is of bifurcated structure whose formation necessitates the use of an additional U-shaped vinyl piece in addition to the two original vinyl layers previously discussed. The two legs of the bifurcated structure of this medial compartment **14** are generally designated as an after-portion **48** and a forward-portion **49**. Each of the bifurcate portions **48** and **49** is formed with a throat portion **51**. These throat portions **51** provide dual access openings into the interior of medial compartment **14** which is otherwise closed off at its upper end by the fusion-lines **52** which link up with the respective vertically-extending fusion-lines **46** and **47**. Each of the bifurcate portions **48** and **49** is formed with a pair of oppositely disposed appendage flaps **53** and **54**, respectively, each of which, in turn, has a metallic eye **56** fixedly securely therein.

Sac member **13**, which is adapted to both hold the given blood culture under treatment and act as the dialysing membrane, is a permeable, normally-flaccid, readily-expandable, tube-like member being open at its respective ends. It is composed of cellophane, or some like material, and is positioned in a substantially U-shaped position within medial compartment **14** with its respective open ends extending out of the respective throat portions **51** of the medial compartment **14**. Within each of the respective open ends of sac member **13** there is positioned the combination of an access fitting **57** and an access tube **58** running therethrough. These access fittings **57** and access tubes **58** are similar to those employed in the outer compartments **16** and **17**. When sac member **13** is operatively positioned within medial compartment **14**, the respective end portions thereof with their inserted access fitting **57** and access tube **58** combinations will be located within the throat portions **51** of the respective bifurcate portions **48** and **49**. Each of the throat portions **51** is encircled by an annular resilient fastening member **59** which holds both the underlying throat portion of medial compartment **14** and the underlying portion of sac member **13** in fluid-tight connection with the access fitting **57**.

Chamber **11** is intended for use in a temperature-controlled environment and, accordingly, is suitably supported in an incubator **61** by means of any suitable supporting members **62** which attach to the various metallic eyes hereinbefore cited.

After the blood culture has been introduced into sac member **13** via one or both of access tubes **58**, the one access tube of sac member **13** is connected to a gas-bearing line **63** and the other access tube of sac member **13** is connected to a gas-bearing line **64**. The respective gas bearing lines **63** and **64** pass through openings therefor in incubator **61** and on to an automatically-controlled valve **66**. From this valve **66** there extends a line **67** to a gas source **68** and another **69** which vents to atmosphere. Gas source **68** provides a gaseous mixture of air and carbon dioxide which is borne to valve **66** by the line **67**. Valve **66** is time-controlled to alternately connect the respective gas-bearing lines **63** and **64** to gas source **68** by way of the line **67** and to atmosphere via line **69**. When one of the gas-bearing lines **63** and **64** is connected to gas source **68**

via valve **66**, the other of gas-bearing lines **63** and **64** will be vented to atmosphere. The aforedescribed procedure keeps the blood in sac member **13** in continuous motion.

In like manner access tube **32** of left-hand outer compartment **16** of wash fluid bag **12** is connected to a gas-bearing line **71** and access tube **39** of right-hand outer compartment **17** is connected to a gas-bearing line **72**. Each of these gas bearing lines **71** and **72** passes through an opening provided therefor in incubator **61** to an automatic time-controlled valve **73**. Also connected to valve **73** is a line **74** which vents to atmosphere and a line **76** which leads to the gas source **68**. The gas pressure derived from gas source **68** is employed to keep the wash liquid (blood plasma) in bag **12** in continuous motion back and forth between the respective outer compartments **16** and **17** by way of medial compartment **14**. Valve **73** acts in such a fashion that, when gas-bearing line **71** is vented to atmosphere via line **74**, gas bearing line **72** bears gas under pressure from gas source **68** via line **76** and vice versa. Time-controlled valve **73** controls the application of gas pressure to these outer compartments **16** and **17** so that when compartment **16** is under pressure from gas source **68**, compartment **17** will be vented to atmosphere and then the cycle is reversed. Pressure from gas source **68** applied, first to one of the outer compartments **16** and **17** and then to the other, forces the wash liquid (i.e., blood plasma) back and forth between these outer compartments to induce movement of the wash liquid (blood plasma) to and fro relative to sac member **13**. This induced movement of the blood plasma wash liquid with respect to sac member **13** improves the efficacy of the dialysis process which takes place between the blood in sac member **13** and the wash liquid (blood plasma) in bag **12** via the dialysing membrane, i.e., sac member **13**.

Wash fluid bag **12** can be constructed of either soft or rigid plastic. Access fittings **28**, **38**, and **57** and access tubes **32**, **39**, and **58** are made of glass or nylon or some other suitable material which will introduce no undesirable reaction effects into the blood in sac member **13** or into the wash fluid (i.e., blood plasma) in wash fluid bag **12**.

Heparin, an anti-coagulant, is employed in both the blood specimen introduced into sac member **13** and in the blood plasma introduced into wash fluid or dialysing bag **12**. The employment of heparinized blood is essential to the satisfactory maintenance of blood herein in vitro. Even heparin can be toxic to blood and, accordingly, the method herein employs the minimum amount of heparin which will prevent clotting. This amount varies with the given individual, one person clotting more readily than another. For the normal person this minimal amount is on the order of .01 to .015 milligram of heparin per milliliter of blood. This minimum dosage of heparin is employed in both the blood specimen in sac member **13** and in the blood plasma in wash fluid or dialysing bag **12**. Even .02 milligram of heparin per milliliter of blood is too toxic and will lead to destruction of red cells of the blood. This toxicity brings about hemolysis which is characterized by a breaking down of the envelope of the blood cell with consequent loss of the hemoglobin which is normally contained by the envelope. It is the continuous motion induced in both the blood specimen in sac member **13** and in the blood plasma in wash fluid or dialysing bag **12** which allows use of the minimal amount of heparin in both the blood specimen and in the blood plasma. Previous attempts to maintain blood cultures in vitro, where there was no such continuous motion imparted to the blood specimen or culture and the wash liquid (i.e. blood plasma), necessitated the use of an amount of heparin which is a gross amount compared to the small amount of heparin required herein and therefore unfortunately toxic.

With a view toward achieving the in-vivo status of the blood as much as possible, heparinised autologous whole blood was employed herein as the tissue culture medium. The next best thing to using autologous heparinised whole blood is to employ hemologous heparinised whole blood.

In-vivo mammalian blood temperature is 37° C. and accordingly the dialysing process herein takes place with incubator **61** maintained at this 37° C. temperature. A slight variation of this temperature is permissible, actual body temperature also being susceptible to slight variation. It is generally known that blood deteriorates rapidly if incubated at 37° C. I have found however a way to prevent the named deterioration. This is achieved by my invention described herein, by heating the blood plasma which is to serve as the wash fluid to a temperature of 56° C. for a period of 15 to 20 minutes, then cooling said blood plasma to 37° C. at which temperature it is then introduced into wash fluid bag **12**. The specified pre-heating of the blood plasma releases a dialyzable factor which promotes the maintenance of the blood specimen in sac member **13** when the blood plasma containing this dialyzable factor is employed as the wash fluid in wash fluid bag **12**. Along with other aspects of the method employed herein, this releasing of the dialyzable factor in the wash fluid blood plasma avoids the blood deterioration previously incident to incubation at 37° C.

A satisfactory ratio of the volume of the blood plasma (introduced into wash fluid bag **12**) to the volume of blood (introduced into sac member **13**) is from about three to one to about six to one, the latter limit being a preferred ratio. When the blood specimen in sac member **13** is to be maintained over a period of time, the dialysing plasma in bag **12** should be exchanged (i.e. renewed) periodically every three or four days.

The blood cells in the blood specimen contained by sac member **13** require feeding. When the blood specimen is introduced into sac member **13** and the blood plasma is introduced into wash fluid bag **12** as the dialysing liquid, care must be taken to ensure that the sugar concentration in the blood specimen and in the blood plasma wash fluid are the same. Otherwise osmotic exchange due to any difference in sugar concentration between the blood specimen and the blood plasma will take place and will be destructive to the blood specimen. As time passes, the blood cells in the blood culture in sac member **13** will metabolize (i.e., consume) sugar with the result that water will go out of the blood into the blood plasma by way of sac member **13** which serves as the dialysing membrane and the blood specimen in sac member **13** accordingly will get concentrated, that is, hemoconcentration will take place in the blood specimen in sac member **13**. This normal consequence of the passage of time (during which the blood specimen in sac member **13** is being maintained) is compensated for by adding sugar to the blood specimen in sac member **13**. This can be done by taking daily checks of the blood specimen in sac member **13** and adding sugar, as necessary, to bring the blood specimen back to its original concentration. The entire process of checking the blood specimen and taking remedial action can be accomplished through the use of hypodermic needles which will readily pass through the tap opening of the sac member **13** and the wall of the wash fluid bag **12**, the hypodermic needles after removal leaving no permanent damage to either of these elements.

Gas source **68** supplies a gaseous mixture of air with 5 to 10 percent carbon dioxide. With this gaseous mixture alternatingly applied to and removed from the blood column contained in sac member **13**, as described above, the blood specimen in sac member **13** is maintained in continuous motion and because of the constituency of the gaseous mixture, the blood in sac member **13** is both oxygenated and kept at a proper pH value. Both of these results are necessary to the proper in-vivo-like in vitro environment created herein. The proper pH value for the blood specimen in sac member **13** is a pH of 7.3. A satisfactory frequency for the inflation-deflation cycle employed with each of the legs of sac member **13** is on the order of 5 to 6 times per minute. The pressure of the

gaseous mixture from gas source **68** is optimally operable at the pressure of in-vivo body capillary or small artery pressure with systolic and diastolic aspects maintained. A representative value of such gaseous pressure is about four centimeters water pressure. In regard to the pH value, too much CO_2 will unfavorably depress the proper pH value and too little CO_2 will unfavorably raise the proper pH value.

While it is absolutely essential herein that the blood specimen in sac member **13** be maintained in motion, it is also preferable (though not essential) to keep the dialysing plasma (blood plasma) in motion as well. This is achieved as described supra.

The electrolyte equilibrium between the blood specimen in sac member **13** and the dialysing fluid (blood plasma) in wash fluid bag **12** is maintained by osmotic exchange. Herein the preferred dialysing fluid, as noted above, is pooled homologous plasma. Aseptic conditions are maintained throughout the dialysing and circulating procedures defined herein to avoid contamination. As an aid in this regard, a small amount of penicillin is added to both the blood specimen in sac member **13** and to the blood plasma in wash fluid bag **12**. The amount used is 100 units of penicillin, or less, for each milliliter of blood or blood plasma.

Certain persons are found to have a high percentage concentration of red cells in their total blood count. This information is readily available from a hematocrit reading. For instance, such individuals will average 45 points on the hematocrit reading scale. When the blood specimen which is introduced into sac member **13** is derived from such individuals, better results will be achieved in the process defined herein if the blood specimen is diluted by autologous plasma to bring the hematocrit reading to something on the order of 20 to 25 points.

An essential requirement of the process herein employing blood circulating-and-dialysing chamber **11** is the maintenance of electrolyte equilibrium within this blood chamber **11**. When the blood specimen is initially introduced into sac member **13** and the blood plasma is likewise introduced into wash fluid bag **12**, provided that the blood specimen and the blood plasma were obtained from the same individual, the blood specimen and the blood plasma will be in electrolyte equilibrium with one another. Where the blood plasma is obtained from some other source other than the individual whose blood specimen is being employed, remedial compensation can be employed to bring these two liquids into this proper initial electrolyte equilibrium. However, as the process of maintaining the blood specimen continues, because of continuous evaporation of water from the blood plasma in wash fluid bag **12**, the initial electrolyte equilibrium which existed between the two liquids, which are in osmotic exchange via sac member **13** which serves as a dialysing membrane, is disrupted. To remedy this natural consequence of the passage of time, manual or automatic addition of distilled water into the blood plasma in wash fluid bag **12** will have to be performed at various intervals in order to restore the electrolyte equilibrium which initially existed between the blood specimen and the blood plasma.

Summing up the above listed aspects which variously contribute to the method herein for producing an in vitro environment which sufficiently approximates the in vivo environment of the blood involved, it is noteworthy to point out that the following items are of critical requirement: (1) maintenance of proper electrolyte equilibrium, (2) maintenance of proper sugar concentration, (3) employment of a "minimal" amount of heparin anti-coagulant for keeping the blood in a fluid state, maintenance of proper pH value in the blood specimen, (4) oxygenation of the blood specimen and (5) maintenance of the blood specimen in motion and suspension.

Other factors previously mentioned, but not listed in the aforesaid grouping, are desirable, but not necessarily essential to the maintenance of the in vivo-like in vitro environment defined herein.

Obviously many modifications and variations of the present invention are possible in the light of the above teachings. It is intended to cover all changes and modifications which may be made without departing from the spirit and scope of this invention.

What is claimed is:

1. A method for maintaining blood in vitro comprising the following steps:

(a) placing said blood in a dialysing chamber in osmotic contact with a dialysing liquid capable of receiving toxins from said blood and of supplying blood-conditioning factors to said blood by means of osmotic exchange;

(b) circulating said blood in an oscillating motion during dialysis to controlled application of an oxygen and carbon dioxide gas mixture under pressure for the combined purposes of oxygenating said blood, keeping said blood in motion, and buffering said blood to a pH of approximately 7.3; and

(c) maintaining osmotic equilibrium between said blood and said dialysing liquid.

2. A method for maintaining blood in vitro comprising the following steps:

(a) placing said blood in a dialysing chamber in osmotic contact with a dialysing liquid capable of receiving toxins from said blood and of supplyng blood-conditioning factors to said blood by means of osmotic exchange;

(b) subjecting said blood during dialysis to controlled application of an oxygen and carbon dioxide gas mixture under pressure for the combined purposes of oxygenating said blood, keeping said blood in motion, and buffering said blood to a pH of approximately 7.3;

(c) maintaining osmotic equilibrium between said blood and said dialysing liquid;

(d) said dialysing liquid being blood plasma which has been preheated to a temperature of about 56° C. to release a dialysable factor therein, said blood plasma and said blood being maintained at a temperature of about 37° C. during dialysis.

3. The method of claim **2** wherein said blood plasma has been preheated to a temperature of 56° C. for a period of from about fifteen to about twenty minutes and then is cooled to 37° C. for its use at the dialysing liquid with said blood.

4. The method of claim **3** further characterized by the step of adding sugar to said blood as necessary to meet the amount of sugar initially within said blood which has been consumed and metabolized by the cells within said blood as time passes.

5. The method of claim **3** wherein said blood has been heparnized by the addition thereto of a minimal amount of heparin, said minimal amount of heparin being defined by that amount thereof which will keep said blood in a fluid state when in motion.

6. The method of claim **5** wherein said oxygenating gas is a mixture of approximately 90 to 95% by volume of air and approximately 5 to 10% by volume of carbon dioxide.

7. The method of claim **6** wherein said oxygenating gas is applied to said blood at a pressure of substantially 4 centimeters of water.

8. A method for maintaining blood in vitro comprising the following steps:

(a) forming said blood into a pair of substantially vertically-extending columns which are freely interconnected;

(b) maintaining said columns of blood in osmotic exchange with a dialysing liquid capable of receiving toxins from said blood and of supplying blood-conditioning factors to said blood;

(c) applying a mixture of oxygen and carbon dioxide gas under pressure to the top of one of said blood columns while at the same time venting to atmosphere the top of the other of said blood columns and periodically alternating this application of gas from one blood column to the other, with the column not under pressure from the oxygenating gas at any given time being vented to atmosphere, the cyclic application and venting of said oxygenating gas relative to each of said blood columns accomplishing the combined functions of oxygenating said blood, inducing sustained circulation of said blood, and maintaining said blood at its normal in-vivo pH value;

(d) periodically introducing distilled water into said dialysing bag for maintaining electrolyte equilibrium between said blood and said dialysing liquid;

(e) maintaining the initial sugar concentration within said blood;

(f) said blood being provided with the smallest amount of heparin which will keep said blood in fluid state while in motion;

(g) said dialysing liquid being blood plasma which has been preheated to a temperature of 56° C. for a period of from about fifteen to about twenty minutes and then cooled to 37° C.; and

(h) maintaining both said blood and said blood plasma at a temperature of substantially 37° C. during the process of osmotic exchange.

9. The method of claim **8** wherein said oxygenating gas is a mixture of substantially 90 to 95% by volume of air and 5 to 10% by volume of carbon dioxide.

10. The method of claim **9** wherein said oxygenating gas is applied to said blood at substantially a pressure of 4 centimeters of water.

11. A method for maintaining blood in vitro comprising the following steps:

(a) heating blood plasma which is compatible with the given blood to be maintained in vitro to a temperature higher than that of the in vivo temperature of said blood and at which a dialysable factor is released from said blood plasma;

(b) cooling said heated blood plasma to the normal in-vivo temperature of said blood;

(c) dialysing said blood against said blood plasma in and carbon dioxide under pressure both to said blood plasma are both maintained at the in-vivo temperature of said blood, and applying in a pulsating manner a mixture of oxygen and carbon dioxide under pressure both to said blood and said blood plasma.

12. A method for maintaining blood in vitro comprising the following steps:

(a) heating blood plasma which is compatible with the given blood to be maintained in vitro to a temperature of 56° C. for an interval of about fifteen to twenty minutes for releasing a dialysable factor from said blood plasma;

(b) cooling said heated blood plasma to the normal in-vivo temperature of said blood;

(c) dialysing said blood against said blood plasma in an environment wherein said blood and said blood plasma are both maintained at the in-vivo temperature of said blood and;

(d) applying in a pulsating manner a mixture of oxygen and carbon dioxide under pressure both to said blood and said blood plasma.

13. A chamber, adapted for the maintenance and treatment of blood in vitro comprising:

(a) an impermeable wash fluid bag comprising a medial compartment and a pair of outer compartments one of which is located on each side of said medial compartment, said medial and said pair of outer compartments being in free fluid communication with one another at the respective lower portions thereof;

(b) each of said outer compartments being formed in its upper portion with an access opening therein and said medial compartment being formed in its upper portion with a pair of access openings therein;

(c) fluid conduit means, individual to each of said outer compartments and passing into its associated outer compartment by way of the access opening therein and in fluid-tight sealing engagement with the access opening involved, for permitting external fluid communication with the given outer compartment;

(d) a permeable normally-flaccid readily expandable tubular sac member having access openings in its respective longitudinal ends and being substantially disposed in said medial compartment of said wash fluid bag in a generally U-shaped attitude therein and with each of the longitudinal ends of said tubular sac member positioned in one of said access openings of said medial compartment; and

(e) means, associated with each of the longitudinal ends of said tubular sac member and with the medial compartment access opening in which the given longitudinal end of said sac member is disposed, for closing off in a gas-tight seal both the given medial compartment access opening and the access opening of the given longitudinal end of said permeable sac member and for providing a fluid communication pathway into said permeable sac member via the given longitudinal end thereof;

(f) said permeable sac member being adapted to hold blood and to act as a dialysing membrane and said wash fluid bag being adapted to hold wash liquid for a dialysis process to be performed via the dialysing membrane represented by said permeable sac member;

(g) each of said fluid conduit means being adapted to adapted to connect alternately to a source of oxygenating gas under pressure and then to atmosphere for the purpose of inducing relative movement of the wash liquid in said wash fluid bag with respect to the blood contained by said permeable sac member and said respective means which provide a fluid communication pathway into said permeable sac member via the given longitudinal end thereof being adapted to connect alternately to a source of oxygenating gas and then to atmosphere for the combined purposes of inducing continual circulation in the blood in said permeable sac member, properly buffering said blood to an appropriaate pH value and oxygenating said blood.

References Cited

UNITED STATES PATENTS

2,652,831	9/1953	Chesler	23—258.5
3,026,811	3/1962	Thomas	23—258.5
3,276,589	10/1966	Jankay	210—175

OTHER REFERENCES

Koff et al.: J. Lab., and Clin., Med., vol. 47, pp. 969–977 (June 1956).

Koff et al.: Brit. Med. Jour., No. 5180, pp. 1149–1153 (April 1960).

Kupfer et al., J. Lab. and Clin. Med., vol. 54, pp. 746–755 (November 1969).

Skeggs et al.: Proc. Soc. for Exper. Biol. and Med., vol. 72, pp. 539–543 (December 1949).

JOSEPH SCOVRONEK, *Primary Examiner.*

U.S. Cl. X.R.

23—258.5; 210—175, 253, 321