

Oct. 4, 1966

L. JANKAY

3,276,589

APPARATUS FOR MAINTENANCE AND TREATMENT OF BLOOD IN VITRO

Filed Dec. 18, 1961

2 Sheets-Sheet 1

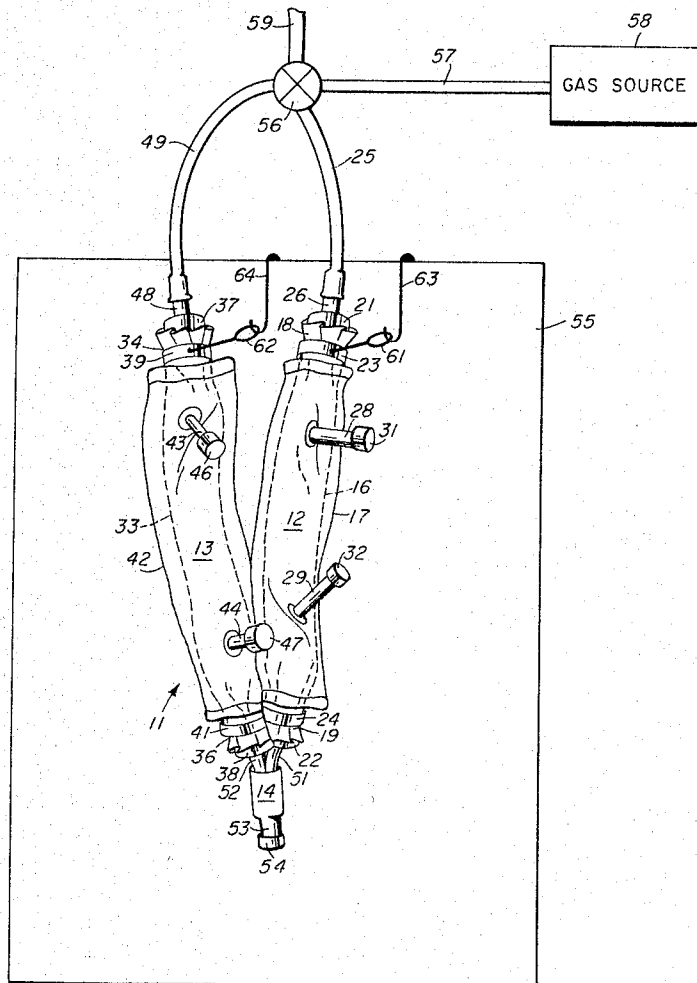


FIG. 1

INVENTOR.
LESTER (NMI) JANKAY

BY

Paul W. Gilkey
Richard J. Horn AGENT
ATTORNEYS

Oct. 4, 1966

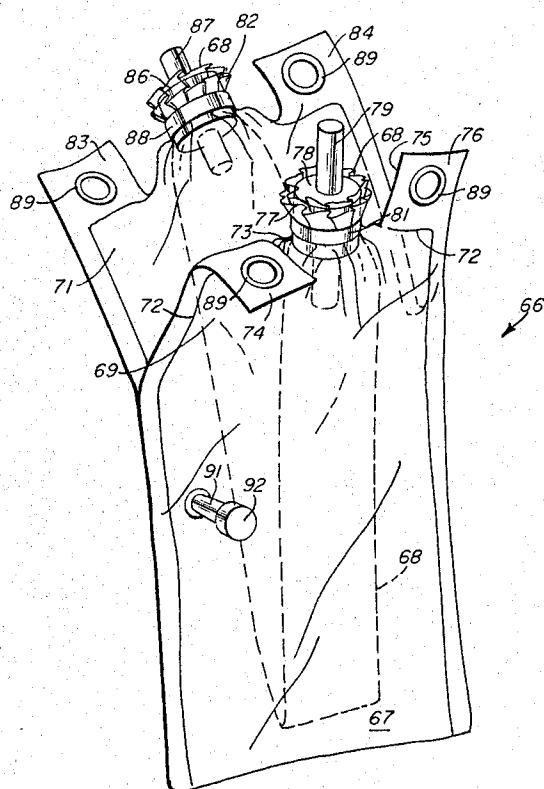
L. JANKAY

3,276,589

APPARATUS FOR MAINTENANCE AND TREATMENT OF BLOOD IN VITRO

Filed Dec. 18, 1961

2 Sheets-Sheet 2



INVENTOR.
LESTER (NMI) JANKAY

BY *Richard P. Howard Jr.* AGENT

James R. Gitcher
ATTORNEYS

1

3,276,589

APPARATUS FOR MAINTENANCE AND TREATMENT OF BLOOD IN VITRO

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

Filed Dec. 18, 1961, Ser. No. 160,376

4 Claims. (Cl. 210—175)

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.

The present invention relates to an apparatus for the maintenance and treatment of blood in vitro and, more particularly, to such method and apparatus where the given blood culture is continuously circulated, oxygenated, dialysed and fed.

Effective maintenance of blood outside of the body has been a long-sought goal for a variety of purposes. In vitro blood studies, reconditioning of human blood affected by disease and extracorporeal maintenance of blood as an aid to surgical procedures are but a few. The main obstacle to such a goal has been the failure to create an "in vitro" environment for blood which satisfactorily approximates its natural "in body" ("in vivo") environment. Previously-used techniques have failed to produce an "in vitro" environment wherein the blood cells can be effectively maintained with their normal behavior preserved. In these prior "in vitro" environments the behavior of the blood cells differs markedly from their normal "in body" behavior; this fact leads to a plurality of manifest difficulties when such "in vitro" environments are employed.

The present invention involves means which effectuate an improved "in vitro" environment for blood which satisfactorily approximates the natural "in vivo" environment for such blood. To cite a few of the uses of such an "in vitro" environment as is produced by this invention: it lends itself to use as a culture medium for effective cultivation of tissues such as cancer tissues and bone marrow, among others; previously used environments for such purposes are generally chemically-defined media which produce an "artificial" environment thus requiring that such cultivations be carried on in a "foreign" environment with all the difficulties that such imposes. Another illustrative application of the in-vivo-like "in vitro" environment herein created is that it renders feasible the study of radiation effects on human blood without the necessity of exposing the human body to such radiation.

The invention means employed herein utilizes a simple-construction and easily-portable blood chamber wherein the blood is kept in continuous circulation to maintain suspension of the blood cells, is continuously oxygenated and, through dialytic action, has deleterious factors removed therefrom and compensating factors added thereto. The apparatus herein is characterized by controlled temperature and pressure aspects for the blood environment; the temperature maintained is substantially the same as the blood would have "in vivo" and the blood is subjected to an oxygenating, circulation-inducing gas which maintains the blood at a pressure comparable to that of "in vivo" body capillary or small artery pressure with systolic and diastolic aspects maintained.

An object of the present invention is means to effectively maintain blood in vitro.

Another object is means to treat blood in vitro.

A further object is means to produce an "in vitro" environment for blood which successfully approximates the natural "in vivo" environment of the blood.

Still another object is to provide an apparatus for performing the combined functions of circulating, oxygenating, dialysing and feeding blood culture.

2

A further object is to provide an apparatus for performing any single one or any combination of the above-cited functions.

An additional object is the provision of a blood circulating and dialysing chamber.

Another object is the provision of a dialysing chamber wherein the liquid being dialysed is maintained in controlled circulation.

Still another object is the provision of a cheap, simple, readily-portable combined blood circulating and dialysing chamber.

Other objects and many of the attendant advantages of this invention will be readily 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 chamber is shown in perspective and the balance of the apparatus is generally shown schematically; and

FIG. 2 illustrates another embodiment of the blood chamber employable in the subject invention.

There is shown in FIG. 1 a bifurcated blood circulating-and-dialysing chamber 11 consisting principally of two identical legs 12 and 13 interlinked at their bottommost portions by a Y-tube fitting 14.

Leg 12 consists principally of a permeable, normally-flaccid, readily-expandable tubular sac member 16 which is surrounded throughout most of its length by an outer, tubular, expandable, impermeable sac member 17. At its upper and lower ends impermeable sac member 17 is fused or otherwise suitably joined to underlying permeable sac member 16 to define a sealed-off compartment whose inner wall is formed by permeable sac member 16 and whose outer wall is formed by impermeable sac member 17. Permeable sac member 16 extends above and below the areas of union between sac member 16 and sac member 17 to form neck-like portions 18 and 19. Insertedly positioned in these terminal neck-like portions 18 and 19 of permeable sac member 16 are a pair of bored cylindrically-shaped access fittings 21 and 22. Annular fastening members 23 and 24, disposed outwardly of respective neck-like portions 18 and 19, hold these neck-like portions 18 and 19 in tight contact with the outside of access fittings 21 and 22 thus creating a fluid-sealing closure between the access fittings 21 and 22 and their companion neck-like portions 18 and 19. Leading into the bore of access fitting 21 and fixedly positioned therein is an access tube 26. This access tube 26 is adapted to connect to an external gas-bearing line 25 for enabling a gaseous mixture (later to be described) to be introduced into the upper portion of permeable sac member 16.

Access to the compartment formed between permeable sac member 16 and impermeable sac member 17 is provided by conduit tubes 28 and 29 which are secured to impermeable sac member 17 and pass therethrough. These conduit tubes 28 and 29 are closed respectively by removable stopper members 31 and 32 affixed to their outer ends.

Left-hand leg 13 is identical in structure with right hand leg 12. It has an inner permeable sac member 33 suitably joined at its upper and lower neck-like portions 34 and 36 to respective cylindrically-shaped bored access fittings 37 and 38 by fastening members 39 and 41.

Leg 13 has an outer impermeable sac member 42 joined to the permeable sac member 33, as described for leg 12. This impermeable sac member 42 is provided with upper and lower conduit tubes 43 and 44 for establishing access to the compartment defined between the inner permeable sac member 33 and the outer impermeable sac member 42. Removable stopper members 46 and 47 affixed to the outer ends of conduit tubes 43 and 44

enable closing off of the compartment. Leading into the bore of upper access fitting 37 and affixed to the access fitting 37 is an access tube 48 which is adapted to connect to a second gas-bearing line 49 for enabling entry into the upper portion of permeable sac member 33 of the same gaseous mixture as referred to above with respect to gas-bearing line 25.

Interconnecting the bottoms of permeable sac members 16 and 33 so as to permit free flow of liquid from one permeable sac member to the other is the Y-tube fitting 14 whose upper portions 51 and 52 are connected respectively to access fittings 22 and 38 and whose lower portion 53 is closed by a removable stopper member 54.

Access fittings 21, 22, 37 and 38, access tubes 26 and 48, conduit tubes 28, 29, 43 and 44, and the upper portions 51 and 52 and lower portion 53 of Y-tube fitting 14 are made of glass or nylon or some other suitable material which will introduce no undesirable reaction effects into the blood or wash fluid (e.g., plasma).

A suitable material for the stopper members 31, 32, 43, 44 and 54 is a material which goes under the trade-name of Tygon which describes a series of vinyl compounds that are unaffected by oils, water, oxidants, acids, bases and salts.

Dialysing chamber 11 is maintained in an incubator 55 which is controlled to keep the fluids in the dialysing chamber 11 at a temperature of 37° C.

Gas bearing lines 25 and 49 which lead to the respective legs 12 and 13 of dialysing chamber 11 pass through openings in incubator 55 and on to an automatically-controlled valve 56. From this valve 56 there extends a line 57 to a gas source 58 and another line 59 which vents to atmosphere. Gas source 58 provides a gaseous mixture of 95% air and 5% carbon dioxide, by volume, which is brought to valve 56 by the line 57. Valve 56 operates to alternately connect the respective lines 25 and 49, leading to the respective legs 12 and 13, to the gas source 58 via line 57 or to atmosphere via line 59. When one of the gas-bearing lines 25 and 49 is conducting gas from gas source 58 to its chamber leg, the other of the gas-bearing lines is vented to atmosphere via valve 56 and line 59. The gas in gas source 58 is under such pressure as to deliver the gaseous mixture to the chamber legs at a pressure of substantially 4 centimeters of water. The periods of alternation are such that once per minute each permeable sac member is subjected to an inflationary-deflationary cycle wherein the 95% air—5% CO₂ gas is first admitted to the permeable sac member at a pressure of 4 cm. of H₂O and then the permeable sac member has the gas source 58 blocked from it and this permeable sac member is vented to atmosphere. During this period of inflation followed by deflation the other permeable sac member (in the other leg of the chamber 11) undergoes a concurrent period of deflation followed by inflation. A satisfactory inflation-deflation cycle for each permeable sac member is in the order of a minute.

Eyes 61 and 62 are affixed to fastening members 23 and 39, respectively, to enable the legs 12 and 13 to be suitably supported at desired locations within incubator 55, as by hooks 63 and 64, for example.

As a portrayal of the efficiency of the in vitro environment developed herein, rat blood, which is noted for its instability in an in vitro environment, has been successfully maintained in vitro by the subject invention.

With legs 12 and 13 suspended in substantially vertical position in incubator 55, fresh heparinized rat blood is introduced into either or both of permeable sac members 16 and 33 via their access tubes. The introduced blood will flow back or forth between the permeable sac members to bring the blood in each permeable sac member to a common level.

With the blood in the permeable sac members 16 and 33, gas-bearing lines 25 and 49 are affixed to their respective access tubes 26 and 48.

The "wash fluid," preferably consisting of heated compatible blood plasma at 37° C., is introduced via conduit tubes 28 and 43 in equal amounts to give substantially the same level of plasma in each leg as of blood. Prior to its introduction into chamber 11 the blood plasma has been heated to and kept at a temperature of 56° C. for 15–20 minutes and then cooled to the 37° C. temperature at which it is introduced into chamber 11. This heating of the blood at 56° C. releases from certain of the blood proteins a dialysable factor which promotes maintenance of the blood cells. With the blood located within permeable sac members 16 and 33 and the interconnecting Y-tube fitting 14, the blood plasma is located in the outer compartments defined between the respective permeable and impermeable sac members of each of legs 12 and 13. A satisfactory ratio of volume of blood plasma to volume of blood is provided by the use of at least three times, and, preferably, six times as much blood plasma as blood. When the blood is to be maintained over a period of time, the blood plasma should be exchanged, i.e., renewed, periodically every three or four days.

The blood-containing permeable sac members 16 and 33 are made of cellophane, or some like material, which has such permeability as to permit the desired osmotic exchange between the blood and the wash fluid (plasma). The outer impermeable sac members 17 and 42 are made of a material such as vinyl, or the like, which will not be reactive with the wash fluid at any time during the dialytic process and will successfully contain and support the same.

With the operative amounts of blood in both permeable sac members and of the heated plasma (wash fluid) in both of the surrounding compartments and the appropriate stopper members in their closing position, valve 56 will alternately introduce the 95% air—5% CO₂ mixture into each of permeable sac members 16 and 33 where this gaseous mixture under 4 cm. of H₂O pressure is brought into direct contact with the blood culture. As the column of blood in one leg is brought under pressure of this gaseous mixture the column of blood in the other leg is being relieved of pressure from this gaseous mixture (by the venting via valve 56 and line 59). As this application of gaseous pressure (followed by release of the gas) takes place alternately first in one leg and then in the other, the blood culture is moved back and forth between the respective permeable sac members 16 and 33 via Y-tube fitting 14 in response to this pressure. With this inflation-deflation cycle occurring in tandem in the respective legs once per minute the blood is kept in "circulation." The direct contact between the blood and the gaseous mixture of 95% air—5% CO₂ causes the blood to be oxygenated.

By virtue of its contact with the wash liquid (plasma) via the "permeable membranes" offered by the permeable sac members, the blood is dialysed to have added to it nutrients and any other selected compensating factors and to have subtracted from it into the wash liquid any deleterious factors. The induced circulation employed here effects oxygenation of the blood, furthers the dialysis process and in its own right obviates the deterioration effects associated with stationary cultures. Although diluents other than plasma may be utilized, plasma has been found to be preferable. The use of heated plasma at 37° centigrade as opposed to non-heated plasma, yields a minimum of hemolysis and essentially no change in the hematocrit value from the normal, thus materially contributing to the stability and viability of the blood being processed.

It will be noted that, though each leg of the chamber has its permeable sac member alternately undergoing inflation and deflation periods, the cycles in each of the legs 12 and 13 complement each other so that the gaseous mixture is practically in continuous contact at all times with the blood culture.

The entire process is performed at a controlled temperature of 37° C. by suitable control of the incubator 55 so

5

that both the plasma and the blood are maintained at this temperature. The reason for the temperature of 37° C. is because this is the temperature of the blood in its natural, in-body environment. A slight variation of this temperature is permissible, actual body temperature also being susceptible to slight variation.

The 4 cm. H₂O pressure for the gas can be varied somewhat. The pressure criterion is that it create a small pressure similar to that existing in the capillary or small blood vessels in the body.

FIG. 2 portrays another embodiment of the invention employing a modified form of blood circulating-and-dialysing chamber 66. This chamber comprises essentially a wash fluid bag 67, of vinyl or some like appropriate material, and a substantially U-shaped readily-expandable tubular sac member 68 which is substantially disposed within the bag 67 and extends therefrom at its terminal ends. Sac member 68 is composed of cellophane, or like material, and like the sac members 16 and 33 of the FIG. 1 embodiment, serves both to hold the blood culture and as the dialysing membrane. Bag 67, which is adapted to hold the heated blood plasma, is of unitary construction and bifurcates at its upper end to form a forward portion 69 and an after portion 71.

Lines 72 as seen in the forward portion 69 represent upper limits of the interior portion of the bag 67 in such forward portion 69 and are lines of fusion of opposing layers of the vinyl material. The bag 67 is similarly formed in the after portion 71. This forward portion has been cut along the lines 73 and 75 to form left and right handed flap appendages 74 and 76, respectively, and a central throat portion 77. One upper terminal of the U-shaped tubular sac member 68 projects out of bag 67 via the throat portion 77. Disposed in this projecting terminal of sac member 68 is a bored cylindrically-shaped access fitting 78 in which is fixedly positioned an access tube 79 which extends above and below the access fitting 78. By means of resilient annular fastening member 81, disposed outwardly of throat portion 77 and resiliently bearing against access fitting 78, bag throat portion 77 and the intermediate adjacent terminal end of sac member 68 are maintained in tight contact with the external surface of access fitting 78 to create a fluid closure of bag 67 in the region of throat portion 77 and a like fluid closure of the projecting terminal end of sac member 68, leaving access available to this terminal end of sac member 68 only via access tube 79.

In like manner the after portion 71 of bag 67 is formed with a central throat portion 82, respective left and right appendages 83 and 84, an access fitting 86, an access tube 87 and a resilient fastening member 88 whose action, in union with the encompassed access fitting 86, closes off the other projecting terminal of sac member 68 and the after bag portion throat 77, leaving access to the sac member terminal via access tube 87.

Each of the four appendages 74, 76, 83 and 84, respectively, is fitted with a metallic eye 89 by which the chamber 66 can be suitably supported.

Like the chamber 11 portrayed in FIG. 1, this chamber 66 of FIG. 2 likewise will be supported in an incubator such as 55 and the access tubes 79 and 87 respectively are adapted to connect to such gas-bearing lines as 25 and 49 when the chamber 66 is in use.

Access to bag 66 is available through conduit tube 91 affixed to and penetrating bag 66 and closeable by stopper member 92.

The mode of operation involved with chamber 66 corresponds exactly with that defined above for chamber 11.

Still another embodiment of the blood-wash fluid chamber would employ a wash fluid bag provided with a single throat opening therein and a rectilinearly-extending permeable sac member disposed therein which is sealed off at its lower end and has its upper end open for access thereto and which extends out of the wash fluid bag via the throat opening thereof for linkage with such a gas-

6

bearing line as 25 or 49. The fluid access structure will be similar to that reflected in the FIGS. 1 and 2 embodiments. Such a wash fluid bag-permeable sac member combination would be fixedly supported on a platform which would be continually oscillated back and forth between positions of zero and ninety degrees to provide the necessary circulation to the blood culture disposed in the permeable sac member.

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. Apparatus for the maintenance and treatment of blood in vitro comprising a first permeable normally flaccid readily expandable tubular sac member having access openings at its upper and lower ends and being adapted to being positioned in a substantially vertical position; a first tubular expandable impermeable sac member in substantially enclosing position around said first permeable sac member, said first permeable sac member and said first impermeable sac member being joined together at their respective upper and lower portions to define a first closed compartment whose inner wall is defined by said first permeable sac member and whose outer wall is defined by said first impermeable sac member; operator-closeable first conduit means extending into and affixed to said first impermeable sac member for furnishing access to said first closed compartment; a second permeable normally flaccid readily expandable tubular sac member having access openings at its upper and lower ends and being adapted to being positioned in a substantially vertical position alongside said first permeable sac member; a second tubular expandable impermeable sac member in substantially enclosing position around said second permeable sac member, said second permeable sac member and said second impermeable sac member being joined together at their respective upper and lower portions to define a second closed compartment whose inner wall is defined by said second permeable sac member and whose outer wall is defined by said second impermeable sac member; operator-closeable second conduit means extending into and affixed to said second impermeable sac member for furnishing access to said second closed compartment; fluid transfer means connected respectively to said first and said second permeable sac members at their lower ends so as to define a fluid passage way between the inner portions of said respective first and second permeable sac members; said first and said second permeable sac members being adapted to contain blood and said fluid transfer means being adapted to freely pass blood back and forth between said first and said second permeable sac means; each of said closed compartments being adapted to contain a wash liquid suitable for osmotic exchange with the blood contained by the respective permeable sac members, each of said permeable sac members serving as a dialysing membrane; inlet-and-outlet means connected to said respective permeable sac members at their upper portions; a source of oxygenating gas under pressure; gas-bearing tube means interconnecting said gas source and each of said respective inlet-and-outlet means; automatic valve means disposed in said gas-bearing tube means; gas venting means connected to said valve means; said valve means functioning to admit said oxygenating gas under pressure from said gas source to one of said permeable sac members via said gas bearing tube means and the inlet-and-outlet means of that particular permeable sac member and at the same time to vent said other permeable sac member to atmosphere via said gas bearing tube means and said gas venting means, and then to repeatedly alternate the above-described process as it relates to each of said permeable sac members so that each of said permeable sac members alternately undergoes inflation with said oxygenating gas and then is relieved of said gas which is

vented, the resulting inflation-deflation cycles experienced by each permeable sac member causing both substantially continuous oxygenation of the blood carried by said permeable sac members and said fluid transfer means and continuous circulation of said blood in said apparatus.

2. The apparatus of claim 1 wherein said oxygenating gas is composed of substantially 95% by volume of air and 5% by volume of carbon dioxide.

3. A chamber adapted for the maintenance and treatment of blood in vitro comprising a wash fluid bag, adapted to hold a wash fluid, said bag being bifurcated at its upper portion to form a first upper portion and a second upper portion, each of said upper portions being formed with a pair of end appendages and a medially-located throat section, said appendages being adapted to connect to a supporting member for supporting said wash fluid bag in an upright position; a substantially U-shaped readily-expandable permeable tubular sac member substantially disposed in said fluid bag and having its terminal portions extending through the respective throat sections of said wash fluid bag; conduit means disposed in the terminal portions of said U-shaped tubular sac member for permitting flow of fluid therethrough; fastening means disposed outwardly of the terminal portions of said tubular sac member and outwardly of the throat sections of said wash fluid bag for closing off said throat portions and for fastening each of the terminal portions of said U-shaped tubular sac member to its respective conduit means so that fluid flow into or out of said U-shaped tubular sac member can be effectuated only through the respective conduit means associated with each of the terminal portions of said tubular sac member; and operator-closeable means affixed to and penetrating said wash fluid bag for enabling wash fluid to be deposited in or removed from said bag, as desired.

4. Apparatus for the maintenance and treatment of blood in vitro comprising;

first and second permeable sac members formed of a material capable of permitting a dialytic osmotic exchange between blood and a blood plasma wash fluid,

an impermeable sac in substantially enclosing position around each of said first and second permeable sac members,

means for introducing said blood into said first and second permeable members,

means for introducing said wash fluid into said impermeable sacs,

said first and second sac members being tubular and being normally disposed in a substantially vertical position,

passage means intercommunicating the lower ends of said first and second permeable members whereby said introduced blood can flow freely from one member into the other,

a pressurized source of oxygenating gas, gas conduit means coupling said source to the upper ends of each of said first and second permeable members,

valve means disposed in said conduit means for alternately applying said gas under pressure to said first and second permeable members and for alternately venting said permeable members whereby said introduced blood can be oxygenated and maintained in a constant transfer motion between said permeable members,

incubator means encasing said impermeable and permeable sacs, and

means for supporting said sacs within said incubator in said substantially vertical positions.

References Cited by the Examiner

UNITED STATES PATENTS

2,969,150	1/1961	Broman	210—321
2,982,416	5/1961	Bell	210—321
3,002,887	10/1961	Zilliken	167—74
3,003,918	10/1961	Sanders	167—74
3,052,238	9/1962	Broman	128—214
3,064,647	11/1962	Earl	128—214
3,110,308	11/1963	Bellamy	128—214

OTHER REFERENCES

Astrada: Chem. Abst., vol. 54, p. 4732(c), 1960.

Gardner: J. Am. Med. Assoc., vol. 162, vol. 43, No. 2.

Kay: J. Am. Med. Assoc., vol. 162, No. 7, pp. 639—641, 1956.

Kolff: Brit. Med. J., No. 5180, pp. 1149—1153, 1960.

Kupfer: J. Lab. Clin. Med., vol. 54, pp. 746—755, 1955.

Kolff: J. Lab. Clin. Med., vol. 47, pp. 969—977, 1956.

Kolff: J. A. Med. Assoc., vol. 164, No. 15, pp. 1653—1660, 1957.

Rosenak: Proc. Soc. Exp. Biol. Med., vol. 76, pp. 471—475, 1951.

Schechter: Military Medicine, vol. 126, pp. 593—603, 1961.

Skeggs: Proc. Soc. Exp. Biol. Med., vol. 72, pp. 539—543, 1949.

REUBEN FRIEDMAN, *Primary Examiner*.

JULIAN S. LEVITT, MORRIS O. WOLK, *Examiners*.

SAMIH ZAHARNA, P. SABATINE,

Assistant Examiners.