

normal saline is prepared by adding 5 grams of said hemoglobin to physiological saline, which is 0.85% sodium chloride in water, with q.s. to 100 ml. These compositions are administered in the manner commonly employed in the blood transfusion art as disclosed in *Practical Blood Transfusion*, by Hustis, Bove and Busch, 1969, published by Little, Brown and Company, Boston.

Other applications for the hemoglobins of the invention include their use as artificial oxygen exchange solutions in conventional oxygenators, such as cardiac bypass extracorporeal circulatory assist devices, hollow fiber and sheet-type membrane devices, as used for assisting circulation in ill patients. For these conditions, oxygenators are widely used to mechanically oxygenate venous blood extracorporeally. In operation, an oxygenator has one or more pumps for maintaining circulation and for perfusion of oxygen by the exchange of gases between blood in an isolated vascular bed and oxygen across an oxygenation membrane.

An oxygen exchange membrane is prepared according to the invention by saturating a porous synthetic semipermeable membrane, such as cellophane or a Millipore® filter having a pore size of 0.45, 0.30 or 0.22 microns, a porosity of 75% and a thickness of 150 mm by soaking the membrane in an aqueous solution of the hemoglobin of the invention to immobilize it within the micropores. The saturated membrane is next mounted in an oxygenator, attached to a source of oxygen having a gas flow meter to control the volumetric rate of oxygen delivered by the hemoglobin oxygenation membrane to blood in the vascular system.

The intramolecularly cross-linked, stromal-free deoxyhemoglobin and oxyhemoglobin can be used as a source of protein and oxygen in the microbiological assay of foods for aerobic bacillus and staphylococcus to insure the food is safe for animal and human consumption. The hemoglobins can be used for the storage and preservation of viable isolated perfused mammalian organs for their eventual transplant into a recipient, as a substitute for the oxygen-carrying capacity of red cells in mammals, and the hemoglobins can be used to supply oxygen as an aid in uranium leaching as disclosed in U.S. Pat. No. 3,003,848, and as a scavenger of noxious gases such as carbon monoxide and nitrogen oxide, and as a means for the purification of oxygen.

In summary, it will be appreciated by those versed in the art that it has now been found that both intramolecularly cross-linked, stromal-free deoxyhemoglobin and intramolecularly cross-linked stromal-free oxyhemoglobin can be prepared according to the mode and manner of this invention; and that they are endowed with the similar beneficial properties of macromolecular cross-linked, stromal-free hemoglobin disclosed in applicants' copending application Ser. Nos. 553,514 and 554,051. For example, intramolecularly and intermolecularly cross-linked stromal-free hemoglobins will have the following therapeutic benefits for treating circulatory shock, compared to the corresponding use of noncross-linked hemoglobin: (a) an increased half-life dependent on the molecular weight of the cross-linked stromal-free hemoglobin, (b) variable oxygen affinities dependent on the method of preparing the product, and (c) increased chemical stability.

Additionally, the cross-linked hemoglobins can be made iso-osmotic by the addition of pharmaceutically acceptable electrolytes, mainly sodium chloride. The oncotic pressure exerted in vivo by each of these is

a function of molecular weight, since oncotic pressure is a colligative property directly dependent on the number of macromolecules in a given solution. For instance, albumin which has a molecular weight of about 69,000 normally supplies the required in vivo pressure to prevent circulatory collapse. In conditions where there is a loss of blood, there is an accompanying loss of albumin, which is usually treated by intravenously administering a plasma expander. Thus, the treatment of blood loss is two-fold, (a) replacement of fluid and (b) replacement of oxygen carriers. Both intramolecularly cross-linked stroma-free oxyhemoglobin and deoxyhemoglobin and intermolecularly cross-linked stroma-free oxyhemoglobin and deoxyhemoglobin are effective replacements for blood loss, and properties exhibited by these beneficial products make available a broader choice of blood replacement therapy than previously known. For example, macromolecular, intermolecularly cross-linked stromal-free hemoglobin might be the preferred therapeutic agent during those surgical procedures and replacement therapies where a long in vivo plasma half-life is desired. The less pronounced oncotic activity exhibited by such macromolecular hemoglobin is simply accommodated by addition of the traditional plasma expanders, for example dextran or albumin. In such cases polyhemoglobins serve primarily as long-lived oxygen carriers. Under other circumstances, the preferred therapeutic method of treating blood loss might require a combination of both enhanced oncotic activity and oxygen delivery and in these cases the intramolecularly cross-linked stromal-free hemoglobins having a molecular weight equal to albumin might be the indicated therapeutic agent, even though the half-life of these materials are less than those of intermolecularly cross-linked hemoglobin. The final choice allowed the practitioner of blood replacement therapy is that of oxygen binding functionality. Each polyhemoglobin exhibits its unique binding functionality which is reflected in the P₅₀ value, determined from the oxygen dissociation curve. Presently, such a choice is not available to practitioners of blood replacement therapy. The therapeutic advantages of blood replacement with polyhemoglobins lies mainly in the versatility of their physical properties, and intramolecularly cross-linked stromal-free polyhemoglobins adds to this versatility.

It will be further appreciated that this invention provides products that are reliable means for transporting and supplying the ligand oxygen to vital tissues and organs in animals including domestic animals such as dogs and cats, farm animals such as cows and pigs, and to mammals including warm-blooded mammals without obtaining unwanted effects. And, while the invention has been described with reference to certain presently preferred embodiments thereof, those skilled in the art will appreciate that various modifications, changes, omissions and substitutions can be made without departing from the spirit of the invention.

We claim:

1. A pharmaceutical composition useful as a blood substitute and blood plasma expander comprising a therapeutically effective amount of intramolecularly cross-linked, stromal-free deoxyhemoglobin, soluble in aqueous and physiological fluids, capable of reversibly binding a ligand, having a molecular weight of about 64,500, and mixed with a pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to claim 1 wherein the intramolecularly cross-linked, stro-