

THE VAPOR PRESSURE OF DOG'S BLOOD AT BODY TEMPERATURE.

By ARTHUR GROLLMAN.

(From the Physiological Laboratory, School of Medicine, The Johns Hopkins University, Baltimore.)

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I.

INTRODUCTION.

An evaluation of the *colligative* properties of the blood is of paramount theoretical importance, for such properties enter as fundamental factors in all physicochemical considerations based either on the kinetic theory or on thermodynamics. Despite this importance, no direct measurements of the colligative properties of blood have been made at body temperature.¹ Our knowledge of these functions is based almost exclusively on freezing point determinations made at 0°. However, the importance of the colloidal constituents of the blood and the magnitude of the unknown change which temperature may produce in their properties renders the application of such measurements to conditions obtaining in the intact organism rather hazardous. Because of the lack of a direct determination of any of the colligative properties of blood at body temperature, it was deemed worth while to make such a measurement.

To obtain a value for the colligative properties of a solution four possible methods are principally used, *viz.* the determination of the raising of the boiling point, the lowering of the freezing point, the osmotic pressure, and the vapor pressure. The first of these methods is impossible in the case of blood solutions. Objections to applying freezing point data to blood at body temperature have already been

¹ Friedenthal (1) attempted to obtain such data by using the tensimetric method for determining vapor pressure. This method involves the evacuation of the system under investigation, a process objectionable in the study of blood. Moreover, it is not capable of yielding a high degree of accuracy.

noted. We are thus restricted to direct osmotic pressure measurements or to the determination of the vapor pressure, in order to obtain exact data concerning the condition of the blood in the state in which we are interested in its manifestations—*viz.* at body temperature. Direct osmotic pressure measurements are beset with many experimental difficulties and pitfalls which often render the results of doubtful value. This is particularly true of attempts to measure the osmotic pressure of solutions of electrolytes as has been elsewhere discussed (2). The recently described method of Frazer and Patrick (3), assuming its validity, would be inapplicable to blood solutions, since it requires evacuation to remove all dissolved gases, which are essential constituents of normal blood.

The determination of the vapor pressure is, therefore, the only remaining method applicable to blood at body temperature. This method was consequently used in this investigation. As we shall see later, relationships exist by which the other colligative properties may in turn be calculated from vapor pressure data. The most exact vapor pressure measurements which have been made by the static method are those of Frazer, Lovelace, and their associates (4). The necessity of having gas-free solutions, obtained by long boiling and repeated evacuations, makes this method inapplicable to blood solutions. The original dynamic method of Ostwald and Walker (5) has also been modified, to give very exact results, by Berkley, Hartley, and Burton (6) and by Washburn, Gordon, and Heuse (7). In the dynamic method it is possible to maintain the blood under the same conditions as exist in the organism. The necessity of passing a stream of gas over the solution to be investigated permits the use of a gas of the composition of the *alveolar* air, thus maintaining the blood in equilibrium with the same gaseous mixture with which it normally comes into equilibrium in the body. For the purpose of the present investigation, therefore, the dynamic method as modified by Washburn and Heuse (7) was followed.

II.

EXPERIMENTAL.

In the dynamic method, as used here, a large volume of air is passed over the solution investigated with which it comes into equilibrium. The gas saturated

with the vapor from the solution is then led over an absorber which removes the water vapor. The dried gas is next led through a similar train in series with the first where it becomes saturated with water vapor from pure distilled water and it then gives up this water vapor to a second absorber. By weighing the absorbers before and after the experiment, a differential measure of the vapor pressure of the solution as compared with pure water, whose vapor pressure at the temperature of the experiment is known, can be obtained.

To simulate as closely as possible the conditions obtaining in the animal body, a gas mixture approximating the composition of the alveolar air with which the arterial blood of the body is in equilibrium was used.² This was obtained by forcing CO₂ under pressure into a steel cylinder containing compressed air. This gas was led through a needle valve to a coil of about 20 feet of copper tubing, contained in the thermostat, where the gas assumed the temperature of the thermostat, before entering the saturators. The saturators were of the type described by Washburn and Heuse (7). They consisted of a train of glass tubes placed on a rocking table. The other parts of the apparatus were mounted on shelves of the same rocking table and the whole was rocked to and fro at a rate of ten per minute. The saturators were about half filled with liquid and the movement of the rocker stirred their contents, thus facilitating saturation of the gas with the vapor. The apparatus was kept in a water bath maintained at 37.50° ±.05° by the usual methods of thermostatic control.

The blood used in these experiments was obtained by bleeding normal mongrel dogs. In order not to introduce any foreign substances in the form of anesthetics into the blood stream, a mode of local anesthesia, for which I am indebted to Professor E. K. Marshall, Jr., was employed. This consisted in infiltrating the region of the femoral artery with distilled water, which procedure combined with freezing with ethyl chloride permitted cannulation of the artery and bleeding of the animal to death. In this way about 1 liter of blood could be obtained from a 15 kilo dog, an amount sufficient for several determinations.

To determine the nature of the blood obtained in the above procedure, the following experiment was performed. A mongrel weighing 14.00 kilos was bled as described. The blood was collected in five successive vessels containing heparin. The total amount collected in this way was 855 cc. An additional quantity measuring 105 cc. was obtained by opening the chest and emptying the heart and large vessels of the thorax. Thus a total of 6.8 per cent of the body weight of the dog constituted the minimal blood volume. Even assuming a conservative estimate of the blood remaining in the tissues, one obtains a figure

² The importance of this procedure is illustrated by the work of Kovács (8) who showed that aeration of blood with CO₂ caused an increase in the value of its freezing point lowering while oxygenation of the blood, both *in vivo* and *in vitro* caused a decrease in the value of the freezing point lowering. Similar findings are reported by Bottazzi (9).

indicating the accuracy of the higher values obtained for the blood volumes of the dog (25). The corpuscular volume, the red blood cell count, the hemoglobin, and the protein content of the plasma, were determined in fractions of the collected blood. The corpuscular volume of the first fraction of blood collected, as determined by the hematocrit, was 48.5 per cent as compared with the value 47.5 per cent obtained on the last fraction of blood obtained. The red blood cell counts of these two samples likewise differed by only 3 per cent. The *hemoglobin* content of the last collected sample, as determined by the acid hematin method against that of the first collected sample was 97 per cent. The *protein* content of the plasma of the blood last collected was 99 per cent of that first obtained, as determined by the refractometric method. It is thus obvious that in the method involving the use of a local anesthetic, no appreciable dilution (greater than 2 or 3 per cent) of the blood occurs in bleeding an animal to death through a large femoral cannula. The rapidity with which the bleeding takes place, no doubt, does not permit the passage of much fluid from the tissues into the blood with its consequent dilution.³ The blood obtained by this method may therefore be considered as representative of the mixed total blood of the animal.

To prevent coagulation, *heparin* was added; and to lessen the rapidity of decomposition by bacterial invasion, *acriflavine* was added. An equal amount of heparin and acriflavine was also added to the distilled water against which the vapor pressure of the blood was determined, to counterbalance the minimal lowerings which these substances produced. The decomposition of blood at 37.5° is one of the most serious difficulties which one encounters in experimental procedures prolonged over many hours as in the case of these experiments. An aseptic technique is difficult to attain. Bacterial decomposition of blood takes place very rapidly with a rapid increase in the freezing point lowering, as shown in the experiments of Carrara (10). The addition of some preservative such as acriflavine is, therefore, essential to avoid the erroneous and fantastically high results otherwise obtained. Another serious difficulty encountered in the study of blood which does not enter into vapor pressure studies of simple solutions is the tendency of blood to foam. Although the aerating gas in the method here employed does not pass through the blood, but merely over it, this tendency for the blood to foam persists. A bubble carried over to the absorbers would naturally give a very high result. Traps were therefore inserted in the system to prevent this. When the rocking mechanism was so adjusted as to work with least jarring the frothing was minimal.

The possibility of obtaining supersaturation of the gas as a result of frothing and the effect of decomposition are two factors which render the accuracy of this method when applied to blood much less than that obtained when simple solutions

³ This finding is contrary to the conceptions found in the current text-books (*cf.* Starling, E. H., Principles of human physiology, Philadelphia, 4th edition, 1926, 866).

are used. Test runs with water in the saturators are therefore deceiving as regards the ultimate accuracy obtained. Thus Washburn and Heuse (7) obtained an average deviation of only 0.4 per cent in the value of the relative vapor pressure lowering of a molar sucrose solution. No such high degree of accuracy is obtained in similar experiments on blood solutions.

III.

EXPERIMENTAL RESULTS.

The results obtained on blood plasma are given in Table I. The plasma was prepared by centrifuging heparinized blood obtained as described above. The plasma thus obtained was always perfectly clear and was free of the hemolysis so commonly obtained from dog's blood that has been defibrinated.

In the first column of Table I are given the experimentally determined vapor pressures, which are calculated in the following manner (7). If a volume of gas, v , is equilibrated with a liquid, whose vapor pressure is p , the mass of water vapor, m , taken up by the gas is given by the relation:

$$m = K p v$$

where K is a constant. The ratio of the masses of water absorbed from two absorbers will be

$$\frac{m_1}{m_2} = \frac{p_1 v_1}{p_2 v_2} \quad (1)$$

If the same volume of gas passes over two liquids, the volumes v_1 and v_2 respectively emerging from the saturators containing the liquids are inversely proportional to the partial pressures of the air in the saturators, *i.e.*,

$$\frac{v_1}{v_2} = \frac{p_2}{p_1}$$

where p_2 and p_1 , the partial pressures of the gas in the saturators, are equal to the barometric pressure minus the vapor pressure of the water in the saturator minus the difference between the barometric pressure and the pressure within the saturator, *i.e.*,

$$\frac{v_1}{v_2} = \frac{B - p_2 - \Delta p_2}{B - p_1 - \Delta p_1} \quad (2)$$

where B is the barometric pressure and Δp_2 and Δp_1 are the differences obtained by a manometer connected to the exit tube of the saturator and open to the atmosphere. Combining equations (1) and (2) gives

$$p_1 = \frac{m_1 p_2 (B - \Delta p_1)}{m_0 (B - p_2 - \Delta p_2) + m p_2} \quad (3)$$

By placing water in one saturator and the solution to be investigated in another, one can determine the vapor pressure of the latter from the weights of the water absorbed from each saturator, the barometric pressure, and the difference in pressures between the interior of the saturators and the atmosphere. Throughout this paper the vapor pressure of pure water at 37.5° is taken as 48.38 mm. of mercury,⁴ as found by Heuse and Scheel (11).

TABLE I.
The Colligative Properties of the Blood Plasma of the Dog.

I	II	III	IV
Vapor pressure observed	Freezing point lowering observed	Vapor pressure calculated from freezing point data	Osmotic pressure at 37.5°C . calculated from the vapor pressures observed
<i>mm.Hg</i>	$^\circ\text{C}$.	<i>mm.Hg</i>	<i>atmospheres</i>
48.09	0.616	48.09	8.5
48.12	0.605	48.10	7.6
48.11	0.604	48.10	7.9
48.08	0.620	48.09	8.8

IV.

Calculation of the Vapor Pressure Lowering from Freezing Point Values.

Since the great mass of existent data on the osmotic pressures of physiological solutions are based on freezing point measurements, determinations of the latter were also made by the usual Beckmann technique on all of the solutions studied. These values are recorded in Column II of Table I. It is of interest and importance, moreover, to compare the vapor pressures as calculated from freezing point data with those obtained by direct measurement.

One may calculate the vapor pressures of solutions from freezing

⁴ Holborn, Scheel, and Henning (23) give the value 48.36.

point data by the use either of Callendar's equations (12) or that of Washburn (13). The latter's equation is

$$\log_{10} \frac{p}{p_0} = \frac{\Delta C_p}{R} \log_{10} \frac{T_F}{T_{F_0}} - \frac{0.4343 (L_{F_0} - \Delta C_p \Delta T_F)}{RT_F} + \frac{0.4343 L_{F_0}}{RT_{F_0}}.$$

In this equation ΔC_p is the difference in the molar heat capacities of the solvent in the liquid and solid states; R is the gas constant; L_{F_0} is the molar heat of fusion of the pure solvent at the freezing point of the solvent; T_{F_0} is the absolute freezing point of the solvent; T_F is the absolute freezing point of the solution; ΔT_F is the lowering of the freezing point and p and p_0 are the vapor pressures of solution and solvent respectively. The values of ΔC_p , R , L_{F_0} , and T_{F_0} are 9.04, 1.985, 1435.5, and 273.1, respectively. When ΔT_F is less than 1.0, as in the case of blood solutions, one may with sufficient accuracy utilize the simplified equation proposed by Callendar

$$\log_e \frac{p_0}{p} = \frac{L_{F_0} \Delta T_F}{RT_0^2} = 0.009696 \Delta T_F.$$

The calculations of the vapor pressure of blood from the freezing point determinations are given in Column III. It will be seen that the agreement between the experimental and calculated vapor pressures are as good as the experimental errors involved allow. In calculating vapor pressures from freezing point data for temperatures other than the freezing point, as is done here, there are two factors which are neglected *viz.*: the change in the heat of dilution of the solution with temperature and the change in the degree of dissociation with temperature. In so far as sodium chloride, the chief electrolytic constituent of the blood, is concerned, however, these factors would not be expected to greatly influence the results of our calculation. Thus a comparison of the highly accurate vapor pressure data of aqueous sodium chloride solutions at 20° as measured by Norris (14) with values calculated by Callendar's simplified equation from the freezing point data of Jahn (15) and Rodebush (16) show perfect agreement up to concentrations of 1 M. The results may therefore be taken to indicate that the freezing point determinations of plasma may be considered as at least fairly accurate measurements of the true colligative properties at body temperature.

V.

Calculation of the Osmotic Pressure from the Vapor Pressure Data.

Recent developments in the application of thermodynamics to solutions have reduced the important status which was previously assigned to osmotic pressures. Its place has been taken by a much more useful and generally applicable function, the *activity*. For physiological purposes, however, the concept of osmotic pressure remains of paramount importance, for it is the chief and most tangible force associated with the movement of fluids within the organism. Moreover, the complexity—as regards the number of constituents—and the paucity of our knowledge, render application of the concept of activity to blood impossible at present.

To calculate the osmotic pressures from the vapor pressure data obtained in this investigation we utilize the thermodynamically derived relation (17),

$$P = \frac{RT}{V_o} \log_e \frac{p_o}{p}.$$

In this equation P is the osmotic pressure at the absolute temperature T ; V_o is the molecular volume of the solvent under the standard pressure; p_o is the vapor pressure of the solvent; p is the vapor pressure of the solution; and R is the gas constant. This equation neglects the compressibility of the solution and assumes that the vapor follows the gas laws. In the case of blood solutions, the experimental accuracy would not warrant consideration of these factors which, moreover, are very small and hence may be neglected. Substituting 82.07 for R , 310.6 for T , 18.016 for V_o , and converting to Briggsian logarithms, the above equation becomes

$$P = 3258 \log_{10} \frac{p_o}{p}.$$

The osmotic pressures in atmospheres calculated by means of this equation from the experimental vapor pressure determinations are given in Column IV, Tables I and II.

VI.

The Vapor Pressure of Dog's Blood.

The results obtained with dog's blood are given in Table II. Calculations similar to those described for plasma were also carried out for the whole blood and are recorded in the table. In general, the vapor pressures of the whole blood are seen to be the same as that of plasma.

Collip (18) found that the lowering of the freezing point of the blood corpuscles of various species was always less (from 0.02° to 0.07°) than that of the serum. Whole blood, likewise, gave a lower freezing point lowering than the plasma, as had previously also been found by other workers (19). This phenomenon, if real, is rather difficult to explain. Assuming permeability of the corpuscular cell wall to

TABLE II.
The Colligative Properties of Dogs' Whole Blood.

Vapor pressure observed	Freezing point lowering observed	Vapor pressure calculated from freezing point data	Osmotic pressure at 37.5°C . calculated from the vapor pressures observed
<i>mm.Hg</i>	$^\circ\text{C}$.	<i>mm.Hg</i>	<i>atmospheres</i>
48.08	0.602	48.10	8.8
48.12	0.585	48.11	7.6
48.09	0.590	48.11	8.5

water, the activity of the water within the cell and that of the plasma must be the same when equilibrium is attained. Conversely, the freezing points of the cells and plasma must be the same. It would therefore seem that determinations of the freezing point of such systems as blood cells or of whole blood are in error, and that the existence of the two phase system *ice-solution*, in the case of the red corpuscle, is not clearly recorded by the ordinary methods for determining freezing points. On the other hand, it is possible that the aqueous phase within the corpuscle is not comparable to that in the plasma. In other words we have to deal with a two phase system analogous to that of the system—*water saturated with phenol and phenol saturated with water*. Such a view is quite contrary to the usually accepted data concerning the equilibrium between corpuscles and plasma (20).

As far as vapor pressure measurements are concerned, however, it must be remembered that the observed vapor pressure from a heterogeneous system will always be that of the more volatile phase.

VII.

The Vapor Pressure Lowering of Blood Plasma Compared to That of Its Ultrafiltrate.

It is the consensus of opinion that the colloids of blood plasma exert only a minimal osmotic pressure compared to that of the other constituents. Direct determinations of the osmotic pressure of plasma measured against the ultrafiltrate of the plasma give values between 30 and 50 mm. of mercury (21). The difficulty attendant upon such direct measurements and the assumptions frequently made concerning the existence of a high degree of water binding by the blood colloids made a redetermination of the osmotic pressure of the blood by some new and direct method seem worthy of further investigation. Neuhäuser (22) attempted the solution of this problem by the original Ostwald-Walker method (5). In the present investigation, blood plasma, obtained as already described, was ultrafiltered according to the technique and with the precautions previously described (24) as essential for obtaining true ultrafiltrates from colloidal solutions. Such ultrafiltrates from blood plasma were put in the saturators previously occupied by distilled water and their vapor pressure determined against that of the plasma part of which had been used in preparing the ultrafiltrates. The average of three such determinations gave a vapor pressure value corresponding to about 0.06 atmosphere for the osmotic pressure of the blood colloids. This value will be seen to be in accord, so far as the experimental accuracy permits one to judge, with the commonly accepted values for the osmotic pressure of the blood colloids. An exact evaluation of this function can be obtained only by direct osmotic pressure determination.

VIII.

SUMMARY.

The vapor pressures of dog's blood and blood plasma were determined at 37.5° by the dynamic method and the osmotic pressures

calculated from the experimental data. The vapor pressures calculated from experimentally determined freezing point data agreed, within the experimental error, with the values obtained from direct measurement. The vapor pressure lowering produced by the colloid constituents of the blood was also determined and found to be minimal compared to that of the other constituents.

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