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THE VAPOUR PRESSURE OF NORMAL HUMAN BLOOD.

By R. MARGARIA[1].

(*From the Department of Physiology and Biochemistry, University College, London.*)

Introduction.

Though the osmotic pressure of mammalian blood is generally held to be very constant, the values given by various authors are not strikingly uniform. In the case of man there are (*a*) the single observations of Dreser [1892], Hamburger [1893], Winter [1896], and v. Koranyi [1897], who all found a value corresponding to a freezing point of 0·56° C.; (*b*) the observations of Veit [1900] on ten normal people, with variations in the freezing point from 0·501° to 0·605°, mean 0·551°; (*c*) those of Viola [1901] on eight people, with a mean value of 0·562°, and variations from 0·544° to 0·57°: those of Krönig and Fueth [1901] who give a mean for the freezing point of the blood of − 0·507°, with variations from − 0·490° to − 0·509°. Stewart [1899] gives a Δ for hirudinized blood of 0·628°, and Collip [1920] for defibrinated human blood under normal conditions, values varying between 0·555° and 0·586°.

Other authors have tried to determine the osmotic pressure of blood by other methods. Thus Eykman [1897], by the hæmatocrit method, based on the change of volume of blood corpuscles immersed in solution of NaCl of various strengths, and assuming as the osmotic concentration of the plasma that of the NaCl solution in which the volume of the blood corpuscles was unchanged, found values, expressed in g. of NaCl per 100 c.c. of solution, from 0·84 (Δ = 0·537°) to 0·89 (Δ = 0·568°), the mean of fourteen observations being 0·856 (Δ = 0·546°). By a similar method Koeppe [1896] found in normal men an osmotic pressure equivalent to that of a solution 0·24–0·25*M* of cane sugar (corresponding to a Δ of 0·546°–0·570°), and he observed on the same person daily variations from 0·225 to 0·27*M* (Δ = 0·513° to 0·615°). The same author describes variations in the concentration of the blood following the

[1] Travelling Fellow of the Rockefeller Foundation.

ingestion of water or of saline solution. After drinking 750 c.c. of water he found a decrease of 6 p.c. in the concentration of the blood, and after taking 10 g. of NaCl in 200 c.c. of water, an increase of 16 p.c. (Δ observed 0·65°).

We see (i) that the data of the osmotic pressures of normal men, given by the various authors, differ widely, viz. from 0·49° to 0·628°, expressed in Δ values, and showing maximum variations of 28 p.c.; and (ii) the data given by the same author differ generally at least by 5 to 10 p.c. This is probably due partly to the fact that the accuracy of the cryoscopic method and of the other methods employed is not sufficient, and also partly to the fact that certain sources of error were not considered.

This paper is concerned with the determination of the osmotic pressure of normal human blood by a measurement of its vapour pressure, by the method recently described by A. V. Hill [1930]. The object was not primarily to get values of the osmotic pressure at a temperature different from that of the freezing point; it has been shown that the strength of the isotonic solution (as determined by the hæmatocrit method) is independent of the temperature. The intention was rather to employ a method which, as will be shown later, is much more sensitive and accurate than any other previously used.

An attempt to measure the vapour pressure of blood was made by Grollman [1928]: he weighed the quantity of water taken out by a known volume of dry air of the same composition as alveolar air, passing over a given quantity of blood. This method, however, besides requiring a large amount of blood, is not very sensitive, certainly less than the freezing point method. He observed a depression of vapour pressure of dogs' whole blood or plasma from 0·26 to 0·30 mm. Hg at 37·5° C., with maximum deviations of about 15 p.c., values corresponding within rather wide limits to those obtained in parallel determinations of the freezing point, which gave more consistent results.

Method.

Observations were made on eighteen men and sixteen women, between 20 and 45 years of age, all presumably healthy, visiting or working at University College. About 2–3 c.c. of blood were taken from the vena mediana of the arm in a syringe previously well cleaned with distilled water and dried with alcohol. It was put at once into a clean dry glass tube containing small pieces of glass rod, closed with a rubber stopper and shaken to defibrinate.

Hill's method of measuring the vapour pressure is based on the

difference of temperature caused by the different rates of evaporation of (or condensation on) two solutions with which two equal and similar pieces of filter paper are soaked. These are laid on the two sets of junctions of a symmetrical thermopile, which is put in a moist chamber and placed in a water thermostat. The method has not yet been described in a physiological journal: since it may have considerable application in the biological sciences, requiring very small quantities of liquid (about 0·2 c.c.), and being, as will be shown later, very accurate, I will briefly describe the principle and the technique.

The thermopile, as shown in Fig. 1, consists of 70 to 80 silver-

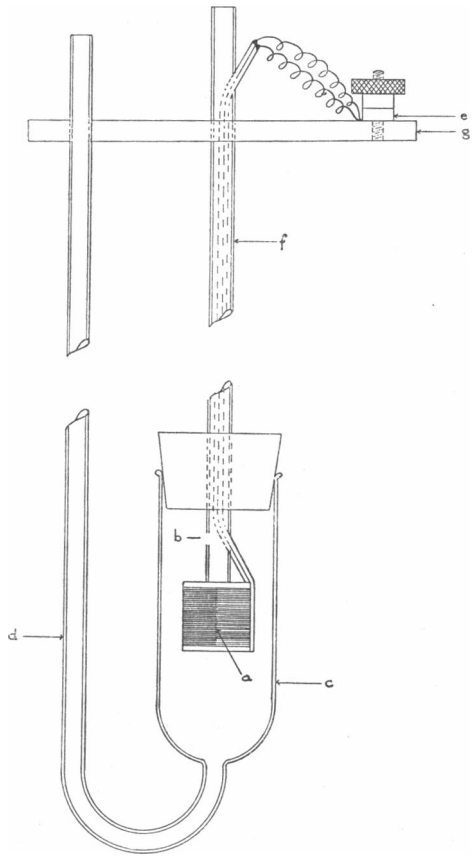

Fig. 1. Thermopile and chamber for thermal measurement of vapour pressure. *a*, one face of thermopile, showing line of junctions; *b*, hole in brass tube; *c*, glass chamber, with *d*, outlet tube; *e*, copper terminals; *f*, brass tube carrying insulated copper wires; *g*, vulcanite platform.

constantan elements, made by electroplating a wire of constantan wound upon a brass rectangular frame insulated with bakelite. The two sets of junctions are each in a line in the middle of two parallel faces, as symmetrically placed as possible. The wires are insulated with bakelite varnish: the thermopile is trimmed up with dissolved shellac, and is finally covered with a thin waterproof layer of a mixture of paraffin wax and beeswax by dipping it for a few seconds in the melted mixture. Fuller details of the construction of a similar instrument are given by Hill elsewhere [1928].

The thermopile is mounted on a brass tube which passes through a rubber stopper. The copper leading-in wires, carried in insulated covers, lie in this same brass tube; this is different from the original model, in which they passed in a separate glass tube. The new arrangement has the advantage of eliminating all glass from the instrument and of avoiding strain and possible breakage of the fine wires coming from the thermopile, when the chamber is being forced on to the rubber stopper.

The leads end under two copper terminals on a vulcanite platform. A small hole is drilled in the thermopile frame to make contact between the insides of the thermopile and the tube, and another in the tube to allow equalization of pressure between the chamber and the outside. Thus the inside and the outside of the thermopile are both at atmospheric pressure.

The chamber is a large glass tube of about 150 c.c. capacity, forced on to the rubber stopper during use. At the bottom it ends in a small curved pipe connected with a rubber tube which ends above the vulcanite platform. Such an arrangement makes it possible to fill the chamber with a gas mixture of known composition by a stream of gas entering at the top through the brass tube and leaving at the bottom by the rubber tube. During the determinations no gas currents must occur in the chamber, and one of the two tubes must be closed, the other remaining open to allow pressure equalization to occur with the outside.

Suppose that on one face (A) of the thermopile is laid a strip of filter paper, just a little smaller than the face itself, dipped in solution a, on the other face (B) a similar strip of filter paper dipped in solution b; the chamber is kept moist by covering its walls with a large strip of filter paper dipped in a solution c. The solution c being in large excess, the water vapour pressure in the chamber will be that of this solution except in the immediate neighbourhood of A and B, where the vapour pressures will be respectively those of the solutions a and b. Calling the vapour pressures of the three solutions p_a, p_b and p_c, the rate of condensation on

face A will be $k(p_c - p_a)$, and similarly on face B, $k(p_c - p_b)$. The excess temperature attained after some time, when equilibrium is reached, by face A will be $k'(p_c - p_a)$, and by face B, supposing the instrument to be perfectly symmetrical, $k'(p_c - p_b)$, where k' is a constant depending upon the temperature, pressure, and thermal conductivity, etc., of the instrument. The final difference in temperature between the two faces, therefore, will be $k'(p_b - p_a)$, which is independent of p_c. Knowing the value of k' for a given instrument at a given temperature and pressure, which can be determined by calibration with two solutions of known vapour pressure, and knowing the vapour pressure of one of the solutions, we can easily calculate the vapour pressure of the other solution.

Since, however, it is very difficult to obtain thermopiles perfectly symmetrical at least in their thermal conductivity, k' will not have the same value for the two faces of the thermopile, k'_A being different from k'_B. The difference of temperature between the two faces of the thermopile will then be

$$k'_A(p_c - p_a) - k'_B(p_c - p_b).$$

The effect due to asymmetry may be far too great to be neglected in very accurate work. It may, however, be eliminated by making with the same instrument two successive determinations, reversing in the second the positions of the two solutions on the thermopile, without removing the solution on the walls of the chamber, and taking the mean of them. The mean of two such determinations, as is easily calculated, is

$$\frac{k'_A + k'_B}{2} \cdot (p_b - p_a),$$

where we see that the mean difference of temperature caused by the different rates of condensation (or evaporation) of the water vapour on (or from) the two faces of the thermopile is again independent of the vapour pressure of the solution on the walls, and is given only by the difference of vapour pressure of the two solutions multiplied by the mean value of the two constants k'.

The thermopiles were suspended in their chambers in a large water bath, the temperature of which, as observed by a Beckmann thermometer graduated in 0·002°, was kept constant to 0·001° by means of a large chloroform-mercury gas regulator immersed in the bath. A very constant temperature is necessary since, as was noted by Hill, an increase of about 1° C. in the temperature implies an increase of about 7 p.c. in the sensitivity of the arrangement, and a fluctuating temperature causes irregular readings. The temperature of the bath in the earlier experiments was 20° C., in the later, with a higher room temperature, 24° C.

The thermopiles were calibrated every day, owing to the daily variations of the barometric pressure. The deflection of the galvanometer was observed when one face of the thermopile was covered with filter paper moistened in a 0·92 p.c. NaCl solution[1], and the other with filter paper moistened with distilled water. The filter papers were of exactly the same size and carefully dried before being moistened with the solutions.

Since the difference of vapour pressure within the range of concentrations involved is practically proportional to the difference of concentration, and the difference of temperature caused by evaporation or condensation of the solvent is proportional to the difference of vapour pressure, the deflection of the galvanometer is proportional to the difference in strength between the two solutions, or, in the case in which the pure solvent was put on one side, to the strength of the NaCl solution on the other. On the walls of the chamber was also put the same 0·92 p.c. NaCl solution.

The readings for the blood were always made against the 0·92 p.c. NaCl solution, with 0·92 p.c. NaCl solution on the walls of the chamber, so that there was in the chamber a vapour pressure equal, or very nearly equal, to that of the solutions on the thermopile. These, therefore, were not subjected to sensible evaporation or condensation, and their composition remained very constant for a long time, much longer than was required for the determination, which was usually not more than 45 minutes.

The solution of NaCl was accurately prepared in large amount by dissolving 0·92 parts by weight of NaCl, dried over a flame, in 100 parts by weight of distilled water. Two controls of the water content of this solution made by drying, one before the beginning, the other at the end of the experiments, gave assurance of the constancy in its composition. The same standard solution was used throughout the work.

The advantage of taking the measurements of the vapour pressure of the blood against that of a solution about isosmotic with it, was to reduce to a minimum the difference of temperature between the two sides of the thermopile, and so to diminish possible errors in calibration. Moreover, to use on the walls of the chamber a solution of nearly the same vapour pressure as that of the solutions on the thermopile has the advantage of reducing to a minimum the effect due to asymmetry of the thermopiles. In fact, supposing that the same solution is on the two sides of the thermopile, then $p_a = p_b$ and the difference of temperature is

[1] Throughout this paper concentrations are given in g. of NaCl per 100 g. of water, not per 100 c.c. of solution. The term "p.c." is used always in this sense.

$(p_c - p_a)(k'_A - k'_B)$. This value will be greater the greater the factor $(p_c - p_a)$, *i.e.* the greater the difference of vapour pressure between the solution on the walls of the chamber and that on the thermopile. It follows also that when two different solutions are placed on the thermopile, the effect of asymmetry of the instrument will be greater the greater the difference of vapour pressure between solution *c* and solutions *a* and *b*.

To avoid completely the effect due to asymmetry, as explained above, for every thermopile two determinations were made, reversing the positions of the two papers moistened with the NaCl solution and with the blood, the mean of the two determinations being taken. This had also the advantage of eliminating the effects of possible constant currents originating in the thermopile.

The chamber was filled with a 5 p.c. CO_2 mixture in oxygen, so that the defibrinated blood was in equilibrium with a gas mixture whose partial pressure of CO_2 was about the same as that of the arterial blood. At least two litres of this mixture were passed through the chamber to fill it. The mixture was previously saturated with water vapour at the temperature of the bath, passing through a moist chamber filled with pieces of glass hanging in the bath itself.

The galvanometer used was a Zernicke moving-coil instrument (*Zc* by Kipp) with a sensitivity of about 3 to 4×10^{-10} amp. per mm. Between the thermopiles and the galvanometer a reversing key allowed the direction of the current to be reversed in the galvanometer. The sum of two reverse readings was taken so that (*a*) the sensitivity was doubled, (*b*) any effect of an E.M.F. originating between the key and the galvanometer was eliminated, and (*c*) errors due to non-uniformity in the sensitivity of the galvanometer for different sizes of deflection were avoided. The sensitivity of the system was such that 1 mm. deflection of the galvanometer corresponded to a difference in strength of the two solutions equal to 0·001 to 0·0014 p.c. of NaCl.

The E.M.F. developed in the thermopiles could be compensated if desired by a potentiometer. As the potentiometer was accurately calibrated it was easy to calculate, from the values obtained with it, the corresponding millimetres of deflection of the galvanometer. The potentiometer, however, was used very rarely, only in case the current produced by a thermopile was too large to allow the readings to be made on the galvanometer scale.

When a steady state is reached in the instrument, in from 30 to 45 minutes, the readings still show small variations, due perhaps to local

fluctuations of temperature in the bath. Five readings, therefore, were made for every thermopile, and the mean of them taken. The following example of four determinations made with four different thermopiles on the same sample of blood from subject J. L. P. will illustrate the method.

	Side *A* of thermopile: blood. Side *B* of thermopile: NaCl 0·92 p.c. solution.				Side *A* of thermopile: NaCl 0·92 p.c. solution. Side *B* of thermopile: blood.			
Thermopile no.	1	2	3	4	1	2	3	4
Deflection of galvanometer (mm.)	30	24	5	19	12	15	14	19
	29	25	7	20	14	15	13	20
	27	25	5	20	13	15	14	17
	28	23	7	18	12	14	13	20
	27	25	6	19	13	14	14	19
Mean	28·2	24·4	6	19·2	12·8	14·6	13·6	19

Mean of the two reverse readings:

Thermopile no.	1	2	3	4
Deflection (mm.)	19·8	19·5	9·8	19·1

The sensitivities of the thermopiles, previously measured, expressed in g. of NaCl p.c., per mm. deflection of the galvanometer, were as follows:

Thermopile no.	1	2	3	4
Sensitivity	0·001120	0·001024	0·001085	0·001118

So that the deflections of the galvanometer, given above, correspond to a difference in concentration of the solutions on the two sides of the thermopiles of

Thermopile no.	1	2	3	4
	0·0222	0·0199	0·0106	0·0214

From the direction of the current in the galvanometer the blood was known to be stronger than the NaCl solution, so that the values obtained must be *added* to that expressing the concentration of the solution of NaCl, to obtain the value of the concentration of the blood expressed in terms of g. of NaCl in 100 g. of water.

The CO_2 introduced in the chamber of the thermopile was normally dissolved in the NaCl solution in amount corresponding to its partial pressure. The increase in the gas content of this solution due to the introduction of the CO_2 at a partial pressure of 5 p.c., expressed in c.c. of CO_2, is about 4 c.c. per 100 g. of water, which corresponds to an increase in the osmotic pressure about equal to that of half the same quantity, in g. equivalents, of NaCl, or to 0·005 g. p.c. of this salt. The true value therefore of the equivalent concentration of the blood is given by adding to the difference in concentration, observed above, the value 0·925. Then we have the following values:

0·9472, 0·9449, 0·9356, 0·9464; Mean, 0·9435.

It is more convenient to express the concentrations of the blood in g. of NaCl per 100 g. of water in an isosmotic solution of this salt, than in terms of the depression of freezing point: we can easily calculate the corresponding value of Δ, this being practically in this range of solutions (from 0·5 to 3 p.c. of NaCl) a linear function of the NaCl, with the formula $\Delta = 0{\cdot}006 + 0{\cdot}579x$, where x is the concentration of NaCl in g. per 100 g.

of water, or, more quickly, with the nomogram of Fig. 2. The values for these calculations were taken from Landolt-Börnstein's tables.

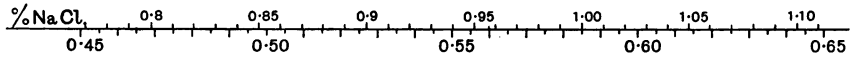

Fig. 2. Nomogram showing the relation between the concentration of a sodium chloride solution expressed in g. of salt in 100 g. of water, and the corresponding depression of freezing point.

For every set of four double determinations the probable error of one double determination was calculated, and the probable error of the mean is given in the following tables.

TABLE I. The osmotic pressure of normal human blood exposed to 5 p.c. CO_2 in air or oxygen, expressed in terms of g. of NaCl in 100 g. of water.

MEN.

1.	Parkinson	British	0·9439 ± 0·0017
2.	Hukuda	Japanese	0·9473 ± 0·0011
3.	Margaria	Italian	0·9622 ± 0·0017
4.	,,	,,	0·9318 ± 0·0009
5.	Macve	British	0·9588 ± 0·0010
6.	Clark	,,	0·9431 ± 0·0012
7.	Hill	,,	0·9432 ± 0·0013
8.	Endres	German	0·9389 ± 0·0008
9.	Teorell	Swedish	0·9511 ± 0·0020
10.	Gilding	British	0·9447 ± 0·0051
11.	Shaw	,,	0·9435 ± 0·0020
12.	Hammouda	Egyptian	0·9457 ± 0·0011
13.	Eggleton	British	0·9482 ± 0·0010
14.	Young	,,	0·9416 ± 0·0005
15.	Blow	,,	0·9332 ± 0·0024
16.	Popa	Roumanian	0·9491 ± 0·0034
17.	Shore	American	0·9437 ± 0·0034
18.	Bhatia	Indian	0·9458 ± 0·0040
19.	Verney	British	0·9341*

* Two double observations only.

WOMEN.

1.	Melville	0·9214 ± 0·0009	9.	Wright	0·9326 ± 0·0007
	,,	0·9208 ± 0·0008	10.	Hetherington	0·9196 ± 0·0008
2.	Taylor	0·9337 ± 0·0019	11.	Jameson	0·9231 ± 0·0014
3.	Eggleton	0·9227 ± 0·0019	12.	Girling Smith	0·9298 ± 0·0017
4.	Grant	0·9378 ± 0·0010	13.	Zeal	0·9260 ± 0·0008
5.	Tansley	0·9320 ± 0·0017	14.	Pickford	0·9065 ± 0·0009
6.	Kerly	0·9353 ± 0·0002	15.	Jackson	0·9142 ± 0·0016
7.	Marrian	0·9401 ± 0·0013	16.	Barrie	0·9248 ± 0·0025
8.	Bell	0·9311 ± 0·0010			

Each individual value is the mean of four double determinations by four separate instruments on the same sample of blood. The probable error of each mean is given.

The average values for all the subjects are:

Men, 0·9447, probable deviation of an individual value = ±0·00495,

Women, 0·9269, ,, ,, ,, ,, = ±0·0059.

From the values of the probable errors obtained in all these determinations we can gain an idea of the reliability of the method in measuring the osmotic pressure of such fluids as blood, and of its sensitivity. The average of the probable errors of the mean, from all determinations, is 0·0015. As the number of every set of determinations was 4, the average value of the probable error of a single observation is twice this, viz. 0·0030, or about 0·3 p.c.; and, if we omit from the calculations some determinations which were made during very hot weather, when the manipulations of thermopiles and solutions were made in a cold store, so introducing new causes of error, this value decreases to about 0·2 p.c. Such an accuracy is not possible by any other method of measuring the osmotic pressure of the blood.

Results.

The results are given in Table I. The mean value[1] for men is 0·9447 with a probable deviation for a single value of 0·0049, or 0·52 p.c. The mean for women is lower, being 0·9269, with a probable deviation for a single value of 0·0059 or 0·64 p.c. The difference between the mean concentrations of the blood of men and women is 0·0178, not a great absolute quantity, but very definite if we take account of the probable deviation from the mean, which has a very low value. This is clear if we compare the two curves of frequency (Fig. 3) constructed from the data of Table I. The area common to the two curves represents 27 p.c. of the total area of each curve, which means that only 27 p.c. of the subjects of one sex have an osmotic pressure which falls within the range of the opposite sex: and, from the measurements of the areas a and b in the figure, we can calculate that only 0·7 p.c. of men have an osmotic pressure which is as low as the mean of the women, while only 2·1 p.c. of women have an osmotic pressure as high as the mean of the men.

Though the number of observations is not very large, the fact that a difference exists between the mean values of the osmotic pressure for men and for women is statistically certain. The probable error of the mean for the blood of men is 0·0011, for the blood of women, 0·0015: the probable error of the difference is 0·0019. The difference observed being 0·0178, there is no probability that the difference is really nothing.

From the values of the probable deviations from the mean and from the frequency curves it appears that there is more uniformity in the readings of the osmotic pressure for men than for women. This phenomenon, however, is not quite certain: the standard deviation for the men (σM)

[1] For conciseness and clearness the osmotic pressure is given in terms of the equivalent solution of NaCl, g. in 100 g. of H_2O, without other designation.

and the standard deviation for the women (σW) with their probable errors have the following values:

$$\sigma M = 0{\cdot}0074 \pm 0{\cdot}00080,$$
$$\sigma W = 0{\cdot}0088 \pm 0{\cdot}00105,$$
$$\text{Diff. } (\sigma W - \sigma M) = 0{\cdot}0014 \pm 0{\cdot}00132.$$

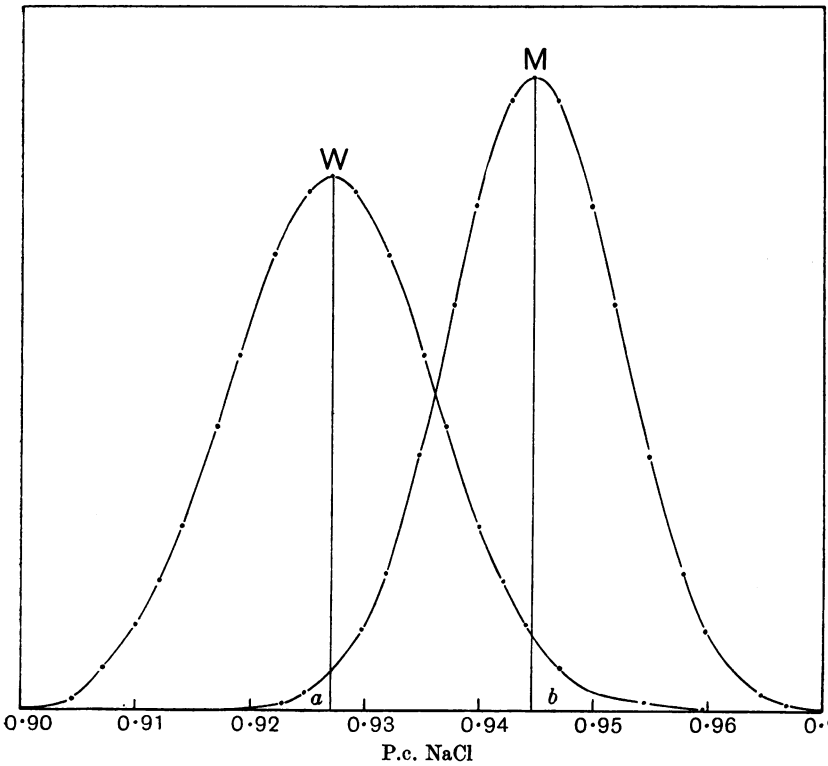

Fig. 3. Curves of frequency, calculated from the data of Table I, of the osmotic pressure of the blood of men (*M*) and women (*W*). The osmotic pressure is expressed in terms of the equivalent sodium chloride solution: g. in 100 g. of water.

The probable error of the difference between the standard deviations is about equal to the difference itself, which means that there is a probability of about 1/4 that the difference is really nothing.

A difference in the average composition between the blood of men and of women is well established in regard to the blood corpuscles: women have about 10 % less blood corpuscles than men, and a correspondent amount less of hæmoglobin. In respect of other constituents, various minor differences also have been found.

From the data collected by Gettler and Baker [1916] in 23 men and 7 women, Dunn [1929] calculated (i) that the difference in concentration of uric acid in the blood between the two sexes is $1{\cdot}426 \pm 0{\cdot}1632$ mg. in 100 c.c. of blood, a difference statistically sure; (ii) in the

alkali reserve of the plasma a difference, expressed in c.c. of CO_2 in 100 c.c. of blood, of $2{\cdot}5807 \pm 1{\cdot}3989$ (a 94 % probability of the real existence of a difference). On the same data I have calculated a difference (iii) of the ammonia N content of blood between men and women of $0{\cdot}13 \pm 0{\cdot}025$ mg. in 100 c.c. of blood (a 99·98 % probability of the real existence of a difference).

A sensible difference in the urea content of the blood between men and women was pointed out by Klisiecki [1926], from whose data, obtained on 26 men and 12 women, I have calculated a difference, expressed in mg. of N in 100 c.c. of blood, of $4{\cdot}85 \pm 0{\cdot}375$ (a zero probability that this difference is zero).

The increase of osmotic pressure of the blood due to the differences in concentration of the substances named above, except the CO_2 of the plasma, expressed in g. of NaCl in 100 g. of water, may be calculated as follows:

Uric acid	0·00033
Ammonia	0·00036
Urea	0·0065
Total	0·0072

The difference in the hæmoglobin content may cause a sensible difference in the osmotic pressure of the blood, when this is equilibrated with a CO_2 mixture. From the data of the osmotic pressure of blood serum, reported below, a difference in the osmotic pressure of men of $0{\cdot}0522 \pm 0{\cdot}0015$, due to the equilibration with a 5 % CO_2 mixture, was observed, this being practically due entirely to the buffer effect of the hæmoglobin: assuming that such an increase in the osmotic pressure is proportional to the hæmoglobin content of blood, since women's blood has 10 % less hæmoglobin, one would expect to have a correspondent smaller increase in the osmotic pressure, say about 0·005.

So that this effect due to the hæmoglobin, together with the effect of the difference in concentration of the substances named above, would bring about a difference in the osmotic pressure of 0·0122, *i.e.* two-thirds of the difference observed.

The smallness of the probable deviation of a single reading, viz. 0·5 to 0·6 p.c., is an index of the great constancy of the osmotic pressure of the blood. This constancy is particularly remarkable when we consider the different nationalities of the male subjects, and the fact that the samples of blood were taken not under scrupulously constant conditions, but from people engaged in ordinary work in the laboratory. It may well be that if the samples of blood had been taken from subjects completely at rest and fasting for several hours a still greater constancy would have been found[1]. The osmotic pressure of the blood may, in fact, be caused to

[1] Such a constancy is so much more remarkable, for it appears that the individual variations are mostly due to variations in the hæmoglobin content (CO_2 effect), and in the urea content, the individual variations due to the other components, especially fixed electrolytes, being much smaller: that is because the probable deviation of the hæmoglobin content in human blood is 5 % [Dunn, 1929], and of the urea content is 2 % (data from Klisiecki).

This fact, in regard to hæmoglobin, appears also evident on comparing the value of probable deviation of men's blood, equilibrated with the 5 % CO_2 mixture, with the one given below for men's serum.

undergo variations, plus or minus, far outside the range of those shown in Table I and Fig. 3.

A preliminary attempt has been made to study such variations, on the low side by drinking quantities of tap water, on the high side by muscular exercise.

(*a*) *Water drinking.* Four experiments were performed as follows:

3. vii. 30. *Subject R. M.* No lunch. 2–2.15 p.m.: 2 litres of tap water drunk. 3 p.m.: blood drawn for analysis. Readings with five thermopiles: 0·9128, 0·9067, 0·8985, 0·9035, 0·9096; mean, 0·9062 ±0·0016. The mean is 4 p.c. less than the normal mean value for men.

4. vii. 30. *Subject T.* 11–11.30 a.m.: 2 litres of tap water drunk. 12 noon: blood drawn for analysis. Readings with four thermopiles: 0·9226, 0·9173, 0·9172, 0·9259; mean, 0·9207 ±0·0015. The mean is 2·5 p.c. lower than the normal mean value for men.

7. vii. 30. *Subject H.* 10.40–10.50 a.m.: 1500 c.c. of tap water drunk. 10.50 a.m.: subcutaneous injection of 2 units of "pituitrin" (Parke-Davis). 12 noon: blood drawn for analysis. Readings with four thermopiles: 0·9148, 0·9088, 0·9030, 0·8969; mean, 0·9059 ±0·0025. The mean is 4 p.c. lower than the normal mean value for men. None of the values given falls within the possible range for normal men.

11. vii. 30. *Subject V.* 9.55–10.1 a.m.: 1500 c.c. of tap water drunk. Samples of blood drawn at 9.50, 10.20, 10.52, 11.35 a.m., 1.30, 2.35 p.m. The osmotic pressure was determined with two thermopiles for each sample of blood and the following values obtained:

Hour of sampling	9.50 a.m.	10.20 a.m.	10.52 a.m.
Osmotic pressure	0·9350 0·9331	0·9007 0·9118	0·8746 0·8987
Mean	0·9341	0·9062	0·8867
Hour of sampling	11.35 a.m.	1.30 p.m.	2.35 p.m.
Osmotic pressure	0·8897 0·8741	0·8917 0·9067	0·9075 0·9069
Mean	0·8819	0·8992	0·9072

In subject V. a very low value was obtained 1½ hours after the drinking: this is 5·6 p.c. lower than his initial value, and was not expected to be so low on the assumption that the water ingested was distributed uniformly in the tissues. In fact, if this assumption were true, we should be forced to conclude that the total water content of this subject, assuming a complete absorption of the water drunk and taking account of the 570 c.c. of urine eliminated between 10 a.m. and 11.30 a.m., was $\frac{930 \times 100}{5 \cdot 6} = 16{,}600$ c.c. This value is evidently far too low for a man of about 60 kg. body weight, so that we must argue that the blood was subjected to a greater dilution than the other tissues.

This experiment, from which a regular curve of the osmotic pressure in human blood can be constructed, demonstrates a further application of the method.

(*b*) *Muscular exercise.* To see to what extent the osmotic pressure may increase, a few experiments have been made after hard work. A. V. Hill

and Kupalov [1930] have recently shown that the osmotic pressure of frog's muscle, stimulated to exhaustion under anaerobic conditions, increases to an extent equivalent to an increase of 0·35 g. of NaCl per 100 g. of water. Considering the relatively great mass of the muscles of the body we should expect as the result of rapid muscular exercise to exhaustion (a great oxygen debt), that there would be a production of osmotically active substances, which would raise the concentration of the body fluids to a far greater extent than is possible by the ingestion of salts.

In an experiment on R. M., after 1 minute of standing running, the blood drawn 1 minute after the end of the exercise gave the following values:

0·9779, 0·9967, 0·9919, 0·9877; mean, 0·9886 ± 0·0024.

The mean is 4·7 p.c. higher than the normal mean value for men. The subject, however, was not trained to this type of work and could not exhaust himself too far as he intended to withdraw his own blood.

A much greater increase was observed in a runner, V. E. M., after a steeplechase of 2 miles, with 36 hurdles and 9 water-jumps, which he covered in 11 min. 15 sec. The blood drawn 1 min. 15 sec. after the end of the race gave the following values:

1·0542, 1·0485, 1·0359, 1·0542; mean, 1·0482 ± 0·0026.

This mean is 11·1 p.c. greater than the normal mean value for men. Assuming a uniform increase of the osmotic pressure in all the liquids of the body, an assumption probably not quite correct, particularly in view of the experiment above described on V., and assuming that the total water content of the body is 40 litres, we have a production of osmotically active substances in the whole body equivalent to 41·8 g. of NaCl, or to 1·47 g. mols. of a non-ionizable substance.

It was shown by A. V. Hill and Kupalov [1930] that the production of lactic acid is responsible for 1/2·8 of the increase of the osmotic pressure in the isolated frog's muscle; assuming the same value in the case of V. E. M. we may calculate that such a production of osmotically active substances corresponds to the presence in the body of 0·525 g. mols. of lactic acid, *i.e.* 47 g. This is probably lower than the real value, the subject being in good training and in a state of complete exhaustion when the blood was drawn.

Similar experiments were made on other subjects with the results tabulated below: every value represents the mean of two double determinations on the same blood.

1. A. V. H. ran for about 2 miles, moderately fast, and then "sprinted about" 300 yards, fatigued:

Blood drawn 35 sec. after exercise	0·9839
„ 3 min. „	0·9645

2. D. K. H., aged 15, before breakfast, similar run, fatigued:

Resting value under similar conditions	0·9115
Blood drawn 1 min. after exercise	0·9609
„ 4 min. „	0·9380

3. K. H. standing running at maximum speed for 1½ min., almost completely exhausted:

Blood drawn 45 sec. after exercise	1·0330
„ 2½ min. „	0·9960
„ 4½ min. „	0·9825

4. K. H. standing running next day at maximum speed for 1½ min., unable to exert the same effort as previous day, very exhausted:

Blood drawn 1 min. after exercise	0·9844
„ 4 min. „	0·9528

5. A. V. H. standing running at maximum speed for 1 min., very exhausted and slowing up rapidly:

Blood drawn 1 min. after exercise	1·028

6. K. standing running at maximum speed for 1 min., very exhausted:

Blood drawn 2½ min. after exercise	0·9841
„ 7 min. „	0·9739

7. Y. standing running at maximum speed for 1 min., very exhausted and slowing up rapidly:

Blood drawn 45 sec. after exercise	1·0032
„ 2½ min. „	0·9777

It is clear that extremely high values of the osmotic pressure are easily reached by short intervals of very violent exercise. The fact may have some application in the study of the phenomena associated with fatigue.

Such great increases in the osmotic concentration of the blood are not to be expected after muscular exercise of long duration [Chiatellino and Margaria, 1929]; the production of osmotically active substances in the muscles being an anaerobic phenomenon, the extent of the increase of the osmotic pressure of the body fluids may be expected to run parallel to the oxygen debt. Now the maximum values of oxygen debt are obtained in exercise of very short duration. That is illustrated by the two experiments on A. V. H., in whom the increase in blood concentration after 1 minute of standing running was more than twice as great as that observed after a 2 miles' run. The rapid onset of recovery is presumably the reason why the increase observed is of such short duration, tending to disappear after a few minutes.

The equilibration with CO_2.

The previous equilibration of the blood with a 5 p.c. CO_2 mixture was necessary because the effect of loss of CO_2 from the blood is far greater than the errors of the method.

Some observations of the osmotic pressure of serum or blood exposed to air gave the values collected in Table II. All the subjects were men, and most of them appear also in Table I. The difference due to equilibration with the CO_2 mixture is $0{\cdot}0522 \pm 0{\cdot}0015$, a very sensible amount.

Table II. The osmotic pressure of normal human blood or serum exposed to air, expressed in terms of g. of NaCl in 100 g. of water.

1. K. H.	$0{\cdot}8962 \pm 0{\cdot}0017$	5. R. A. K.	$0{\cdot}8865 \pm 0{\cdot}0018$
2. G. F. M.	$0{\cdot}8865 \pm 0{\cdot}0002$	6. Y.	$0{\cdot}8918 \pm 0{\cdot}0013$
3. H. B.	$0{\cdot}8960 \pm 0{\cdot}0015$	7. J. M. W.	$0{\cdot}8909 \pm$ —
4. A. V. H.	$0{\cdot}8940 \pm 0{\cdot}0006$	8. R. M.	$0{\cdot}8982 \pm 0{\cdot}0012$

The average value is 0·8925; probable deviation of an individual value = $\pm 0{\cdot}0028$.

The effect of CO_2 on the osmotic pressure of blood will be further discussed in a separate paper. The effect of CO_2 should be readily measurable by the freezing point method, corresponding to a difference of about 0·03° in the freezing point. It has never been considered before, which may explain in part the divergences in the values of the osmotic pressure obtained by previous authors.

Another cause of error not considered before is muscular work; this error also may have been very sensible, since human blood is usually drawn from the veins of the arm after stasis and, in order to make the veins more obvious, after energetic contractions of the muscles of the forearm.

For other mammals values are generally given 10 to 15 p.c. greater than those for man. I have examined some ox blood from the slaughter-house and observed a mean value of about 1·01, not very far from that of man, especially if we consider that violent muscular movements may have occurred. The concentration of lactic acid in the serum, once kindly examined for me by Miss Kerly, was very high, namely 0·098 p.c. Such a quantity of lactic acid, together with the other osmotically active substances which appear during muscular contraction, corresponds approximately to that necessary to produce the observed excess of osmotic pressure.

Summary.

1. Measurements have been made of the vapour pressure of defibrinated human blood with Hill's thermoelectrical method. The

accuracy of this method as used is such that the probable error of a single determination is about ± 0·2 to 0·3 p.c.

2. The average value, expressed in terms of g. of NaCl in 100 g. of water, is for men (nineteen observations) 0·9447, with a probable individual deviation of ± 0·00495, for women (sixteen observations) 0·9269, with a probable individual deviation of ± 0·0059.

3. The reality of a difference between the values of the osmotic pressure for men and for women is statistically certain; the difference is 0·0178 ± 0·0019. Such a difference is mostly due to the difference in bicarbonate and urea content.

4. The values of the probable individual deviations are an index of the great uniformity of the osmotic pressure of human blood under normal conditions.

5. A preliminary study was made of the maximum variations which may occur in the osmotic pressure of human blood. After drinking 1500 to 2000 c.c. of water, values were found as low as 0·88: after severe muscular exercise a value as high as 1·048 was observed.

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