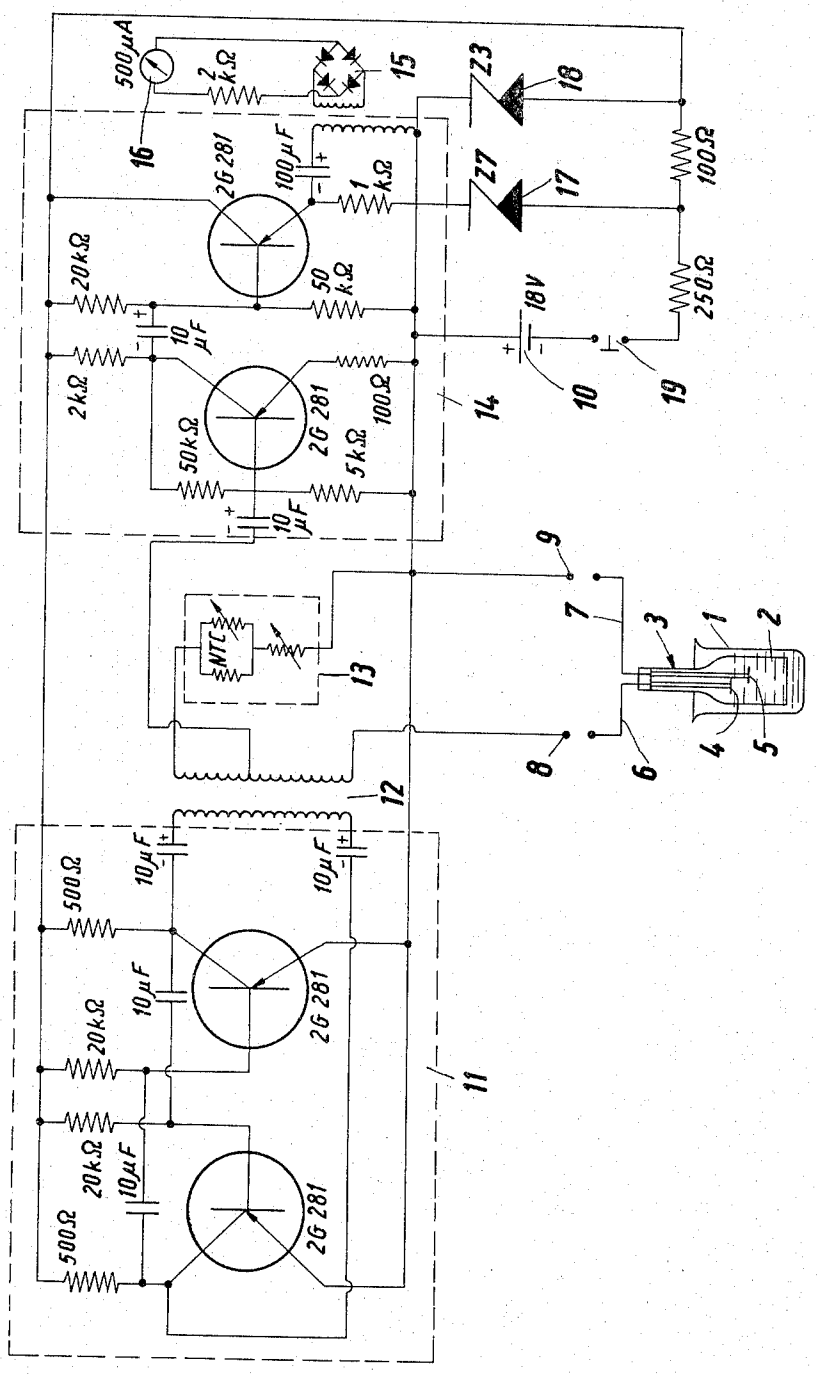
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METHOD OF DETERMINING HEMOGLOBIN IN BLOOD

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METHOD OF DETERMINING HEMOGLOBIN IN BLOOD

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ABSTRACT OF THE DISCLOSURE

The hemoglobin content of a blood specimen is determined by mixing the same with hydrochloric acid and measuring the decrease in conductivity of the acid solution which results from formation of practically non-conductive acid hematin and is proportional to the hemoglobin originally present. Other electrolytes which form non-conductive compounds with hemoglobin may be substituted for hydrochloric acid. The generated signal modified in response to the sensed resistance may further be modified in response to the temperature of the mixed specimen.

This invention relates to the determination of hemoglobin in blood and more particularly to a novel method for determining hemoglobin and to an apparatus for performing the method.

The hemoglobin content of the blood is a basic diagnostic criterion in human as well as in veterinary medicine. The method most commonly employed for determining hemoglobin is the Sahli-method in its various modifications in which a blood sample is mixed with hydrochloric acid whereby the hemoglobin is converted into acid hematin. Various colorimetric methods are used for comparing the color of the acid hematin sample with a standard, and the hemoglobin content of the original blood sample is calculated from the result of the colorimetric evaluation.

Those known devices for determining hemoglobin in blood which are relatively low in cost rely upon the judgment of an operator in comparing the colors of a specimen and of a standard, and are subject to human error. It is well known that the hemoglobin values visually determined by some laboratory workers are always higher than those of others. The instruments which replace the eye of an operator by a photoelectric cell, are relatively costly.

It is a common source of errors in both known types of colorimeters that the mixture of blood specimen and hydrochloric acid does not maintain a stable color. The color increases in depth with time, and the reading of color intensity must be taken at a precisely fixed time after mixing of blood and acid, usually six minutes, if correct values are to be obtained.

Another source of errors which is common to all colorimetric methods is constituted by colored components of the plasma, such as bilirubin in case of jaundice. An additional minor source of inaccuracies in the known methods is the temperature of the specimen. Although all specimens are normally at ambient temperature, the variations in room temperature with the seasons and with other factors are sufficient to influence the results of the colorimetric tests. Some colorimetric methods are additionally affected by variations in the intensity and the wavelength distribution of the light used in evaluating the color of the specimen.

The primary object of the invention is a method for determining the hemoglobin concentration of blood which avoids the sources of error in the known colorimetric methods.

Another object is a method for determination of hemoglobin which yields precise results almost instantaneously.

An additonal object is such a method which is virtually foolproof and requires only minimal skill on the part of an operator.

I have found that the conductivity of a mixture of acid hematin with water is so small, as compared to a mixture of water with the corresponding amount of a strong acid, such as hydrochloric acid, as to be entirely negligible. When a blood specimen is mixed with a known excess amount of a strong acid, the loss of conductivity due to reaction of a portion of the acid with hemoglobin is uniquely correlated with the amount of hemoglobin originally present. The reaction goes to completion practically instantaneously. There are no significant side reactions which would consume acid and could affect the accuracy of the hemoglobin values derived from the conductivity of the mixture of acid and blood.

The invention, in one of its aspects, therefore resides in a method in which a blood specimen is mixed with an excess of an electrolyte adapted to react with the hemoglobin in the specimen to form a substantially non-conductive reaction product. The electrical resistance of the reaction mixture is sensed, and a signal indicative of the hemoglobin content of the blood is generated in response to the sensed resistance.

The exact nature of this invention as well as other objects and advantages thereof will be readily apparent from consideration of the following specification relating to the annexed drawing in which the sole figure is a wiring diagram of a hemoglobinometer of the invention and of associated elements.

In the drawing, there is seen a test tube **1**, partly filled with a mixture **2** of a blood specimen and of dilute hydrochloric acid. A dip cell **3** is inserted in the test tube so that the electrodes **4**, **5** of the cell are fully immersed in the liquid mixture. The two leads **6**, **7** of the electrodes may be attached to terminals **8**, **9** of the hemoglobinometer which includes all other elements illustrated.

An 18-volt battery **10** energizes a square wave oscillator section **11** whose output is fed to the primary winding of a transformer **12** equipped with a center tap in its secondary winding. The two halves of the secondary transformer winding, the dip cell **3**, and a resistor assembly **13** constitute the four legs of a Wheatstone bridge circuit. The assembly includes one resistor having a negative temperature coefficient (NTC) and two variable resistors respectively arranged in series with and parallel to the NTC resistor. The square wave output of the bridge circuit is amplified in a two-stage amplifier section **14**. The sections **11** and **14** each have two transistors of Type 2G281.

The amplified signal is fed through a full-wave rectifier **15** to an ammeter **16**. The ammeter which swings full scale at a current of 500 microamperes is calibrated in grams hemoglobin per 100 milliliters blood.

The voltage output of the battery **10** is precisely stabilized by two Zener diodes **17**, **18**. A normally open push-button switch **19**, the dial of the ammeter **16**, and the terminals **8**, **9** are the only elements of the hemoglobinometer which are normally accessible to the operator. The variable resistors of the resistor assembly **13** are adjustable for calibration of the instrument. If so desired, thermal contact between the test tube **1** and the NTC resistor in the assembly **13** may be enhanced by a heavy metal bracket on the non-illustrated instrument housing. A recess in the bracket outside the housing receives the test tube **1** whereas the NTC resistor is mounted on another portion of the bracket within the housing. The ambient air, however, usually provides adequate thermal contact.

The aforedescribed apparatus is operated as follows: A blood specimen is drawn to the first mark into a Sahli pipette graduated at 30 cubic millimeters and two cubic centimeters. It is then filled to the second mark with N/100 hydrochloric acid. The pipette is shaken to mix its contents, and the mixture is discharged under air pressure into the test tube **1**. The dip cell **3** is inserted, the pushbutton switch **19** is closed, and the hemoglobin content of the blood specimen is read from the scale of the ammeter **16**.

The conductivity signal indicated by the ammeter **16** is not affected by the time interval between the mixing of the blood specimen with the acid and the sensing of the conductivity of the mixture. A correct reading is available at once, but may be taken at any reasonable later time. The NTC resistor assembly **13** is at ambient temperature under all normal conditions of operation. The solution whose conductivity is being sensed mainly consists of dilute hydrochloric acid, which normally is at the same ambient temperature as the resistor assembly **13**. The reaction between the acid and the hemoglobin does not develop significant caloric energy. By proper adjustment of the variable resistors in the assembly **13** the instrument can be made insensitive to the common temperature of the mitxure **2** and of the assembly **13** over any practical range of operating temperatures. The resistance measured and indicated is a generally linear function of the hemoglobin content in the blood specimen. The square wave generator avoids errors due to varying ambinet temperature which an otherwise suitable generator of sinusoidally alternating current may introduce.

The method relies for precision and accuracy on the skill of an operator only for filling a pipette to the graduation marks. The method otherwise is not affected by human error if the indicating ammeter **16** is replaced by a recording instrument in a conventional manner.

Hydrochloric acid is one of the least expensive laboratory chemicals. In N/100 solution it is safe to use under almost any conditions. Hydrochloric acid therefore is preferred in the method of the invention, but the invention is not limited to this specific strong acid, nor actually to the use of an acid. Any electrolyte adapted to form a practically non-conductive reaction product with hemoglobin upon mixing may be employed with the apparatus illustrated.

Suitable non-conducting products also results from the well known reaction of hemoglobin with soluble cyanides, such as sodium or potassium cyanide, to form cyanmethemoglobin, and the conductivity loss of a cyanide solution upon admixture of hemoglobin is a linear function of the amount of hemoglobin added. The known alkali hemin and carboxyhemoglobin procedures are also readily modified for use in the method of this invention if for any reason the use of a hydrogen choride electrolyte is not desired. Other acids which are analogous to hydrochloric acid in the method of the invention, but less desirable at this time are hydrogen iodide and hydrogen azide, also hydrogen sulfide. The loss of conductivity of their aqueous solutions is indicative of the admixed hemoglobin.

It should be understood that the foregoing disclosure relates to only a preferred embodiment of the invention, and that it is intended to cover all changes and modifications of the example of the invention herein chosen for the purpose of the disclosure which do not constitute departures from the spirit and scope of the invention set forth in the appended claims.

What is claimed is:

1. A method of determining the hemoglobin content of blood which comprises:

(a) mixing said blood with an excess of an electrolyte adapted to react with hemoglobin to form a substantially non-conductive reaction product;

(b) sensing the electrical resistance of the reaction mixture; and

(c) modifying a generated signal in response to the sensed resistance, the modified signal being correlatable to the homoglobin content.

2. A method as set forth in claim **1**, wherein said electrolyte is hydrogen chloride.

3. A method of determining the hemoglobin content of blood which comprises:

(a) mixing a measured specimen of said blood with a measured amount of dilute hydrochloric acid in an amount at least sufficient to convert the hemoglobin in said sample to acid hematin;

(b) sensing the electrical resistance of the resulting mixture;

(c) modifying a generated signal in response to the sensed resistance;

(d) further modifying said sensed resistance-modified signal in response to the temperature of said mixture; and

(e) measuring the magnitude of the modified signal and correlating the magnitude of the modified signal to the hemoglobin content.

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