Physiology. — *"On the resisting power of the red blood corpuscles"*. By Dr. H. J. HAMBURGER.

(Read March 31, 1900).

Since DUNCAN [1]) had in 1867 called attention to the fact that in chlorosis the red bloodcorpuscles lose colouring matter in a solution of salt, in which this does not take place under normal circumstances, MALASSEZ [2]) as a consequence of his study on the counting of the red bloodcorpuscles determined the so-called resistance of these cells, by mixing blood with a strongly diluted salt-solution and by examining at regular intervals how many bloodcorpuscles were left. The sooner the bloodcorpuscles were destroyed, the less the resistance.

Later on determinations of resistance were given by CHANEL [3]) equally by counting, although in a different way. Both methods are rarely cited even in French literature and still less put into practice. This is also the case with reference to the method of LANDOIS, LAKEN and also of others, who determined the power of resistance in regard to electric discharges, desiccation and other influences.

A more favourable reception was accorded to a method of investigation, originally only intended for the study of the laws of isotony in the bloodcorpuscles [4]) but which was first applied in 1890 by VON LIMBECK [5]) to investigate the resistance of the bloodcorpuscles during disease. It consists in the determination of the particular Na Cl-solution, in which the first bloodcorpuscles are about to lose colouring matter. If this happens for instance in a Na Cl-solution of 50 pCt., then 0.50 pCt. is called "the resisting power of the least resistant bloodcorpuscles".

If the dilution of the salt-solution is continued, a certain number of the more resistant bloodcorpuscles also lose their coloured contents, and finally all the bloodcells, even the most resistant, have lost these. In a salt-solution, somewhat stronger than the last one mentioned, the most resistant can thus still exist. It is this salt-solution which then represents the "maximum resistance" (MOSSO [6]),

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[1]) DUNCAN, Sitzungsber. d. Wiener Akad. d. Wissensch. 11 April 1867.

[2]) MALASSEZ, Mém. de la Soc. de Biol. 1873, p. 134; Compt. rend. de la Soc. de Biol. 1895, p. 2.

[3]) CHANEL, Sur la résistance des globules rouges. Thèse. Lyon 1886.

[4]) HAMBURGER, Kon. Akad. v. Wetensch. Proces-Verbaal der Zitting van 29 Dec. 1883. Archiv. f. (Anat. u.) Physiol. 1886.

[5]) VON LIMBECK, Prager med. Wochensch. 1890, No. 28 u. 29.

[6]) A. MOSSO, Archives Italiennes de Biol. 1887. T. VIII, p. 257.

VIOLA [1])). With the methods here mentioned (HAMBURGER-MOSSO-VIOLA) a relatively large number of resistance-determinations have been made, but whether they have increased our knowledge of the physiological and pathological conditions, to the study of which they were applied, is very doubtful.

To a certain extent an exception might be made for cyanosis and feverish conditions. The observation that in cyanosis "decrease of resistance" is observed can at least be referred to the fact, that the same is seen in bloodcorpuscles treated with CO_2 [2]), and for this last symptom we have a good explanation [3]).

That the resistance must diminish in feverish conditions is evident when it is taken into consideration that in fever the proportion of alkali in the serum is lowered, which decrease also involves that the bloodcorpuscles belonging to it already begin to lose colouring matter in a higher concentration of salt than those which have sojourned in normal serum [4]).

The reason why the resistance-determinations referred to, have thus far had little success may be sought in the circumstance that it was not duly taken into account what was indeed obtained by determination of the resistance, and what was the physiological meaning to be attached to it. Even in 1895 one could read in the conclusions of the dissertation of URCELAY: "Sur la résistance de globules rouges", Thèse de Paris: "La cause de la résistance des globules rouges nous est inconnue", and this at a time, when most of the resistance-determinations thus far known had been performed and URCELAY had contributed some himself.

As regards myself, I never felt induced to use my method of investigation otherwise than for more circumscribed aims, and on purpose I have thus far avoided to use the *in casu* unfit word "resistance" when colouring matter disappeared under the influence of certain salt-solutions and other mixtures.

Being invited to read a paper on this subject in the International Medical Congress to be held in Paris next August, I find a welcome opportunity to study the question at the present time, the

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[1]) VIOLA, Gazette degli Ospedali 1894, p. 115; Archives de Physiol. et de Pathol. générale 1895, p. 37.

[2]) HAMBURGER, Versl. en Meded. Kon. Akad. v. Wet. 3e Reeks. Vol. IX. 1891, p. 197. Zeitschr. f. Biol. B. 28. 1892, S. 105.

[3]) HAMBURGER, Zittingsverslag Kon. Akad. v. Wet. 28 Nov., 1896; 24 Febr. 1897. Zeitschr. f. Biol. 1897. S. 252.

[4]) HAMBURGER, Versl. en Meded. Kon. Akad. v. Wet. 3e Reeks. Vol. IX, 1892, p. 354; Archiv f. (Anat. u.) Physiol. 1892, p. 513.

more so because this affords a means of controlling the investigations lately made on the volume-determination of the protoplasmic reticulum of the bloodcorpuscles.

I shall try to analyse the term "resistance" of the bloodcorpuscles in regard to salt-solutions and must in the first place inquire which are the factors on which depends the loss of colouring matter in the bloodcorpuscles by means of salt-solutions. My particular view is, that the bloodcorpuscle consists of a protoplasmatic reticulum, the interstices or meshes of which, closed or unclosed, contain the intraglobular liquid; it is this liquid which solely represents the power of the cell to attract water; the protoplasmatic reticulum has no share in this.

If one now imagines a bloodcell being immersed in a hypisotonic solution, then only the contents of the meshes will swell. The amount of this swelling will be more considerable in a certain hypisotonic solution, the greater the amount of the osmotic pressure of the intracellular liquid and also the greater the quantity of the intracellular liquid in a given cell-volume.

The more considerable the increase in volume is, which the intracellular liquid can be made to undergo without colouring-matter being extruded from the protoplasmatic network, the more resistant the protoplasm may be considered to be.

Taking these matters into consideration, we conclude that when different salt-solutions are allowed to act upon the red bloodcorpuscles, three or perhaps two forms of resistance come forward.

1. *The resistance of the bloodcorpuscle against loss of colouring matter, under the influence of diluted solutions.*

It is this form of resistance, which has been determined until now. It is of a complicated nature.

2. *The relative resistance of the protoplasm against the extrusion of colouring matter during expansion.*

3. *The absolute resistance of the protoplasm against extrusion of colouring matter during expansion.*

Ad I. *Resistance of the bloodcorpuscle against loss of colouring matter in diluted salt-solutions.*

As it was observed above, it is this form of resistance which has hitherto been determined by the so-called method of the bloodcorpuscles.

To salt-solutions of gradually diminishing concentration a few

drops of the same blood are added and it will be observed that in a Na-Cl-solution of 0.49 pCt. some colouring matter has been extruded, which is not the case in a Na Cl-solution of 0.50 pCt. This is called the minimum resistance. It would be more correct anyhow to express it by $\frac{1}{0.50}$, as the resistance is inversely proportional to the limit of concentration referred to; therefore in general $R_{b(loodcorpuscles)} = \frac{1}{C}$; in which C represents the limit of concentration, in which the first bloodcorpuscles are about to lose colouring matter. With regard to the application of this method we take the liberty to propose a modification. It seems to us to be recommendable, also in connexion with the determination of the other forms of resistance to perform the determinations in small funnelshaped tubes of which the capillary part is calibrated and closed with a little ebony stop. They have the same shape as described formerly[1]), but in view of their being used for human blood they are smaller. With a capillary pipette a determined quantity of defibrinated or oxalate blood is measured for the different tubes which contain an equal volume of the different salt-solutions and the mixtures allowed to stand for half an hour; they are then centrifugalized. After a quarter of an hour's moderate rotatory velocity the bloodcorpuscles have already subsided and it can be seen by comparison where colouring matter begins to extrude and where not. This way of experimenting has a threefold advantage.

1. As the relative quantity of blood and salt-solution has been fixed and also the shape and measures of the little funnel-tubes are equal, we can compare the results of different investigators better than could be done hitherto and uniformity is thus enhanced.

2. As the full subsidence need not be waited for, the time for the determination of the resistance will be shortened.

3. Those tubes of which it is desirable, can further be used for the determination of both the other forms of resistance; but later on more of this.

To find the maximum-resistance the same method is followed as for the minimum resistance: the salt-solution is determined, which, mixed with the blood, gives a perfectly pure transparent liquid. The solution, which yet retains a trace of opacity is the sought for C'_g.

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[1]) Verslag Kon. Akad. v. Wetensch. 21 April, 1897.

The maximum-resistance is then $R'_b = \frac{1}{C'_g}$.

The average resistance $\frac{1}{2}(R_b + R'_b) = \frac{1}{2}\left(\frac{1}{C_l} + \frac{1}{C'_l}\right)$, whereas we shall call the difference $R'_b - R_b = \frac{1}{C'_l} - \frac{1}{C_l}$: resistance-breadth. The determination of this value seems important to me.

It ought to be kept in mind, however, that quantities of a complicated nature are here determined, which it can however be important to be acquainted with in certain circumstances.

Ad. 2. Relative resistance of the protoplasm R_{pr}.

It is measured by the proportion of the volume V_l which the intraglobular liquid may attain in maximo before it is exuded by the protoplasm, as compared to the volume (V_n), which it possesses in normal conditions.

This proportion $\frac{V_g}{V_n}$ can be found by means of three methods.

Method a.

This method consists in attempting to determine the limit-concentration of the Na Cl-solution, in which the bloodcorpuscle swells at its maximum (C_g), and is thus about to lose its colouring matter, and also the concentration of the Na Cl-solution, in which its volume remains unchanged, that is: a NaCl-solution C_n, isotonic with the serum. As the attraction exercised by the intracellular liquid towards water, agrees with that of its environment under different circumstances, so $\frac{v_l}{v_n}$ is $= \frac{C_n}{C_l}$, at least when the dissociation of the contents of the bloodcorpuscles and the surrounding Na Cl-solution are left out of consideration [1]).

For the determination of C_n the freezing-point-method can be used, or if only very little blood is available the method of GRIJNS-EYKMAN [2]).

Method b.

According to this method the quantity of water is determined with which the respective blood-serum can be diluted, without the

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[1]) This is permissible here, as will be explained elsewhere. Here this explanation would lead us too far.

[2]) C. EYKMAN, Annual report of the Laboratory for Patholog. Anat. and Bacteriol. at Weltevreden for the year 1894.

blood losing any colouring matter. Let x be the percentage of the water added, then $\frac{v_p}{v_n} = \frac{100 + x}{100}$.

The quantity of serum required can be considerably limited, by centrifugalizing each time blood has been added to the diluted serum and after having waited for half an hour. When by this time the red colour has not yet appeared a known quantity of water is dropped into this serum, it is mixed with the serum, and the serum thus diluted is brought into close contact with the underlying bloodcorpuscles. This is repeated until colouring matter is seen to be extruded. At the utmost 8 cc. of blood is needed for this method.

Method c.

According to a method formerly indicated by me, the volume of the protoplasmatic reticulum of a given quantity of bloodcorpuscles is first determined [1]). Let this be π. If further the volume of the bloodcorpuscles in their own serum be V_n, then the volume of the intra-globular liquid in the normal condition is $V_n - \pi$ and in the condition of maximum swelling $V_l - \pi$, and therefore the relative resistance $R_{pr} = \frac{V_l - \pi}{V_n - \pi}$.

With this method c the *average* relative resistance of the protoplasm is immediately fixed, the three values of V_g, V_n and π having reference to all bloodcorpuscles together.

For the two other methods mentioned sub 2 the resistance must be fixed separately, for the least resistant and for the most resistant.

If for method a the Na Cl-concentration, in which the most resistant bloodcorpuscles are about to lose the colouring matter, be C'_l, then the maximum resistance is $R'_{pr} = \frac{C_n}{C'_l}$, and the average

$$\tfrac{1}{2}(R_{pr} + R'_{pr}) = \frac{1}{2}\left(\frac{C_n}{C'_l} + \frac{C_n}{C'_l}\right).$$

If for method b, x' be the percentage of water that must be added in order to extract colouring matter even from the most resistant bloodcorpuscles, then $R'_{pr} = \frac{100 + x'}{100}$.

The average resistance will then be:

$$\tfrac{1}{2}(R_{pr} + R'_{pr}) = \frac{1}{2}\left(\frac{100 + x}{100} + \frac{100 + x'}{100}\right) = \frac{1}{2}\left(\frac{200 + x + x'}{100}\right).$$

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[1]) Reports of the Roy Acad. of Sciences. Amsterdam May 28, 1898.

The relative resistance breadth of the protoplasm we indicate by $R'_{pr} - R_{pr}$.

This value seems important from a physiological and pathological point of view.

Ad. 3. *Absolute resistance of the protoplasm against the extrusion of colouring matter during expansion* R_{pa}.

Superficially it might be supposed that the relation of the intracellular contents of the bloodcorpuscles in the condition of maximal swelling and in the normal condition, expresses the degree of resistance in an absolute sense. This however is not the case. Imagine two bloodcorpuscles of equal size in their own serum, both have intraglobular contents of equal osmotic pressure, but the volume of the intraglobular liquid is greater in the first bloodcorpuscle than in the second. If it is proved that, nevertheless, both bloodcorpuscles lose colouring matter in the same saltsolution (Cl), in which case the osmotic pressure of the intraglobular contents must necessarily be equal, then the conclusion is inevitable that the protoplasm of the first bloodcorpuscle is more resistant than that of the second, for the absolute increase of volume of the first bloodcorpuscle was more considerable than of the second. With equal C_n and C_l it is therefore not necessary that the resistance should be equal. In order to be able to compare the absolute resistance of the protoplasm of two bloodcorpuscles, the quotient $\frac{C_n}{C_l'}$, which was therefore called relative resistance, must be multiplied by a factor which expresses the percentage of the volume of the intraglobular liquid, a factor which we calculate from π.

$$f = \frac{V_n - \pi}{V_n} \times 100.$$

As we do not know whether this factor may be used separately with the minimum-resistance, or with the maximum-resistance because we do not know whether the relative volume of the protoplasmatic reticulum is the same in all bloodcorpuscles of the same blood, it is undoubtedly safer to use the factor only where it is in all cases applicable, viz. with the average resistance.

Thus in this third method the average absolute resistance of the protoplasm against the transmission of colouring matter when expanded is determined, so that $R_{pa} = f \frac{V_l - \pi}{V_n - \pi}$ (of method 2*a*).

SIMULTANEOUS DETERMINATION OF THE THREE FORMS OF RESISTANCE.

Suppose the three forms of resistance have to be determined during an illness and little blood is thus at our disposal. 1 cc. of blood is taken, defibrinated and strained or made to flow in 0.2 cc. sodium-oxalate of 1.5 pCt. Of this blood equal quantities (measured with a capillary pipette) are transferred to little funnel-tubes, which contain NaCl-solution of 0.30, 0.32, 0.34, 0.36, 0.38, 0.40, 0.42, 0.44, 0.46 0.48, 0.50, 0.52, 0.54, 0.56 pCt. [1]).

These liquids are mixed, allowed to stand for half an hour and then centrifugalized. After this it is determined in which tube colouring begins to show itself. The tube following upon this containing a more concentrated liquid, represents C_l. By determining where the mixture has become transparent, the maximum-resistance C'_l is found. Thus $R_b = \frac{1}{C_l}$ and $R'^b = \frac{1}{C'_l}$ (Method 1).

Now five tubes are prepared with equal quantities of blood.

Tube (1), undiluted defibrinated blood.

" (2) blood + Na Cl 0.9 pCt.
" (3) " + " 0.88 "
" (4) " + " 0.86 "
} to investigate in which Na Cl-solution the volume of the bloodcorpuscles becomes like that in tube (1).

" (5) " + the Na Cl-solution just found, viz. the limit solution C_l in which the bloodcorpuscles are on the point of emitting colouring matter.

" (6) " + Na Cl 1.5 pCt., also for the determination of the protoplasmatic reticulum.

The whole mass is centrifugalized to a constant volume.

We can now calculate the relative resistance $\frac{C_n}{C_l}$ by dividing the concentration of the Na Cl-solution (2), (3) or (4) by that of the Na Cl-solution (5) (Method 2*a*), or also by calculating the protoplasmatic reticulum π from the Na Cl-solution (2), (3) or (4) and the Na Cl-solution (6). Tube (1) gives V_n, tube (5) gives V_g, and therefore relative resistance R_{pr} is also $= \frac{V_l - \pi}{V_n - \pi}$ (Method 2*c*).

All the values are now also known for the calculation of the ab-

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[1]) If so many tubes are not to hand, the same could preliminarily be performed by increasing with 0.4 pCt. Na Cl and seeking whereabout the limits lie for minimum- and maximumresistance and then fix these more accurately later on.

solute resistance, of which only the *average* can be determined. It is $100\, R_{pr} \frac{V_n - \pi}{V_n}$ (Method 3).

If there is reason to believe, in comparing the resistance of two samples of blood, that under normal circumstances the volume of the protoplasmatic reticulum, or, what comes to the same, of the intracellular liquid, does not differ, then the determinations become simpler and the results of 2a, 2b or 2c may prove to be sufficient. If moreover the osmotic pressure of the serum is the same, then the first method suffices.

Chemistry. — "*The behaviour of mixtures of mercuric iodide and silver iodide*". By Prof. H. W. BAKHUIS ROOZEBOOM.

(Read May 26, 1900.)

The double iodide $HgI_2\,2AgI$ is known as one of the finest examples of a solid substance which undergoes a change at a definite temperature, because this substance changes, when heated to 45°, from the pure yellow to orange red.

There was, however, a difference of opinion as to the change which takes place here; some attributed it to the change of the compound itself into another modification; others thought that, at 45° it broke up into the two component iodides.

At my request Dr. STEGER has made a further investigation of the matter and has come to the conclusion that the two iodides mixed in varying proportions and at different temperatures are of a very varying nature. If we start from fused mixtures, it appears firstly that the melting point of HgI_2 is lowered from 257° to 242° by an admixture of 14 mol. pCt. of AgI. On the other hand the melting point of AgI is lowered from 526° to 242° by an admixture of 86 mol. pCt. of HgI_2.

By means of an accurate determination of the temperature-interval in which solidification of a certain mixture takes place, it may be found out what happens during the solidification. To do this with accuracy, a bath was used of melted $NaNO_3 + KNO_3$ which was stirred and which by judicious heating enabled us to maintain any constant temperature between 200°—500°, or to slowly vary it. The course of solidification of the different mixtures shows that two kinds of mixed crystals are formed; on the HgI_2 side with 0—4 mol. pCt. of AgI, on the other side with 18—100 pCt. of AgI. The first series has the type of the rhombic HgI_2, the other