forming the Milky Way, and in order to ascertain more about their structures and distances we have to go on to still lower magnitudes. That is why we do not venture here a comparison between our numbers and the light of the Milky Way. We only want to observe that the views based on former investigations have been rather contradicted than corroborated by this research. What has been found here indicates *that no organic relation exists between the great mass of stars of the 9th magnitude and perhaps as far as the 11th, and the star-clusters forming the Milky Way.* Before putting this down as a certainty, however, it is desirable that we should wait till we have more material available.

The completion and the publication of the photographic *Carte du Ciel* promises important results; it will be some time, however, before the charts fully cover the regions that are to be examined. But however much may be expected from a systematic treatment of the thus completed material, through combining the B.D., the catalogue-plates and the chart-plates of the *Carte du Ciel* and the star countings by HERSCHEL and EPSTEIN for different parts of the sky, still there remains the lack of homogeneity and of exact identity of the celestial regions for which these numbers stand. Another time I hope to describe a method free from these drawbacks.

Physiology. — *"The permeability of red blood-corpuscles in physiological conditions, especially to alkali- and earth alkali metals* [1]). By Prof. H. J. HAMBURGER and Dr. F. BUBANOVIĆ (Croatia).

Introduction.

In a former communication one of us [2]) has demonstrated by means of quantitative chemical determinations that red blood corpuscles are in both directions permeable to Ca. At the same time the conditions were investigated under which this permeation took place. We have now extended our investigations to other Kations viz. magnesium, potassium, and sodium, and have finally connected with it the question whether, under the same physiological conditions under which the permeation of calcium, magnesium, sodium and potassium, was inves-

[1]) More explicit communications on this subject will appear in the *Archives Internationales de Physiologie* publ. par LÉON FREDERICQ.

[2]) On the Permeability of blood cells to Calcium. These Proceedings of March 27 1909. See also a more detailed account in the Zeitschrift für Physikalische Chemie. Bd. 69, S. 663, 1909. (Festband f. Arrhenius).

tigated, it would also be possible to demonstrate a permeation of anions, such as chlorine and alkali. In order to promote the permeation of the above-mentioned ions, the same modifications were made in the composition of the serum, as had been made in the former experiments on Ca. They simply consisted in the blood being made hyperisotonic or hypisotonic, and that, to an extent corresponding to the fluctuations which may daily occur in normal life. These investigations were also made with a view to collecting data which afterwards might serve perhaps to explain a phenomenon, noticed by HEDIN [1]) several years ago and which has hitherto been left unexplained. We mean the fact that the volume of red blood-corpuscles is equal in isosmotic-isotonic solutions of different salts, but unequal in isosmotic-anisotonic solutions. Also on former occasions our attention was drawn by this phenomenon [2])

Method of Investigation.

As has been said the permeability of the blood-cells was investigated by modifying the composition of the serum within physiological limits. The modification consisted in the blood-serum being made anisotonic, that is to say hyperisotonic by an addition of 0.2% NaCl, hypisotonic by an addition of 10% water. To accomplish this in an efficient manner a certain quantity of blood was centrifugalized, the serum was partly removed and mixed with the necessary amount of NaCl, or water; then it was added to the rest of the blood and well mixed with it. The suspension thus acquired was left to itself for an hour, to enable the blood corpuscles to get balanced with their new surroundings. After that time the suspension was centrifugalized, and the serum which was thus removed, could be examined as to its percentage of magnesium, potassium, sodium, etc.; this percentage could then be compared with the relative amount of these substances in the original serum. To control the result of the experiment we have in most cases made a quantitative determination of these substances as found in the red blood-corpuscles, and thus we could easily verify whether a decrease in the amount of certain serum substances was accompanied by a corresponding increase of them in the blood corpuscles or vice versa. For an exact determi-

[1]) HEDIN, Skandinavisches Archiv. f. Physiol. 1895 S. 377.

[2]) HAMBURGER and HEKMA, Zur Biologie der Phagocyten III, Biochem. Zeitschr. 9, 281, 1908.

HAMBURGER and DE HAAN, Zur Biologie der Phagocyten V, Biochem. Zeitschr. 24, 317, 1910.

nation of the increase or decrease it was necessary to know in all cases the volumes of the serum and of the blood corpuscles, as they were modified by anisotony, for it is obviously impossible to obtain and analyse *all* the serum of a certain amount of blood. For, however strongly we may centrifugalize, yet it remains impossible to remove all the serum from the sediment; a thin layer is always left behind. Whenever it was necessary, we have therefore determined the relative volumes of blood corpuscles and serum by centrifugalizing e.g. 0.06 cc blood in our funnel shaped tubes, until the volume of the sediment remained constant.

Finally it must be observed that all the blood, used for these experiments, had been shaken with 5 volume percent carbonic acid. This was done to render eventually a more extensive interchange of substances possible, thus causing permeability when present, to manifest itself in a more marked degree.

Permeability to Potassium and Sodium.

The permeability to potassium and sodium was investigated in the following way. As has been said above, a great volume of blood (3 litres) was shaken with 5 vol. perc. carbonic acid and left to itself for three hours to enable the carbonic acid to act; then 3×12 tubes were each filled with 75 cc. of this blood and closed at the top with india rubber covers; at the same time 0.06 cc. of the same blood were put in 3 funnel shaped tubes provided with well-fitting stoppers in order to determine the relative volumes of blood corpuscles and serum. Then in 12 tubes, part of the clear serum was removed, and Na Cl was dissolved in it. To the serum of the 12 other tubes water was added, then these sera were replaced in their original tubes and well mixed with the rest of the serum and the blood corpuscles. The amount of salt added was just sufficient to cause an increase of 0.2 % NaCl in the serum. The amount required was calculated by means of the comparative volumes of blood corpuscles and serum, as they appeared from volumetrical determinations in the funnel shaped tubes. In the same way the amount of water was determined which had to be added to the serum in the 12 other tubes.

Now the blood corpuscles were left for an hour to get into a state of equilibrium with the surrounding fluid. This fluid was centrifugated and the clear serum was removed as much as possible. The volume of it having been measured, it was evaporated in a platinum basin at 110° and the residuum was exposed to a soft glowing heat.

Then distilled water and HCl were added to turn the metals into chlorides. The solution was filtrated and washed, and $BaCl_2$ and BaH_2O_2 were added to the filtrates to remove sulphuric acid and magnesium. Then the filtrate was mixed with $(NH_4)_2CO_3$ to remove the surplus of barium and also the calcium. These having been removed by filtration, the filtrate could be evaporated in a platinum basin and glowed to remove the superfluous $(NH_4)_2 CO_3$. What was left behind now was only KCl and NaCl. The total amount of it was weighed.

In this mixture the potassium could be determined. HCl and aq. dist. were added; then an excess of H_2PtCl_6, and the whole was concentrated on a water bath into a semi fluid mass, 80% alcohol being added. In this way sodium chloroplatinate (Na_2PtCl_6) remained in solution and yellow crystals were formed of K_2PtCl_6. These were placed on a filter, which had previously been dried and weighed; the crystals were washed with alcohol, dried at 120° and weighed. To determine the amount of sodium we had only to subtract the amount of KCl from the total amount of KCl and NaCl.

In the blood corpuscles the determination of potassium and sodium was carried out in the same way as in the serum. As a matter of course we had to take into account that there was still serum left among the red blood corpuscles. The amount of it was established in the usual way by centrifugalizing the thick suspension in funnel shaped tubes.

The results which we obtained may be summarized in the following table. (p. 262).

From this table it appears:

1. *that when serum is made hyper-isotonic by the addition of NaCl* to an amount which is also observed in normal life, *sodium enters the blood corpuscles and potassium leaves them* (Comp. the first two numbers of column 4*a*, of column 4*b*, 5*a* and 5*b*.

2. *when water is added to the serum* to an amount which is also observed in normal life, *sodium likewise enters the blood corpuscles whilst potassium leaves them.* (Comp. in all cases the first and third numbers of the above mentioned columns).

The entrance of sodium into the blood corpuscles appears not only from a decreased percentage of this metal in the serum but also from an increase in the blood corpuscles, whilst the fact that *K* leaves the blood corpuscles not only appears from a decreased percentage of this metal in the cells, but also from an increased percentage of this metal in the serum.

TABLE I.

Permeability to Potassium and Sodium.

1	2a	2b	3a	3b	4a	4b	5a	5b
900 cc blood	In the 900 cc blood are:		Total amount of K and Na determined as KCl and NaCl, in:		Total amount of K determined as KCl, in:		Total amount of Na determined as NaCl, in:	
	serum	blood corp.	the serum	the blood corp.	the serum	the blood corp.	the serum	the blood corp.
normal	594 cc	306 cc	4.9805 Gr.	1.6577 Gr.	0.3479 Gr.	1.2379 Gr.	4.6326 Gr.	0.4198 Gr.
to the serum *0.2%* *NaCl* has been added	618 „	282 „	4.9323 „	1.7666 „	0.4438 „	1.0761 „	4.4885 „	0.6905 „
to the serum has been added *10% water*	570 „	330 „	4.9170 „	1.7755 „	0.4006 „	1.2132 „	4.5164 „	0.5623 „

Permeability to Magnesium.

With a view to the slight amount of magnesium found in blood, and likewise with a view to its lightness, a great quantity of blood was operated upon viz. 3 × 1200 cc. The experiments were identical

with those made on the determination of K and Na. The ash was treated in a different way, of course. After it had been moistened with some HCl and dissolved in hot water, it was diluted to 100 cc. in a beaker and neutralised with $(NH_4)OH$ till a precipitate was formed. Then it was acidified with acetic acid and a concentrated solution of ammonium acetate was added. After being boiled the precipitate was filtrated and washed.

In the acetic acid filtrate the Ca was precipitated by ammonium oxalate at boiling-heat and the whole was left to itself for 4 hours. The filtrate was then mixed with ammonia and some Na_2HPO_4 and the mixture was left to itself for 24 hours at a low temperature. The resulting $Mg(NH_4)PO_4$ could now be washed, dried, and glowed in a platinum basin.

The following table contains the results obtained.

From this table it appears that *if the serum is made hyperisotonic by an addition of* 0.2% *NaCl, the amount of magnesium decreases* (from 0.1445 to 0.1385) and that accordingly the magnesium increases in the blood corpuscles (from 0.0166 to 0.0221 gr.) *whilst if the serum is made hypisotonic by an addition of water, magnesium acts just the other way, that means to say that it leaves the blood corpuscles.* (Comp. the first and third numbers of column 2*a* and of column 2*b*).

TABLE II.

Permeability to Magnesium.

	1*a*	1*b*	2*a*	2*b*
	In the 1200 cc blood are		Amount $Mg_2P_2O_7$ indicating the amount of Mg. in	
	serum	blood corpuscles	serum	blood corpuscles
a. 200 normal blood	768 cc	432 cc	0.1 45 gr.	0.0166 gr.
b. 200 cc blood (a), to the serum of which was added 0.2% *NaCl.*	804 „	396 „	0.1385 „	0 0221 „
c. 1200 cc blood (a), to the serum of which was added 10% water.	736 „	464 „	0 1319 „	0 0159 „

Permeability to Calcium.

As we said above, former experiments [1]) had shown that calcium can both enter and leave the blood corpuscles. This motion of calcium was discovered to find its cause in a disturbance of the equilibrium between blood corpuscles and serum. This disturbance was, amongst other causes, brought about by an addition of some NaCl or of a slight amount of water to the serum. *We now wished to investigate to what extent a disturbance of the equilibrium caused by 5 vol. perc. carbonic acid would likewise cause a transfer of Ca.*

As we know an addition of 5% CO_2 to arterial blood falls within physiological limits.

After what has been said, it may be esteemed superfluous to enter into technical details as to the method of investigation. Let us only state that the Ca was determined in the ash by ammonium oxalate, that after being heated the oxalate was weighed as CaO, and further that in this case only the serum was examined as to its amount of Ca. This seemed sufficient after the detailed investigations formerly made on Ca [1]).

The result of the experiments now made was that under the influence of 5 vol. perc. CO_2, calcium had entered the blood corpuscles and that owing to a further disturbance in the equilibrium, caused by the addition of NaCl to the blood after it had been shaken with carbonic acid, a new amount of Ca had entered the blood corpuscles

TABLE III.

Permeability to Calcium.

	In the 900 cc blood are	Amount of CaO indicating the amount of Ca in the serum
a. 900 cc blood	600 cc serum and 300 cc bl. corp.	0.1592 gr. CaO
b. 900 cc blood shaken with 5 vol. perc. CO_2	588 cc " " 312 cc "	0.1526 " "
c. 900 cc blood shaken 5% CO_2, to the serum of which 0.2% NaCl has been added	606 cc " " 294 cc "	0.1383 " "

[1]) These Proceedings of March 27 1909; Zeitschr. f. physik. Chemie, Festband. Arrhenius l c.

If we compare the 3 numbers of the third column, *it becomes manifest that under the influence of CO_2 calcium enters the blood corpuscles, and that this is the case to a much greater extent if to this blood a physiological amount of NaCl has been added.*

We may add to this that in connection with these experiments we also investigated the effect of an addition of NaCl to blood which had *not* been treated with CO_2. The amount of CaO now found in the serum was 0.1444 gr. whilst the volumes of serum and blood cells had become 618 cc. and 282 cc. respectively. This may serve as a confirmation of what had formerly been observed in the above-mentioned investigation concerning the permeability of red blood corpuscles to Ca-Ions.

Permeability to Chlorine.

Though at the present moment there is probably no one who doubts the fact that red blood corpuscles are permeable to chlorine, yet we have thought it expedient, in connection with the above-mentioned experiments, to investigate whether a motion of chlorine could be demonstrated under the same conditions under which the kations K, Na, Mg, and Ca passed through the blood corpuscles. Hitherto, indeed, we examined the permeability of red blood corpuscles to this anion almost exclusively by allowing physiological amounts of CO_2, H_2SO_4 and KOH to act upon the blood [1]). Would it be possible to establish likewise a permeation of chlorine if the normal equilibrium between red blood corpuscles and serum was broken by adding to the latter 0.2% NaCl or 10% water?

For this purpose we have experimented in exactly the same way as above, that is to say a known volume of serum as well as a known volume of blood corpuscles were dried, made into ash and in the ash the chlorine was determined. This was done after the method of VOLHARD.

It need hardly be said that to obtain an estimate as to the absolute amount of chlorine in blood corpuscles and serum the volumes of both had to be established.

1 Litre of blood was again shaken with 5 vol. perc. CO_2. Of this quantity we took 3 times 300 cc.

To one of these 3 quantities Na Cl 0,2 % was added, to another

1) HAMBURGER, Zeitschr. f. Biologie 1891, S. 405; Archiv f. (Anat. u.) Physiologie 1892, S. 513; 1893, S. 153; 1893, S. 157; Zeitschr. f. Biologie 1897, S. 352; Archiv. f. (Anat. u.) Physiol. 1898.
HAMBURGER und VAN LIER, Archiv f. (Anat. u.) Physiol. 1902, S. 492.

water, whilst the third as such was examined as to its amount of chlorine.

TABLE IV.

Permeability to Chlorine.

	2a	2b	3a	3b
	In the 300 cc blood are		Amount $^1/_{10}$ n. $AgNO_3$ indicating the amount of Cl in	
	Serum	blood corp.	serum	blood corp.
a. 300 cc. normal blood	189 cc.	111 cc.	110 06 cc.	33.34 cc.
b. 300 cc. blood *a*, to the serum of which 0.2 % NaCl had been added	197 „	103 „	110 34 „	34 16 „
c. 300 cc. blood *a*, to the serum of which 1 % water had been added	181 „	119 „	112.20 „	31.18 „

From these experiments it appears that by the addition of 0.2 % NaCl to the serum a certain amount of chlorine enters the blood corpuscles, and that conversely by the addition of water to the serum chlorine leaves the blood corpuscles. (Comp. of the same columns the first and third numbers).

So it appears that under the same circumstances or in other words by the same equilibrium disturbances which cause blood corpuscles to be permeated by kations, also a permeation of chlorine takes place.

Finally we shall examine whether the same holds good for alkali.

Permeability to Alkali.

Already on the occasion of former investigations as to the permeability of red blood corpuscles and other cells to chlorine and other anions, the permeability to alkali has been set forth. In this paper we have investigated to what extent an addition of slight quantities of NaCl or water to the serum caused the blood corpuscles to yield or to take in alkali. The amount of alkali in the serum was determined by means of lacmoid paper. As we know, in this way the total amount of diffusible and non diffusible alkali is titrated.

We summarise the results in the following table, allowing for the modification of the volumes, mentioned before.

TABLE V.

Permeability to Alkali.

	In the 100 cc blood are	Amount of $1/_{25}$ norm. tartaric acid corresponding with the alk. of the serum
a. 100 cc. normal blood (with 5% CO_2)	61 cc serum + 36 cc bl. corp.	28.8 cc
b. 100 cc. blood (*a*), to the serum of which 0.2% NaCl had been added	67 cc „ + 33 cc „	27 5 cc
c. 100 cc. blood (*a*), to the serum of which 10% water had been added	61 3cc „ + 38 7cc „	27 1 cc

These experiments show that by making the serum hyperisotonic by the addition of NaCl, alkali enters the blood corpuscles, whilst by the addition of water to the serum the same thing takes place, but in a higher degree.

Summary.

The investigations described above have chiefly led to the following results:

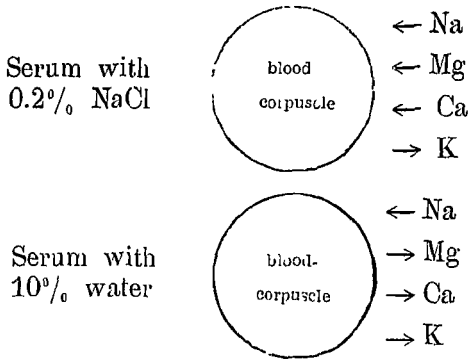
1. If in the composition of blood we bring about a disturbance in the equilibrium between blood corpuscles and serum, falling within physiological limits, a redistribution of the anorganic components takes place over blood corpuscles and serum.

2. This redistribution relates to kations as well as anions.

a. As regards the kations it has been seen that by the addition of 0.2% *NaCl to the serum, Na, Mg, and Ca enter the blood corpuscles, whilst K leaves them.*

When the serum is diluted with 10% *water Na enters, whereas K, Mg, and Ca leave the blood corpuscles.*

A survey of these movements may be given in the following way:



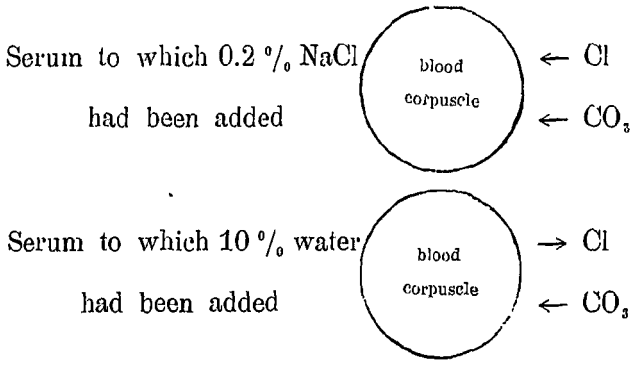
As regards Ca, this result is a confirmation of what had previously been found as an outcome of detailed investigations.

In this paper it has moreover been demonstrated that Ca enters the blood corpuscles too when the blood is shaken with a slight quantity (5 vol. perc.) of CO_2 and that this transfer becomes more considerable still, if to this blood containing CO_2 some NaCl is added.

b. The addition of Na Cl or water to serum as mentioned sub. *a* caused not only a movement of kations, but also of *anions. By the addition of some NaCl to the serum, chlorine was found to enter the blood corpuscles; by the addition of water to the serum chlorine left them.*

The alkali (CO_3) likewise participated in this movement. An addition of NaCl to the serum caused alkali to enter the blood corpuscles, whilst an addition of water had the same effect, but in a somewhat higher degree.

A survey of these movements may be given again in the following way:



These observations have proved again, and that by methods entirely different from those formerly employed by us and by others, that

red blood corpuscles in physiological conditions, are permeable to anions.

3. The conclusion concerning the permeation of Na, K, Mg, Ca and Cl is based upon the results of quantitative-chemical analyses of these substances in the serum; these results were in all cases confirmed by the quantitative determiuations of these substances in the corresponding blood corpuscles.

4. Briefly **the results described above justify us in asserting that blood corpuscles under physiological conditions are permeable to kations** as well as **to anions**, or if we do not wish to view the matter in the light of the ion-theory, **to metals and acid anhydrids.**

As to the kations (metals) this result is opposed to the current view.

The latter is based upon an investigation of GÜRBER according to which the serum retained its amount of sodium and potassium when blood was saturated with carbonic acid; and tacitly the impermeability to potassium- and sodium ions has been extended to calcium and magnesium. If, however, we examine GÜRBER's experiment more closely, it is seen that the blood used by this investigator for his analyses, amounted to only 100 cc., a quantity much too small to arrive at definite conclusions as to the permeability to K and Na. The amount of potassium indeed, contained in the serum of 100 cc. blood (0.018 gr. K_2O), is so small that it is impossible to demonstrate with certainty an increase or a decrease of 5 %. In order to get results which are at all reliable, it is necessary to experiment upon a much greater quantity of blood; besides it is necessary to analyse as a further test not only the serum, but also the corresponding blood corpuscles; this was neglected by GÜRBER.

More explicit critical remarks are found in our treatise in the Archives Internationales de Physiologie. In the same article the grounds may be found which induce us to look upon the permeation of substances as an interchange of *ions.*

5. As to the conditions under which the permeation of kations occurs, we assume also on account of former investigations on Ca, that it takes place only where an exchange is possible with equivalent kations on the other side. And this possibility occurs whenever the equilibrium is disturbed in the normal chemical composition of serum and blood corpuscles. If further we investigate by what

causes again this disturbance may be brought about, then it appears that in the first place this becomes possible by a change in the osmotic pressure of the blood, by which the dissociation is modified. This dissociation occurs, in the blood corpuscles in another way than in the serum. Likewise the equilibrium is disturbed when substances are added to the serum such as CO_2 and different salts. Of these two causes for the disturbance in the equilibrium the change of osmotic pressure was found, at least as regards calcium, to have a paramount influence (l.c.).

6. That the movement of kations and anions through cells is of importance to life has been plainly set forth for instance by comparative investigations on the influence of KCl, NaCl, NaBr, KI, NaI, NaFl, and also of Ca on phagocytosis.

Groningen, June 1910.

Physiology. — "*Experimental researches on the segmental innervation of the skin in dogs.*" By Prof. C. WINKLER from researches made in collaboration with Prof. G. A. VAN RIJNBERK. (VIth Communication).[1])

On form and situation of the dermatomata of the posterior extremity.

It has been for some years now that we have been occupied by attempts to obtain some insight into the manner in which the dermatomata are ranged on the posterior extremity in dogs.

These researches have been made partly at the Laboratory for Neurology in Amsterdam, partly at the Laboratory for Physiology of Prof. LUCIANI in Rome.

Albeit we were acquainted with the work of TURCK, SHERRINGTON, BOLK and others, and though we took from their researches the startingpoint for our experiments, still it has taken a long time before we obtained any reliable result, because we were not prepared for so great a variability in the innervation of the skin as we found.

The first difficulty that presents itself, is of course the definition of the boundary between the posterior extremity and the trunk.

1) The 5 preceding notes are printed in *Proc. Kon. Akad. v. Wetensch. te Amsterdam* 1901, vol: IV. p 266, p. 308, p. 508, and 1903 vol. VI p. 347, p. 392,