Physiology. — "*A direct proof of the impermeability of the bloodcorpuscles of man and of the rabbit to glucose*". By S. VAN CREVELD and R. BRINKMAN. (Communicated by Prof. H. J. HAMBURGER).

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1. *Introduction.*

The question dealing with division of glucose between the red bloodcorpuscles and the bloodplasma, which has been discussed so often already in the literature, has come to the front again through recent research.

In 1919 one of us together with Miss E. VAN DAM published a series of researches [1]) which clearly demonstrated that the permeability of the bloodcorpuscles for glucose is intimately related to the process of coagulation, and that the bloodcorpuscles of the frog and of man are found to be impermeable to gluclose only when the earliest incipiency of coagulation has been prevented. In the case of the frog the physiological impermeability could be shown by direct chemical analysis.

Such a direct chemical proof could at that time not be given for the human bloodcorpuscles. In the osmotic experiments these bloodcorpuscles were invariably found to be impermeable in cases where the blood had not yet coagulated, and it was held that all the authors who had found the bloodcorpuscles to be permeable to sugar had used blood of which the commencement of coagulation had not been prevented.

Shortly after this publication there appeared an article by W. FALTA and M. RICHTER-QUITTNER [2]) on the distribution of sugar, chlorides and residual-N between plasma and bloodcorpuscles in the circulating blood. Also these investigators came to the conclusion that in man the sugar in the blood occurred only in the plasma. The method used by them could be considered as a direct chemical one. They determined the amount of sugar in the blood as a whole and in the plasma, and from these two values calculated the volume of the bloodcorpuscles, taking for granted that all the sugar occurred in the plasma. The volume of the bloodcorpuscles found in this way corresponded in a large number of cases to that determined in the haematocrite.

The results of the Austrian investigators have, during the past year, been contradicted from different quarters by others who had used the same method, but had come to opposite results [1]). This did not surprise us seeing that FALTA and RICHTER-QUITTNER had used hirudine to obtain the bloodplasma. Before them, however, several other investigators had already used hirudine blood and had found the bloodcorpuscles to be permeable.

The explanation of this we thought could be sought in the fact that hirudine does not prevent the first phases of coagulation. Only after this had been prevented in another way it was found by the osmotic experiments that also in the hirudine blood the bloodcorpuscles are impermeable to sugar [2]). If FALTA and RICHTER-QUITTNER in spite of using hirudine blood had obtained the same results, then, we thought, it was to be attributed not to the hirudine but to the separation of the plasma and bloodcorpuscles by direct and rapid centrifugalisation. Whether, by setting to work in this way, the bloodcorpuscles are indeed found to be impermeable to sugar is, however, still subject to grave doubt owing to the many failures of experiments done with hirudine blood by others.

The great theoretical and practical value of the question under discussion demands however direct chemical proof which can be regarded as being absolute. Also this we think cannot be said of the experiments of FALTA and RICHTER-QUITTNER.

According to our train of thought such direct proofs could be given only by examining plasma which was free from bloodcorpuscles and which had been drawn directly from the bloodvessels, or had been obtained outside the body from blood which had remained perfectly fluid without the addition of a single one of the substances which prevent coagulation, for these, after all, do not prevent the first phases of coagulation. The amount of sugar in the plasma ought, if the bloodcorpuscles were impermeable, to be able to be calculated approximately from the total amount of sugar in the blood, and the volume of the bloodcorpuscles [3]).

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[1]) BRINKMAN and v. DAM. Arch. Intern. de Physiologie XV. 105. 1919.

[2]) FALTA and RICHTER—QUITTNER: Biochem. Zeitschr. **100**. 140. 1919.

[1]) See f. i Biochem. Zeitschr. **107**. 246 and 248. 1920.

[2]) BRINKMAN and v. DAM l.c.

[3]) We say "approximately" because we want to take for granted for the time being that the blood corpuscles have a share in the socalled restreduction. This is however very small according to the investigation of R. EGE (Biochem. Zeitschr. **107**, 229, 1920) when determined by the Bang-method which we used.

We have succeeded in obtaining plasma in both of these above-mentioned ways, first from the rabbit and afterwards from man, and in subsequently demonstrating in a direct chemical way the impermeability of the bloodcorpuscles towards glucose.

II. *Determination of the amount of sugar in the bloodplasma of the rabbit obtained from a vein isolated from the body.*

To obtain blood-plasma from a blood-vessel our primary idea was that we could make use of the property of the bloodcorpuscles of female animals (especially pregnant ones) of settling rapidly compared to those of male animals [1]). Accordingly we several times clamped the marginal vein of a she-rabbits ear which did not show apparent anastomoses, the rabbit being bound on a rabbit plank and the ear in question held vertically. We did not succeed, however, in obtaining sedimentation in this way, probably because, after all, there still existed small anastomoses on account of which the blood could still circulate in the clamped vein.

By another method, however, the desired result was obtained with the same animals.

ARTHUS [2]) has found that when blood is kept in a vein which is taken from the body and ligated at both ends, the blood remains fluid in this vein, and, what is of great importance with regard to the question under discussion, shows no glycolysis. This method for obtaining uncoagulable plasma has, practically speaking, up to the present been followed only with the jugular of the horse and is therefore known as the jugular-method.

We have applied it twice to obtain pure plasma from rabbits. Here we set to work in the following way: The jugular on one side was laid open over a length of at least 4 cms. and dissected free from the neighbouring tissues and the greatest length between two of its confluent veins was doubly ligated at both ends. This part which was $\pm$ $2^1/_2$ cms. long in both cases was then removed from the body and held vertically. Seeing that it would take too long to wait for the bloodcorpuscles to settle down when the vein was hung up we placed it in a small centrifugal tube in which the vein just reached to the bottom, and centrifugalized rapidly. After some minutes there could be seen through the wall of the

[1]) FÄHRAEUS. Biochem. Zeitschr. **89**. 355. 1918.

[2]) ARTHUS. Arch. de Physiologie 1891—1892.

vein the distinct division between the dark mass of bloodcorpuscles and the pale yellow translucent plasma. The bloodvessel was then ligated in the lowest layer of plasma and a prick hole made in the top portion from which the oozing plasma was caught up on two pieces of Bang's paper. In both cases we obtained enough plasma to enable us to make a reliable double determination of the plasma sugar. Simultaneously with this blood was drawn from a vein in the ear of the same rabbit and in this the amount of blood sugar and the relative volume of blood corpuscles was determined. The result of both experiments was the following:

	a. Plasmasugar.	*b.* Total bloodsugar.	*c.* Volume of bloodcorpuscles.	*d.* Amount of plasma sugar calculated from *b* and *c*.
Exper. I.	0.266 %	0.194 %	27	0.2657 %
» II.	0.255 »	0.1935	27	0.265

We have therefore obtained with the jugular-method the result almost surprisingly accurate and accordanto that *in the case of the rabbit the blood sugar occurs almost exclusively in the plasma.* It can be remarked here still that both rabbits which were operated upon under a light ether anaesthesia showed a pronounced hyperglycaemia. This *hyperglycaemia* could therefore be reduced totally to a hyperglucoplasmia.

III. *To show the impermeability of the bloodcorpuscles of man towards sugar by the paraffin method.*

To investigate also in the case of man the impermeability of the blood corpuscles towards sugar along directly chemical lines we first used the vein method for obtaining the plasma. Upon the advise of Prof. HAMBURGER the umbilical cord was used as human vein. Through the condencension of Prof. NIJHOFF and the house obstetricians of the obstetrical clinic in Groningen we had for some weeks at our disposal perfectly fresh umbilical cords. We tried repeatedly to bring about in pieces of umbilical cord a division between plasma and bloodcorpuscles in the large vein which could not always be traced distinctly, because this vein could only with great difficulty be dissected free from the neighbouring tissue with which

it was intimately connected. Neither by centrifugalizing in suitable tubes nor by hanging up vertically pieces of ligated cord did we succeed in this however in more than a few cases. The strong contortions of the most umbilical cords and the consequent twistings were the chief reasons for this. Only once up to this did we succeed in bringing about in a cord with few contortions, the division and comparing the plasma sugar with the quantity of sugar in the blood of the cord as a whole and the relative volume of the blood corpuscles. The concentration of the sugar in the plasma was found to be markedly higher that that of the blood in the cord.

It appeared that some of the large veins which are constantly found on the surface of the foetal side of the placenta could be more easily isolated and then centrifugalized. In these the blood remained fluid for a markedly long time. Also in these cases we can up to this boast of only one reliable determination for comparing the plasma and the blood as a whole. This however also proved to be in favour of the plasma. We have not been able to make a sufficient number of determinations by this method to come to a conclusion through them whether human bloodcorpuscles are permeable or impermeable to sugar.

We succeeded in doing this in the meantime by another method viz the paraffin method; one way of keeping blood uncoagulated without adding one of the known substances is by collecting it in tubes which have been thoroughly cleaned and then waxed to make them perfectly smooth. By using thus small and narrow waxed tubes the blood collected in them can by rapid centrifugalization be divided into its corpuscular and plasmatic parts which takes place without the occurrence of coagulation. In larger tubes coagulation took place fairly regularly during the process of centrifugalization. The way in which plasma was obtained now was very simple.

From a carefully cleaned finger tip in which a deep prick was made with a needle, we allowed a few drops of blood to fall into two tubes which had been waxed shortly before the experiment. These were then rapidly centrifugalized for a period of from one to two minutes and the plasma then sucked off by means of a waxed pipette and dropped on BANG's paper. At the same time blood was collected for the determination of the total amount of bloodsugar and the relative volume of the bloodcorpuscles.

From a number of these experiments conducted with different persons at different times of the day the following results were obtained:

Total bloodsugar.	Sugar in the plasma.	Volume of the bloodcorpuscles.	Calculated sugar of the plasma.
0.117 %	0.178 %	38 %	0.188 %
0.103	0.161	39	0.169
0.110	0.165	38	0.180
0.135	0.223	43	0.237
0.137	0.192	38	0.221
0.111	0.188	41	0.190

In our opinion these results afford a direct chemical proof that the bloodcorpuscles of man are free from sugar.

This decision will have to be taken into account in clinical examinations so that besides the determination of the amount of sugar in the whole of the blood the volume of the bloodcorpuscles will have to be determined.

The contradiction which we find with the many authors on this subject [1]) we hold has its origin in the following facts which we will return to in extenso later:

1. Only in blood in which no signs of coagulation have appeared, we find the bloodcorpuscles free from glucose (hirudine and other substances which are supposed to make the blood uncoagulable, do not arrest the very first phases of the process of coagulation).

2. The existence of a glucose-colloid-compound must be taken into account.

3. Experiments which purpose the examination of the permeability of the bloodcorpuscles towards glucose with the aid of the introduction of fresh glucose must be judged with great care because the relative permeabilities of the α- and β-modifications of the α-glucose which result on solution of the latter are by no means equal [2]).

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[1]) See f. i. FALTA en RICHTER-QUITTNER. l.c.
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[2]) HAMBURGER. Proceedings of the Royal Acad. of Sciences XXI. N°. 4, XXVIII p. 318 and 327.