CVI. SOME EXPERIMENTS AND REMARKS ON THE POSSIBLE TRANSFORMATION OF *d*-GLUCOSE IN THE INTESTINE AND ON THE NATURE OF BLOOD-SUGAR.

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IN this article are communicated the results of experiments on carbohydrate-metabolism, which are closely connected with those recently published by a number of English investigators. These experiments deal, namely, with the possible transformation which the stereoisomeric glucose-forms, present in a solution of *d*-glucose in equilibrium, undergo in contact with the intestinal mucosa, and with the nature of blood-sugar.

I began these investigations at the suggestion of Prof. Hamburger as early as July, 1922. At first it was not my intention to communicate the results of these experiments yet, though their number in both problems was a large one. However, as I shall not have opportunity to continue my experimental work on these and allied subjects in the nearest future, I give here a short account of my work, especially as I am informed that the publication of the results reached up to the present may be of value for others, and also, as in more than one respect the methods used are different from those followed by the English investigators.

The reason for undertaking the experiments on the subjects mentioned, was the results of the investigations of Prof. Hamburger and Dr Brinkman on the permeability of the kidney towards isomeric and stereoisomeric sugars, which were published in different papers [Hamburger, 1919, 1, 2; 1921, 2; 1922, 1; Hamburger and Brinkman, 1918, 1], and discussed by Prof. Hamburger in two lectures [Hamburger, 1921, 1; 1922, 2]. The results of these investigations seemed to prove that the permeability of the kidneys for the stereoisomeric forms of the same sugar could be quite different.

Of these numerous experiments I wish to draw attention here only to those concerning the partial retention of the kidney for *d*-galactose [Hamburger, 1921, 1, 2; 1922, 1, 2], which was always about 50 %. Therefore Hamburger concluded that the only reason for this partial retention could be the fact, that of the two modifications of *d*-galactose in solution, namely the α - and the β -variety, one form is retained by the kidney, the other form is not held back.

Similar results were obtained with the two varieties (α - and β -) of another mutarotatory sugar, *l*-xylose. Here 25 % was always retained.

To study the mechanism of this difference, a number of experiments were made. In addition the question was put whether the difference in permeability, found between the isomeric and stereoisomeric forms of the same sugar, might also have a practical significance for the diabetes problem. That means, the question was introduced by Hamburger, whether the quite different behaviour of the normal and diabetic organism towards glucose had as underlying principle a stereoisomeric difference between the glucose of the blood under normal and pathological conditions [Hamburger, 1922, 2].

In the literature we find a few examples of a different behaviour of the organism—of the normal as well as of the diabetic—towards the stereoisomeric forms of the same sugar. Thus a difference was found in the behaviour of the human body towards different stereoisomeric methylglucosides [Lit. cited by Neuberg, 1911]. But that really in the normal organism one special stereoisomeric form of glucose is preferred, seemed to be demonstrated by the well-known experiments of Hewitt and Pryde [1920]. They seemed to indicate with some certainty, that normally by the resorption of carbohydrates in the intestine these are not only all transformed into glucose, but principally into one definite stereoisomeric form of glucose of a very reactive nature and with a negative or weak positive specific rotation (γ -glucose?). They would also show us that we might expect the possibility of the presence of such a reactive sugar, which is in equilibrium with *d*-glucose, in the blood. It is well known to the readers of this *Journal*, that in the recent investigations of Winter and Smith [1922; 1923, 1, 2, 3, 4; also Forrest, Smith and Winter, 1923] the existence of such a reactive sugar in the normal blood is assumed to be very probable.

As both questions, the transformation of *d*-glucose in the intestine and the nature of the blood-sugar, were fundamental and might be closely connected, Prof. Hamburger advised me to take up the problem of the stereoisomeric sugars. At his suggestion a beginning had already been made with a repetition of the experiments of Hewitt and Pryde. I have continued and extended these experiments.

In the first series of experiments in our laboratory only narcotised rabbits were used and aqueous solutions of very pure glucose in equilibrium of a strength of 2 to 3 %.

As the results reached in these experiments wholly correspond to those just published in detail in this *Journal* by Stiven and Reid [1923], I may refrain from a detailed statement of our results. This especially as our technique, as regards filtration and care for accurate polarimetric readings, largely corresponded to that followed by these authors.

About the procedure I only wish to say, that we worked so quickly, that the first polarimetric readings of the clear filtrate were always made within three minutes after the liquid had been removed from the intestine. The polarimeter which we used, in these and the following experiments, was a two-field instrument with which readings to a maximal accuracy of 0.01° could be made. The readings in these experiments had in nearly all a maximal error of 0.04° . The solution remained in contact with the mucosa from 5–15 minutes.

For washing the loop of intestine before it was filled with glucose, we used in two experiments Tyrode's solution made up without glucose, and in four experiments a modified Ringer's solution of the following composition: NaCl 0.7, KCl 0.01, NaHCO_3 0.2, CaCl_2 6 aq. 0.02 %.

Before the beginning of the experiment we passed CO_2 through this solution till $[\text{H}^+]$ was $0.45 \cdot 10^{-7}$ (determined with neutral red as indicator). In this way we had a solution of which the $[\text{H}^+]$ and $[\text{Ca}^{++}]$ correspond to those of the serum of the animal used, and which, from the well-known experiments on the retentive power of the kidney for sugar [Hamburger, 1919, 1, 2; Hamburger and Brinkman, 1918, 2], and from those on the resistance of the red corpuscles [summarised by Hamburger, 1922, 3], may be regarded closely to approach the normal physiological fluid.

I give here as illustration only the shortened report of one experiment.

Exp. A. Rabbit, weight 2500 g. No food was given in the last 24 hours before the experiment began.

Morphine-anaesthesia; 60 mg. HCl-morphine intravenous.

Glucose solution of about 2 %, prepared 18 hours before the beginning of the experiment; rotation in 20 cm. tube $2^\circ.04$ (mean of six determinations).

Beginning operation five minutes after morphine injection.

Intestine loop used, directly behind stomach, of about 40 cm. length, tied off, two canulae in the ends, washed out with Ringer fluid at body-temperature, then with glucose solution and filled with the latter carefully. Glucose in intestine from 10 h. 30–10 h. 44. At 10 h. 44 content of intestine loop filtered and filtrate investigated in 10 cm. tube.

10.45	+1.30°	10.54	+1.28°
10.48	1.32	11.07	1.26
10.49	1.26	11.21	1.26
10.51	1.29		

No alteration after addition of a trace of alkali.

It is seen, that in this case the first reading (in the table is always given the average of several readings) was made within one minute after the liquid had been removed from the intestine. The difference between the first and the following readings was in this and also in the other cases within the limits of permissible error.

Altogether six of these experiments with narcotised rabbits have been done, but like Stiven and Reid, who have done a much larger number of experiments, we have in no case obtained results of the same nature as those of Hewitt and Pryde.

In two of the six experiments we determined the reduction of the filtrate immediately and also after a few hours, without finding a difference of importance. Compared with the polarimetric value, the reduction, determined by Bang's method, was constantly a little higher.

We have repeated these experiments under conditions which in my opinion may be called more physiological than those with the narcotised rabbits; namely, we made use of a dog, in which I had made a *Vella-fistula* of the small intestine (the length of the intestine loop was about 40 cm.). With this dog I have repeated the experiments of Hewitt and Pryde, now of course without anaesthesia, at least 25 times, but, as may be said directly, also with negative result.

In these experiments the fistula was first washed out with the usual Ringer or Tyrode, heated to 37° . We then injected the 2 % solution of glucose in equilibrium, also heated to 37° , and at a certain moment, whilst one person injected the solution slowly into one end of the fistula, another closed the other opening with his fingers. Then the injection-canula was removed and the other opening also closed with the fingers. The dog was a very quiet one and with the openings of the fistula firmly closed with the fingers, remained standing for the whole length of time that the fluid remained in the intestine, *i.e.* for 5–10 minutes. Generally about 50–60 cc. of the glucose solution was in the intestine loop at the moment it was closed. At the end of the determined time we could not wait till a peristaltic action of the bowel removed the unresorbed liquid and therefore it was washed out with a quantity of the modified Ringer or

Tyrode. The liquid obtained was filtered directly and the clear filtrate investigated in the polarimeter tube at a temperature of 37°, although in the first four experiments we made the readings at room-temperature just as in the preceding experiments. The observation-time in the experiments with the dog was much longer than in those with the rabbits.

I give here as illustration a shortened report of one of these experiments, which may serve as a good example of the general results obtained in all. It is seen that also in this experiment we did not get results similar to those obtained by Hewitt and Pryde, though the conditions were, I believe, much more physiological.

Experiment 1 with fistula dog.

Glucose solution of about 2 % in equilibrium. Fistula loop washed out with Tyrode, made up without glucose.

3.21-3.29 glucose solution is in the fistula.

3.29 fistula is washed out with Tyrode.

Polarimetric readings in tube of 10 cm.

3.315	+ 0.74°	3.48	+ 0.70°
3.325	0.70	3.485	0.70
3.345	0.72	3.495	0.67
3.37	0.70	3.53	0.70
3.385	0.68	4.08	0.72
3.40	0.68	4.09	0.71
3.415	0.66	Next morning at 9.15 + 0.68.	
3.44	0.67	After addition of trace of alkali no change.	
3.45	0.68		

After we had read the experiments of Winter and Smith, which will be shortly referred to later on, we thought of the possibility, that *insulin* might play a rôle in the transformation of glucose in the normal intestine and that insulin was absent from the mucosa of the fistula loop. We have therefore, in four experiments with the fistula dog, added a trace of insulin to the glucose solution in equilibrium brought into the intestine, although our experiments in this direction with rabbits had also given negative results. However in these experiments we again did not get indications of mutarotation.

In the experiments with the dog we also very often compared the reduction and polarimetric value of the filtrate directly (within a few minutes after the clear filtrate had been obtained) and after one or two hours, once also after 24 hours. Here again we found no difference of importance.

Although I have often tried by altering the details in the various experiments (small variations of the p_H in the modified Ringer used, etc.) to get a positive result, that is to say, to observe that the liquid obtained from the intestine showed an upperwards tending mutarotation of importance; yet, as already mentioned, and to my regret, like Stiven and Reid, I was never able to manage it, let alone to get a negative reading such as Hewitt and Pryde reported they had obtained once.

In the meantime we had also commenced to study the problem of *the nature of the glucose in the circulating blood*, since dealt with fully by Winter and Smith [1922, 1923, 1-4; see also Forrest, Smith and Winter, 1923]. We expected from the beginning that if the blood-glucose consisted mainly of one stereo-isomeric form of glucose and that if we tried to investigate this by using blood itself, there was reason to expect a rapid change to the α - β -equilibrium; as the

blood had to be deproteinised before the reduction or optical rotation could be determined, which takes time. Then, if the deproteinisation is done chemically, this in itself might have an influence and invalidate the experiment¹. Looking for other possibilities, we thought of the *aqueous humour* and of the *serum ultrafiltrate*; the first of these had been previously used in researches of this laboratory several times [van Creveld, 1921; de Haan and van Creveld, 1, 2].

For the problem in question, the aqueous humour, which may be regarded as an ultrafiltrate or better as a *vivo*-dialysate of the blood-plasma, has several advantages: it is colourless, water-clear, does not clot (the primary liquid at least) and contains only a trace of protein. It is therefore possible to determine reduction and polarimetric value of the liquid of the eye-chamber for the first time directly after puncture. A drawback was of course that the amount of glucose in the normal chamber liquid is only small, smaller than in the blood-plasma [de Haan and van Creveld, 1921, 1, 2]. Notwithstanding this disadvantage we thought it to be of great importance to see in how far with the eye-chamber liquid, which was not treated in any way, indications about the stereochemical forms of glucose in the blood under normal and pathological conditions might be obtained. We made the puncture of the cornea in the same way as in former researches with aqueous humour, namely with a sharp-pointed glass capillary, and in the upper part of the cornea, after cocaineisation with 2 % HCl-cocaine. From each rabbit's eye about 0.3 cc. may be obtained.

First we looked for a qualitative test of the presence of a definite stereo-isomeric form of glucose. As such the *decolorisation of permanganate* may be regarded, as it may point to the existence of a reactive sugar. We compared the rate of decolorisation of a 1/100 *N* weak alkaline solution of potassium permanganate by the eye-chamber liquid at different times after the puncture.

In these experiments with normal animals we found that, whereas *the addition of the eye-chamber liquid immediately after the puncture caused a rapid and intensive decolorisation of the permanganate*, this became slower the longer the time that elapsed between the time of puncture and the moment of addition to the permanganate. Using a proportion of 2 cc. *N*/100 KMnO_4 to 0.2 cc. eye-chamber liquid I found that after 25 minutes to half an hour, the rapidity of decolorisation had decreased in a high degree and was then not much larger than that of a 0.5% solution of glucose in equilibrium, used for comparison. There is a possibility that the rapid decolorisation of permanganate by the fresh eye-chamber liquid is due partly to the presence of auto-oxidisable substances. It is difficult, however, in my opinion, thus to explain the whole decolorisation. The relatively slow decrease of the power of decolorisation is in any case difficult to account for in this way and it is also of importance to mention at this point that the reduction and the refractive index of the eye-chamber liquid, determined at different moments during the first hour after the puncture, appeared not to decrease. The change in the behaviour towards permanganate might thus surely be in favour of the presence of a reactive sugar in the normal aqueous humour.

To find out in how far other findings were in accordance with this, we investigated the polarimetric value of the aqueous humour during the first hour

¹ Compare here also the remarks of Hewitt [1923].

after the puncture. Here we made use of the micropolarisation method as described by E. Fischer [1911]. This was necessary, as the results with the permanganate test seemed to us to indicate that if the eye-chamber liquid showed mutarotation, the changes might take place during the first half-hour after the puncture. We therefore worked as quickly as possible, using only a small quantity of liquid, obtained from one or two eyes. Waiting till we had collected sufficient liquid from several rabbits to fill a common polarimeter-tube would have taken too much time.

Making use of the figures obtained in a former research, concerning the average sugar-content of the normal aqueous humour of the rabbit, we calculated whether the possible mutarotation of this liquid could be determined with sufficient accuracy. We found that, accepting that the specific rotation of the greater part of the glucose present in the eye-chamber liquid was low and that outside the body a specific rotation of 52.5° could be reached, we then should need at least a 10 cm. tube to be able to investigate the existence of a mutarotation accurately enough, although we should be unable to determine this quantitatively. For technical reasons, tubes longer than 10 cm. cannot be used for micropolarisation. Therefore we lack the ability of reading off a greater rotation and herewith the chance of determining a possible mutarotation with greater nicety.

The tubes were filled with the aqueous humour by means of a glass capillary, as soon as possible after the puncture of the cornea had been made. The first readings were usually made within one or two minutes after the punctures. All readings in these experiments were at room temperature. My eyes, as well as those of the persons who were kind enough to control my readings by making these readings by turns with me, were trained in looking through the polarimeter with the capillary tubes in it. The reading error with these tubes was, when we were well adapted, $0.02-0.03^\circ$.

I have done a large number of these experiments with the aqueous humour of normal rabbits. At first we only made as many readings as we could during the first half-hour after the puncture and then took a few readings after a drop of alkali had been added. In later experiments we continued for some hours to make readings at intervals of a few minutes. Because of the great number of such experiments we came to the conclusion, however, that the sugar content of the normal aqueous humour is so small that a possible mutarotation, even qualitatively, could not be determined by this method. We believe, it is true, that we have found with tolerable certainty a few times (in five experiments) that the optical rotation found during the first 5-15 minutes greatly increased, sometimes even to more than 100 % of the original value. That increase differed, however, too little from the reading error which we had fixed. This is possible, as the rotation—not of course the same in all experiments—amounted to an average of only 0.08° . We have never found a pronounced negative original rotation, though we found a few times a value of $0.00-0.02^\circ$, which apparently increased to $0.04-0.06^\circ$. Comparison of the reduction with the optical rotation has, within the bounds of the reading error, always shown a fairly good agreement. The addition of a trace of alkali to the aqueous humour brought about no distinct alteration in the optical rotation.

It occurs to me, that the doubtful result of the experiments mentioned

above need not be a reason why the use of aqueous humour for an investigation on the nature of glucose in the blood and especially of the stereochemical modifications should be given up. If it were possible to determine the optical rotation of the aqueous humour with greater exactness than is now possible, *e.g.* by using a polarimeter with an objective reading, then the doubt which, I must conclude, now exists concerning the mutarotation of the normal aqueous humour would surely be done away with. Perhaps the optical rotation of the aqueous humour might then also be determined *in vivo*.

The above mentioned investigation of normal aqueous humour was accompanied by a similar investigation of *diabetic* aqueous humour. For this we chose the *aqueous humour after adrenaline injection*. It is shown from the experiments carried out by de Haan and myself [1921, 1, 2] that the hyperglycaemia after subconjunctival adrenaline injection, shows itself only slowly in the aqueous humour, though the increase of the sugar content therein is also very great. In consequence of the results obtained in these experiments, I have now investigated in a number of cases the aqueous humour, two hours after a subconjunctival injection of about 0.75 cc. adrenaline, 1/1000. We also made a qualitative examination of the behaviour towards an alkaline solution of permanganate.

We expected a less rapid decolorisation in connection with the supposition that the diabetic sugar is chiefly a different (stereochemical) modification of glucose than the normal, and with the fact that the behaviour of the normal aqueous humour towards permanganate, as described above, seemed to indicate the presence of a reactive sugar in this liquid. It appeared, however, that *the same permanganate solution became decolorised much more quickly and more intensively by the aqueous humour of the adrenaline injected rabbit than by the normal aqueous humour*. Twenty minutes after the puncture the difference was still clearly to be seen. This difference is probably not to be explained simply by the raising of the sugar content of the aqueous humour, as might be deduced from the control experiments with glucose solutions at similar alkalinity. May we, then, perhaps assume that an equilibrium α - β -glucose $\rightleftharpoons$ reactive sugar (γ -glucose?), existing (as supposed by Winter and Smith) normally *in vivo*, is changed after adrenaline injection in the direction of the reactive sugar? The latter would not be in accordance with the hypothesis of Winter and Smith.

The polarimetric investigation, compared with the results of the reduction analyses, might possibly teach us something about this.

Before mentioning the results of such investigations, I wish to draw attention to the fact, that in the literature a test has already been described for distinguishing normal from diabetic blood, which also depends on the reducing properties of the blood (aqueous humour). This test is Williamson's test [Cammidge, 1913]. According to this, methylene blue in alkaline solution is decolorised by diabetic blood (and also by diabetic urine), but not by normal blood. This test, although not wholly in agreement with the difference found in behaviour of normal aqueous humour and this liquid after adrenaline injection, still points in the same direction as regards a possible quantitative proportion between α - β -glucose and the reactive sugar in the normal and diabetic blood.

The result of the polarimetric investigation, compared with the results of the reduction analyses of the eye-chamber liquid after adrenaline injection, have, however, in a large number of experiments, supplied no arguments at all in favour of such an hypothesis, and thus far are more consistent with what Winter and Smith find probable for diabetic blood. The optical rotation now remained, with only a single exception, within the limits of the reading error and this remained so from the moment of the first reading during some hours, after which observations were discontinued. Addition of a trace of alkali did not alter the optical rotation.

The increase of the sugar content, determined by the reduction method, was important in all experiments and in connection herewith also the optical rotation which corresponded closely with the reduction value. The average rotation was $+0.22^\circ$. A possible mutarotation of the same nature as formerly supposed might thus have been determined in every case with greater accuracy than in the normal aqueous humour.

The question arises whether any importance as regards a difference in the glucose modification, may still be ascribed to the difference found in the behaviour towards the permanganate of normal and of diabetic aqueous humour. Of course it is possible that disturbance of the equilibrium suggested above, really takes place, but that this is of such a nature, that it cannot be determined polarimetrically. However it may be, these observations teach us again, that one cannot be too careful in drawing conclusions concerning the nature of the glucose modification from the behaviour of the fluid under investigation towards permanganate.

After the experiments on the nature of blood-sugar, described above, were completed, the first publications of Winter and Smith on the same subject came to our notice. The results obtained by these investigators induced me to extend the experiments. The authors have compared the reduction and the polarimetric value of a concentrated protein-free glucose solution, obtained by special technique—which involves a chemical deproteinisation—from blood, under normal and diabetic conditions. In normal men and animals they found that, whereas the reduction of the solution during some days remained constant, the optical rotation increased in three to four days from an original low value, to a constant value, which corresponded with the specific rotation of the α - β -glucose and with the reduction of the solution.

On reading these investigations, we were struck at once by the *slow* change found by Winter and Smith: the equilibrium was only reached after some days. The possibility of such a slow alteration had never been obvious in examining the aqueous humour. This could, however, be connected with the small rotation of the normal aqueous humour that was only examined in a 10 cm. tube. We have therefore examined greater quantities of aqueous humour in ordinary polarimetric tubes of 20 cm. length, and we have extended the time of observation over 4–5 days. Thereupon, we examined *aqueous humour* (in tubes of 10 or 20 cm. length), *of which the glucose concentration was increased by concentration of the liquid in vacuo*. And, thirdly, we have extended our research to *ultrafiltrates*—either concentrated or not—*of blood serum and of artificial transudates*.

The greater quantities of eye-chamber liquid were obtained either by puncture of the eyes of several rabbits or from cows' eyes, brought fresh on ice from the abattoir.

For the ultrafiltration a large apparatus of Bechhold was used, with collodion filters of 3 % or 6 % strength. The artificial transudates were obtained by injecting 100–200 cc. NaCl 0.9 % or modified Ringer's solution, of which the composition was given above, into the peritoneal cavity and drawing off the unresorbed liquid, which now contains sugar, after some hours¹. From this liquid, one obtains a large amount of ultrafiltrate much quicker than from blood serum, and with about the same sugar content as the serum-ultrafiltrate possesses.

The advantage of using a concentrated ultrafiltrate above that of the concentrated aqueous humour lies herein, that the first-named can be kept absolutely free from protein, whilst by the latter with the increase of the sugar-content the trace of protein may also increase to a disturbing concentration. The advantage of the deproteinisation by ultrafiltration over the chemical deproteinisation as followed by Winter and Smith for the subject in question cannot be denied.

Of the various liquids here named we now compared the reduction and optical rotation for some days, and in a large number of experiments. During the time between the several observations the liquids were kept at low temperature.

I have been unable to obtain with the liquids I have used similar results to those which Winter and Smith described for the concentrated protein-free filtrates prepared from normal blood. Time and again I have found that the reduction of the examined liquids remained the same for 4–5 days and that there was also no alteration in the rotation. There was always a small difference between reduction and optical rotation in favour of the former.

We have also had the opportunity of testing an hypothesis suggested by Winter and Smith in connection with their own experiments and with those of other investigators. They have, namely, expressed the possibility that only the glucose form which in normal men and animals forms the greater part of the blood-sugar (the reactive sugar with a small positive or negative rotation) may be used in metabolism. Further, that in the production of this reactive form, insulin may play an important rôle. It seemed to us that the experiments made by Hepburn and Latchford in Prof. MacLeod's laboratory [1922] formed suitable material from which to get support for that hypothesis, and indirectly also for the nature of blood-sugar. These investigators have found, namely, that in perfusing the mammalian heart by Locke's method [Locke and Rosenheim, 1907], the addition of insulin to the perfusion-fluid caused a quantity of glucose to be withdrawn from the perfusion-liquid, which was four to six times as large as the normal quantity. In how far was this due to an increased formation of the so-called reactive form of glucose? We felt justified in putting this question after the well-known experiments of Clark [1916] and others, and of Winter and Smith themselves, although it was not proved that there is actually an increased sugar-consumption under influence of insulin.

Like Winter and Smith, I had found in *in vitro* experiments that insulin alone has no influence on the optical rotation of *D*-glucose in equilibrium. It was possible, however, that the effect found by Hepburn and Latchford was

¹ Compare Feringa [1922].

due to a co-operation of insulin and heart-enzyme *in vivo*, which resulted in an increased transformation of the glucose of the perfusion liquid into the reactive form. If such a process did not take place intracellularly, it might possibly be determined by investigating the perfusion liquid issuing from the heart.

In the perfusion apparatus, at a short distance under the heart, we have therefore fastened a T-piece, through which at any moment a quantity of liquid coming straight from the heart, could be obtained. The reduction and the optical rotation of this liquid were determined at 37°, with or without filtration through a Gooch filter. (When the heart-perfusion lasts somewhat longer, the perfusion liquid always becomes opalescent, as Locke has stated already.)

In the first two experiments, in which no insulin was added, it appeared that the liquid coming from the heart and investigated within one or two minutes afterwards, did not show mutarotation. Then we have in five other experiments, after the perfusion had lasted about one hour, added to the perfusion liquid bit by bit a small quantity of insulin, dissolved in a little perfusion liquid. In three experiments we have found distinctly an increase of the amount of glucose withdrawn from the perfusion liquid, as compared with the normal condition. In no case, however, did the issuing liquid show any mutarotation, either spontaneously or after addition of alkali.

How favourable were the conditions under which these experiments were carried out, may be seen from the following. We thrice observed, that after the heart had contracted regularly for about eight hours and then had stopped beating after the O₂-supply and the warming of the perfusing liquid had been stopped, and was left for about 20 hours at room-temperature, yet the heart began again to beat rather strongly, when the O₂-supply and the warming of the perfusion-liquid were again set going.

DISCUSSION.

In considering the experiments on the nature of blood-sugar described above and judging from these and comparing them with those of other investigators, I must conclude that the question of the stereochemical forms of glucose present in blood under normal and pathological conditions is not yet settled at all. I obtained in my experiments only very small indications about the existence of definite stereoisomeric forms of glucose in normal and pathological blood. Notwithstanding this, the principle of the question, namely, that in blood different stereoisomeric forms of glucose occur with a different biochemical significance, in our opinion need not be given up. The results obtained in those biochemical experiments in which more or less pure solutions of the stereoisomeric sugars were used, such as those of Hamburger [1919, 1, 2; 1921, 1, 2; 1922, 1, 2] and of Hamburger and Brinkman [1918, 1], and the investigations recently published by Willstätter [Willstätter and Sobotka, 1922] give, in our opinion, much support to this conception. That in the case of blood it is so extremely difficult to give direct proofs, may be partly due to technical difficulties, which might be overcome in future, and of which I could mention some.

Then we must remember that the chemical side of the problem is still very complicated. I tried from the beginning to get information about the sugar

of blood principally by comparing the reducing and polarising power of the liquids used, just as had been done by Winter and Smith. Our idea was that the polarimetric evidence alone would be sufficient. This has since been doubted, especially as far as the detection of the so-called γ -sugars is concerned, from the side of the chemists, whereas they also think it highly probable that γ -sugars play an important rôle in carbohydrate metabolism. If their conception is right, then of course we cannot expect to get more information about the presence of γ -sugars in blood as long as we do not know more about the properties and the detection of these sugars in the organism.

The difficulties also concern the question of the mechanism of the action of insulin, if it becomes more probable that this really is connected with a conversion of the stereochemical forms of glucose into each other. Until now, however, this question also seems not to be settled [MacLeod, 1923].

I hope that if the conditions are more favourable in every respect for further research, the methods and results described here may appear to be of some value in obtaining more information about the nature of the normal and diabetic blood-sugar.

SUMMARY AND CONCLUSIONS.

1. A repetition of Hewitt and Pryde's experiments on the transformation of *d*-glucose in the intestine, with narcotised rabbits and with a dog in which a Vella-fistula was made, has not given any evidence for stereochemical changes of the nature described by these authors.

2. To study the nature of blood-sugar, *aqueous humour* and *serum-ultrafiltrate* have been used. Investigated during the first half-hour after it had been obtained from the eye, the *normal* aqueous humour showed a rapid decolorisation of permanganate directly after the eye-puncture, which decreased in 20–25 minutes in a very important degree. With the technique used, a possible mutarotation of the normal unconcentrated eye-chamber liquid, even qualitatively, could not be determined with certainty.

Reduction and optical rotation of the normal eye-chamber liquid showed a fairly good agreement.

3. After (subconjunctival) adrenaline injection the rapidity of decolorisation by the aqueous humour is increased. With great certainty it may be said that after adrenaline injection the aqueous humour does not show mutarotation of any importance and that the optical value under these conditions closely corresponds to the reduction value.

4. Reduction and optical value of serum-ultrafiltrate, of concentrated serum-ultrafiltrate, of aqueous humour and of concentrated aqueous humour, compared during four to five successive days, may remain the same from the beginning of the experiment, and closely correspond with each other.

5. During the increased removal of glucose from the perfusion-liquid of the heart, observed when insulin is added, no sign of mutarotation can be observed in the issuing liquid, which could point to the formation of a "reactive" sugar.

In conclusion it gives me great pleasure to thank Prof. Hamburger for his constant stimulating help, criticism and advice in the course of these investigations.

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