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A COMPARATIVE STUDY OF THE ROTATORY AND REDUCING PROPERTIES OF PLASMA ULTRAFILTRATES FROM DIABETIC AND NEPHRITIC PATIENTS

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In a previous paper (1) a method for studying the rotatory values present in human blood plasma has been discussed, and a series of comparative reducing and rotatory values have been presented, recording the values obtained from normal individuals and hospital patients, exclusive of diabetics and nephritics. It was found in this series that the rotatory values of plasma ultrafiltrates, if expressed in terms of glucose generally proved to be lower than the reducing values and in fact, good agreement between the two was recorded in only a few instances. These recognized differences between the reducing and rotatory values were designated in the experiments as R-P values (the reducing minus the polariscopic value) and, in the previous series, were found to range between the equivalent of 0 and 0.130 gram of glucose per 100 cc., averaging about 0.040 gram per 100 cc. The rotatory values proved to be somewhat irregular, however, for in studying the ultrafiltrates over a period of seventy-two hours, fluctuations were noted, amounting to an average value of ± 0.022 gram of glucose, whereas the reducing values remained practically unchanged. These findings conform in general to those which have been reported by other investigators whose work has been reviewed in the previous communication.

At the outset, however, it is well to re-emphasize the fact that studies of the R-P values of blood plasma consist essentially in the measurement of a rather complex property of human blood plasma and not

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in the measurement of a substance. Furthermore we are dealing with a property which under the given conditions is in all probability somewhat labile and may be subject to mutarotatory phenomena and may be quite sensitive to certain environmental conditions, such as temperature, the hydrogen ion concentration, the presence of oxidizing or reducing agents, etc. Attention has been called to this fact by other workers in this field who have also pointed out that if we assume that the reducing power and the specific rotation of glucose in the blood is the same as that of an aqueous solution of α,β -glucose in equilibrium, the differences then between the rotatory and reducing values expressed in terms of glucose may be due either to the presence of reducing substances other than glucose, or to the presence of optically active substances other than glucose without giving a clue as to the nature of either type of substances. As a matter of fact both explanations seem to be in part responsible for the R-P values. In the case of the former it is recognized that small amounts of reducing substances which cannot be attributed to glucose may be present in blood. The exact nature of these substances has not been defined, although it is known that creatinine, creatine, uric acid, glycuronic acid, pentoses, disaccharides, purines and adrenalin have the power of reducing the reagents commonly used in blood sugar determinations, and, after the removal of glucose from blood by yeast fermentation or by the glycolytic action of whole blood, the residue of reducing substance has been found to be equivalent to 0.010 to 0.030 gram of glucose per 100 cc. (2). In the case of the other optically active substances it has been suggested that they may be represented by β -oxybutyric acid, amino acids and glycuronic acid (3), or by non-protein sulphur compounds (4) (5), all of which are known to exist in the blood. The quantitative data on this subject are as yet too limited to warrant a very profitable discussion as to their nature.

In extending the work in an effort to determine the nature and significance of these R-P values the number of determinations on clinical cases has been increased in the present study, for the immediate purpose of determining whether these values would show any degree of uniformity if classified according to the type of disease. Although the number of cases which are reported below is small, the findings show that in at least two types of disease, namely, in diabetic and in

nephritic patients with uremia, a certain degree of uniformity has been observed.

It is not within the scope of this paper to explain the general nature of the factors producing these differences between the reducing and rotatory power of the blood but rather to call attention to the values obtained. In the nephritic series, however, a note is made of an obvious factor which has been shown to be in part responsible for the differences.

Although there have been a number of studies in which comparisons between the rotatory and reducing values are recorded, the number of observations of this type, which have been made upon groups of pathological cases, appears to be limited.

Stepp (6) has reported a series of such determinations expressed in terms of glucose, upon a group of seventeen normal individuals, a group of fourteen diabetics in which some of the cases exhibited evidences of acidosis, a group of six nephritic cases, both with and without nitrogen retention, and a small group of five miscellaneous cases. This author worked with blood filtrates from which the protein had been precipitated with phosphotungstic acid and which had been subsequently concentrated by vacuum distillation.

In his original filtrates he reported large differences between the high reducing and the low rotatory values, his attention being largely confined to a discussion of the factors which promote these differences. He considered, however, that the rotatory values were a more accurate index of the true blood sugar content than the reducing values owing to the very appreciable presence of reducing substances other than glucose which could be removed by mere vacuum distillation or by the addition of neutral lead acetate. Although his observations do not easily lend themselves to an analysis such as the one given below, no appreciable degree of uniformity in the reducing-rotatory difference is detectable in any of the particular groups of cases which he studied.

A somewhat similar study of the differences between rotatory and reducing values in the blood of normal individuals and in diabetics has been reported by Winter and Smith (7). They found that after precipitating the blood protein and concentrating the filtrate in vacuo the rotatory values of normal blood filtrates, expressed as above, were considerably below the reducing values, but on standing, these rotatory values rose until in a day or two they had become equivalent to the latter. In diabetic individuals, however, the differences in rotatory and reducing values with a subsequent rise of the former were not observed.

Lundsgaard and Holbøll (8) have also studied the differences between the rotatory and reducing power in dialysates obtained from normal and diabetic blood and have reported findings which show the same general trend as those observed by Winter and Smith.

METHODS

The procedure, including the methods of obtaining the blood and filtering it through collodion sacs, which has been followed in the present study has been practically the same as that outlined in the previous communication (1). A slightly different technique was, however, employed in about half of the observations given below for the polariscopic determinations. The 189 mm. polariscope tube with a Mazda lamp and an appropriate dichromate solution filter as the source of light were replaced by a longer tube and a mercury vapor lamp using the green light with an arc of low pressure. A special polariscope tube measuring 241.8 mm. in length and holding 10 cc. of solution was constructed for this purpose, which utilized the maximum length allowed by the instrument and at the same time did not require the use of an excess of solution. The length of the tube was also gauged to facilitate the calculations for estimating the per cent of glucose which is derived by the well known formula:

$$\text{Per cent of glucose} = \frac{\text{Reading} \times 100}{\text{Specific Rotation} \times \text{length of the tube in dm.}}$$

By employing a wave length of 5461 Å, the specific rotation of glucose is 62.03° (9) giving the final calculation:

$$\text{Per cent of glucose} = \frac{\text{Reading} \times 100}{62.03 \times 2.418} = \frac{\text{Reading}}{1.5}$$

In determining the error by the use of this method readings were made upon a series of standard glucose solutions of a concentration of approximately 0.100 per cent. The readings on the solutions were frequently alternated with readings of the zero point. The average error of each individual determination actually proved to be about the same as that previously reported amounting approximately to ± 0.010 gram of glucose, although theoretically the increased length of the tube should increase the accuracy of the method.

The reducing determinations were run by the method of Folin and Wu (10).

In the previous communication, attention was called to the fact that the rotatory values observed in the plasma ultrafiltrates frequently showed fluctuations from day to day. These fluctuations represent an uncontrolled variable in our hands, but in order to attain a moderate degree of uniformity the rotatory readings, upon which the calculations in this study have been based, were in all instances made on the day following that on which the sample of blood was obtained and filtered.

All averages are followed by the standard deviation of the average calculated by the formula.

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

where σ = standard deviation

x = an observation

\bar{x} = the average

n = number of observations in the average

No averages have been considered as significantly different unless they differ by more than the sum of their standard deviations or by the square root of the sum of the squares of their standard deviations.

EXPERIMENTAL

Normals

The results of a small series of reducing and rotatory determinations with the corresponding differences between the two, expressed in

TABLE 1
Ultrafiltrate reducing and rotatory values obtained in 5 normal individuals

Number	Diagnosis	Reducing value	24-hour rotatory value	R-P
37	Normal	0.085*	+0.051	0.034
38	Normal	0.109	+0.007	0.102
40	Normal	0.086	-0.003	0.089
41	Normal	0.104	-0.028	0.132
42	Normal	0.082	+0.013	0.069
Minimum R-P value.....				0.034
Maximum R-P value.....				0.132
Mean R-P value.....				0.085 \pm 0.036

* All of the reducing and rotatory values in this and subsequent tables are expressed as grams of glucose per 100 cc.

terms of glucose as R-P values, which have been made upon a series of five adult normal individuals are shown in table 1. It will be noted that the rotatory values are appreciably below the reducing values in all five instances and the resulting R-P values are large varying from 0.034 to 0.132 gram per 100 cc., and averaging 0.089 ± 0.036 . These values are also graphically presented in figure 1, in which the rotatory values have been plotted against the reducing ones. It will be seen that the points all fall within a relatively small area, which is within the range of normal fasting blood sugar values. The relatively large R-P values are emphasized by the chart.

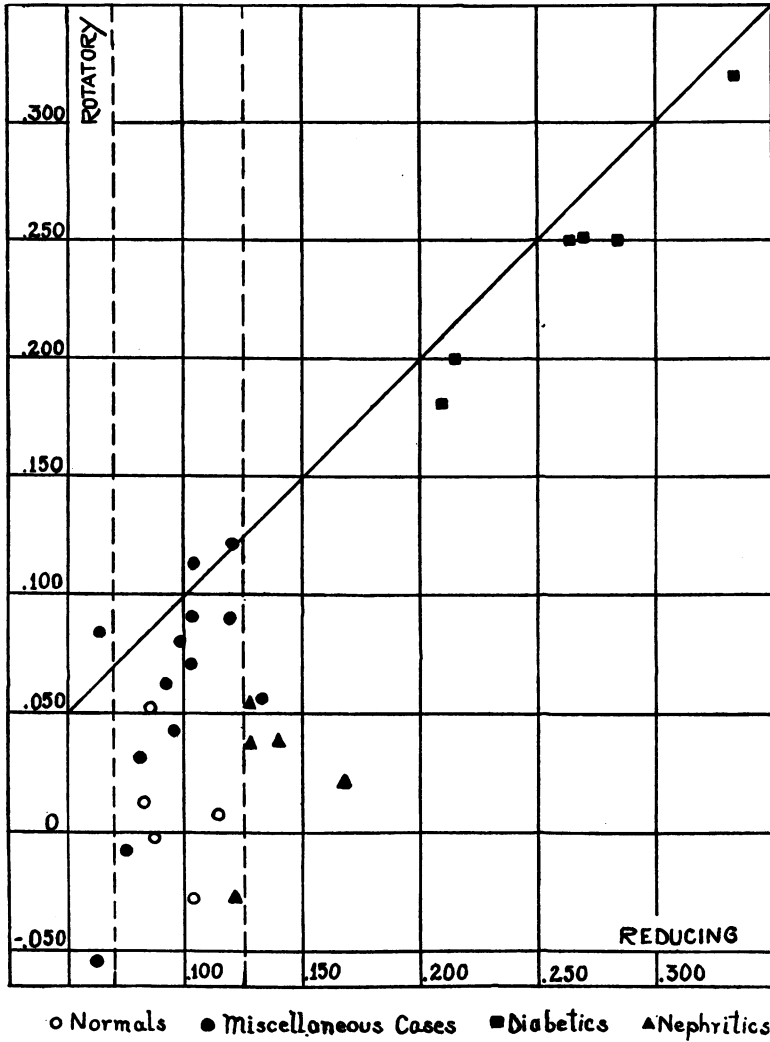


FIG. 1. THE DISTRIBUTION OF THE ROTATORY AND REDUCING VALUES FROM A SERIES OF BLOOD SAMPLES OBTAINED FROM NORMAL INDIVIDUALS, MISCELLANEOUS CASES, DIABETICS AND NEPHRITICS

The oblique line indicates the position of the points of exact agreement between reducing and rotatory values. The limits of usual normal fasting "blood sugar" or reducing values, are marked by the two vertical broken lines.

Miscellaneous cases

The reducing and rotatory values obtained from a group of thirteen miscellaneous cases which represent a variety of clinical conditions are shown in table 2 and graphically in figure 1. Three of the rotatory values are actually larger than the reducing ones resulting in negative R-P values. In general, the R-P values range from a minimum of -0.021 to a maximum of 0.116 with an average value of 0.031 ± 0.037 . Although the points shown in figure 1 cover a rather wide area, they fall for the most part within the range of normal fasting blood sugar values. A striking feature of the cases with higher reducing values is

TABLE 2

Ultrafiltrate reducing and rotatory values obtained in 12 miscellaneous hospital cases

Number	Diagnosis	Reducing value	24-hour rotatory value	R-P
51	Arteriosclerosis	0.102	+0.115	-0.013
52	Arteriosclerosis	0.064	+0.085	-0.021
35	Pyelitis	0.092	+0.063	0.029
27	Cardiac decompensation	0.102	+0.071	-0.031
43	Cardiac decompensation	0.134	+0.105	0.029
62	Cardiac decompensation	0.061	-0.055	0.116
26	Lobar pneumonia, syphilis	0.102	+0.091	0.011
64	Lobar pneumonia	0.071	+0.031	0.040
74	Rheumatic fever	0.119	+0.090	0.029
28	Salvarsan poisoning	0.095	+0.041	0.054
20	Cirrhosis of liver	0.075	-0.008	0.083
67	Pyonephrosis, syphilis	0.118	+0.120	-0.002
68	Pyonephrosis	0.097	+0.080	0.017
Minimum R-P value.....				-0.021
Maximum R-P value.....				0.116
Mean R-P value.....				0.031 \pm 0.037

that the majority of them give rotatory values showing a close degree of approximation to the reducing values, a feature which will be subsequently shown to be quite characteristic of the diabetic series. This fact was so noticeable that the attempt was made to determine whether the group of individuals in which these small R-P values were recorded showed any common clinical features. Unfortunately, the amount of data which had been collected on these patients was small and our information is limited, but it seemed to be a noticeable feature

that all of the R-P values which were below 0.030 in this series occurred in patients who were showing evidence of vascular disease either syphilitic or arteriosclerotic. However, too many variables enter the problem to allow stressing this point which seems rather to be a feature warranting further investigation.

Diabetic cases

All of the diabetic specimens were obtained from ambulatory patients who were attending the Diabetic Clinic of the Out Patient Department of the Pennsylvania Hospital.² The values obtained in six of these diabetic patients are recorded in table 3, also in figure 1 by the points represented as small squares. It will be noted that the

TABLE 3
Ultrafiltrate reducing and rotatory values obtained from 6 diabetic cases

Number	Diagnosis	Reducing value	24-hour rotatory value	R-P
A	Diabetes (insulin treatment)	0.264	+0.250	0.014
B	Diabetes	0.208	+0.179	0.029
C	Diabetes	0.333	+0.320	0.013
53	Diabetes (insulin treatment)	0.270	+0.251	0.019
56	Diabetes (insulin treatment)	0.284	+0.248	0.036
60	Diabetes	0.214	+0.197	0.017
Mean R-P value.....				0.021 ±0.009

specimens were obtained during stages of moderate hyperglycemia in which the reducing values range from 0.214 to 0.333. The consistently close approximation which the rotatory values bear to the reducing values is a striking feature of this series. The R-P values are uniformly small, averaging 0.021 ± 0.009 mgm. per 100 cc. The distribution of the points in relation to the line of equality between rotatory and reducing values is emphasized by figure 1.

This finding of the close approximation between rotatory and reducing values in diabetics has been previously noted by Winter and Smith (7) and Lundsgaard and Holbøll (8). Considerable literature has arisen with regard to its significance. It is not the purpose of this article to discuss the significance of the finding but merely to call

²I am indebted to Dr. E. S. Dillon for the privilege of studying these patients.

attention to our confirmation of it, noting at the same time that some fairly low R-P values are also recorded in some of the miscellaneous, non-diabetic patients. It would seem therefore, that low R-P values, although characteristic of the diabetic series, do not represent a finding which is specific for that disease.

Chronic nephritis with uremia

Early in the course of this study it was noted that large R-P values were encountered in nephritic cases with uremia and consequently a small series of these cases was assembled for further study. This type of case, namely that of advanced chronic nephritis of arterio-

TABLE 4

Reducing and rotatory values obtained from patients with chronic nephritis (nephrosclerosis) and uremia

Number	Initials	Date	Plasma urea nitrogen	Plasma creati- nine	Reduc- ing value	Rotatory value	R-P
			<i>mgm. per 100 cc.</i>	<i>mgm. per 100 cc.</i>			
29	A. R.	April 20, 1926	179	25	0.140	+0.038	0.102
30	A. D.	April 27, 1926	277	20	0.121	-0.028	0.149
31	A. D.	April 28, 1926	277	20	0.168	+0.021	0.147
69	M. N.	May 20, 1927	150	16	0.127	+0.055	0.072
76	L. L.	July 6, 1927	210	16	0.127	+0.038	0.089
Minimum R-P value.....							0.072
Maximum R-P value.....							0.149
Mean R-P value.....							0.112 ±0.035

sclerotic origin (nephrosclerosis) was selected for investigation because the findings proved to be relatively consistent and because we were dealing with a clearly defined clinical entity, in which we succeeded in confirming the diagnosis at necropsy in all of the cases recorded. Furthermore the degree of intensity as evidenced by blood urea nitrogen and creatinine determinations could be easily followed. The studies were made during the stages of terminal uremia.

The results of this series of observations are recorded in table 4, and the points have also been charted as small triangles in figure 1. It will be noted that the R-P values tend to be large, ranging from 0.072 to 0.149 and averaging 0.112 ±0.035. In figure 1 the points

fall in a group which does not coincide exactly with the points registered by the control cases, due in some measure to the fact that the reducing figures occupy a higher range than the controls and also to the fact that the R-P values tend to be large.

The distribution of these points which have been obtained from the nephritic cases, suggests that reducing substances other than glucose may be more responsible for the increased R-P values in this group than in the others. The increase in the reducing power of the blood in cases with nitrogen retention is recognized. It has been shown to be partly due to the presence of reducing substances such as uric acid and creatinine which are present in increased amounts. Hiller, Linder and Van Slyke (2) have found that, after submitting normal blood to yeast fermentation or to the glycolytic action of whole blood, the residue of reducing substance other than glucose was equivalent to 0.010 to 0.030 per cent of glucose. They showed that in a series of five cases of chronic glomerulonephritis with nitrogen retention, four showed abnormally high amounts of total reducing substance and the non-glycolyzable residual reducing substances were increased to about the slight extent that might be expected from the retained uric acid and creatinine, most of the total reduction values being from 0.020 to 0.050 per cent. These authors noted, however, that the important part of the increase in reducing substances in the nephritic cases was due to material which when fermented or incubated lost its reducing power as does glucose.

As the observations just mentioned apparently have a very definite relationship to the R-P values, we have also determined the amounts of non-fermentable reducing substances in the ultrafiltrates from some of our nephritic cases.

The experiments were performed in the following manner. The reducing and rotatory values were first determined. To about 25 cc. of ultrafiltrate a small portion (about a fifth) of yeast cake was added, the suspension was agitated and placed in the incubator at 37° for one hour. The suspension was then refiltered through a collodion membrane and its reducing and rotatory value redetermined.

The results of a small series of such determinations on a filtrate of ox blood and on three filtrates from human nephritic blood are shown in table 5. It will be noted that the values obtained from the non-

fermentable residue are uniformly lower than those reported by Hiller, Linder and Van Slyke, but it is evident none the less, that they show an appreciable relationship to the creatinine content and that reducing substances which include creatinine and uric acid are probably responsible for a definite fraction of the large R-P values encountered in the nephritic cases.

It will also be noted that the R-P values after fermentation are less than those reported before fermentation. The factors tending to produce this latter phenomenon are unexplained and are in contrast to the results obtained after removal of sugar by the glycolytic action of whole blood where it has been shown that subsequent to this procedure the R-P values were increased (1).

TABLE 5

Rotatory and reducing values on animal and nephritic blood, before and after fermentation

Number	Before fermentation				After fermentation			
	Creatinine	R	P	R-P	Creatinine	R	P	R-P
Ox	1.8	0.127	+0.031	0.096	1.7	0.011	-0.040	0.051
A	9.2	0.184	+0.090	0.094		0.015	-0.060	0.075
B	17.0	0.083	-0.100	0.183		0.026	-0.130	0.156
C	19.8	0.048	-0.132	0.180		16.8	0.029	-0.085

Although it is a rough estimate it is noteworthy that if we assume that a maximum value of 0.030 will cover the average non-fermentable fraction of reducing substances in the uremic bloods we still obtain an average R-P value, which is far larger than the average value obtained in the series of miscellaneous cases. Interestingly enough, however, the average nephritic values minus 0.030 approximates the average R-P value obtained in the normal series.

Post mortem samples of blood

Owing to the occasional clinical difficulty of obtaining the large quantities of blood necessary for these determinations, a number of blood samples were secured quite soon (i.e., within two hours) after death and the reducing rotatory determinations were compared with the series of ante-mortem figures. It is to be emphasized at the start, however, that a comparison of post-mortem analyses of this type with

the figures obtained during life should be made with caution as a variety of factors have to be considered which generally do not concern us in the interpretation of ante-mortem values. After death, the stabilizing influences which normally control not only blood volume, but its reaction, the non-protein nitrogen, the chloride content and a host of other substances, break down with considerable rapidity together with the appearance of autolytic phenomena. This breakdown does not necessarily await the death of the patient for it is recognized that the conditions of the terminal hours are often not comparable with those of ordinary life and during these terminal hours or even days a variety of protective and other mechanisms of the body may begin to fail.

The findings in this series of determinations proved to be fairly consistent and somewhat noteworthy. The reducing determinations were frequently found to be well below the range of normal fasting blood sugar values. The rotatory values showed still lower figures and a large per cent of them proved to be markedly levorotatory producing a corresponding increase in many of the R-P values (0.029 to 0.234). These values may be partially explained on the basis of glucose loss from the blood which we know occurs in varying degree at about the time of death (11). However, a notable feature of these observations proved to be that low reducing values were almost invariably associated with high R-P values.

In conclusion I wish to express my appreciation to Dr. J. H. Austin for his helpful criticism during the course of the experiments and to Miss E. F. Herr and Mr. S. L. Wright, Jr. for their assistance in the analytical work.

SUMMARY

A comparative series of the reducing and rotatory values as determined upon plasma ultrafiltrates have been recorded on a group of miscellaneous cases, a group of diabetics, and a group of patients with chronic nephritis and uremia. The differences between reduction and rotation have been expressed in these observations in terms of glucose as R-P values (i.e., the reducing minus the polariscopic values) and the range of these R-P values from blood taken ante-mortem has been found to be from -0.021 to 0.149 .

It has been shown in the group of normal individuals that the R-P values are moderately high (0.034 to 0.132). In the miscellaneous cases the R-P values cover a wide range (-0.021 to 0.116) although most of them are low. On the basis of a clinical classification it has also been shown that the two distinct groups of cases chosen for study, give a series of R-P values which are characteristically different for the two groups. In the group of ambulatory diabetic patients the R-P values proved to be uniformly low (0.013 to 0.036), a fact to which attention has been called by previous investigators (7) (8). In the group of uremic patients the R-P values tend to be high (0.072 to 0.149). This increase has been shown to be in part due to the accumulation in the plasma of increased amounts of reducing substances other than glucose such as creatinine, etc. However, the findings although different for the two groups are not specific, and values which are equivalent to those observed in some of the diabetic and in some of the nephritic patients are also recorded among the normal and miscellaneous cases.

It has been further shown that both levorotation and the R-P values are increased in the blood after death, a phenomenon which is frequently associated with low reducing values.

As a general rule, however, the antemortem R-P values have been found to deviate from the normal range more frequently in those cases in which the blood sugar or reducing values are outside the usual range. Furthermore, two distinct factors have been noted in these experiments as being correlated with abnormally high R-P values. One of these is the presence in the blood of an excess of reducing substance other than glucose, and the other is the presence of an associated hypoglycemia.

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