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Research Article

THE OPTICAL ACTIVITY OF GLUCOSE AS INFLUENCED BY NORMAL AND DIABETIC URINE

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INTRODUCTION

There have been several recent studies upon the influence which tissues and certain body fluids may exert upon the optical rotatory properties of various sugars. More than one investigator in this field has noted that certain changes occur in the optical rotation of glucose which has been brought into contact with body tissues or fluids, and has suggested that these changes may represent an important step in the preparation of this sugar for its utilization by the body.

The first recent important observation was made by Admont Clark (1), who found that on perfusion of the dog's pancreas with Locke's solution containing glucose in approximately physiological quantities the optical activity of this solution became slightly diminished, but its copper reducing power was unaltered. However, after acid hydrolysis this loss in optical activity was partially regained. No change in the optical activity was noted as the result of similarly perfusing the heart, spleen or kidneys. Furthermore, osazones were obtained from the pancreatic perfusate which had slightly lower melting points than glucazone but approached that of glucazone after acid hydrolysis. Clark concluded that these phenomena were due to an enzyme or enzymes obtained from the perfused pancreas which exerted a specific action on glucose, and was responsible for certain essential steps by which glucose was prepared for utilization by the body.

Another interesting set of experiments somewhat along the same line have been reported by Hewitt and Pryde (2). These observers have described the polarimetric changes occurring in glucose solu-

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tions which have been allowed to remain in contact with the intestinal mucosa of the rabbit for a few minutes. Following this exposure they observed mutarotary phenomena in the glucose solutions which consisted in a rapid diminution of the optical rotation of the sugar to values much lower than that of normal equilibrated glucose. Following the withdrawal from the intestine the solutions underwent a slower dextro-rotation to a permanent value corresponding with the specific rotation of α-β glucose in equilibrium.

More recently studies by Winter and Smith (3) have suggested that there may be an actual difference in the type of sugar which is present in normal and diabetic blood. These investigators called attention to the fact that in comparing the rotatory power of sugar obtained from the blood of normal and diabetic individuals a difference in the specific rotation was observed. They found that the sugar from the blood of normal animals and men, when examined after its separation by a rather lengthy process from the blood protein, showed a rotation of polarized light below that corresponding to the ordinary equilibrated α-β glucose. On standing, the optical rotation rose until in a day or two it became constant and agreed with the rotation that would be expected from the amount of glucose indicated by copper reduction determinations. In diabetic individuals, however, this diminution of optical activity and subsequent rise was not observed. It was suggested that these results might indicate the presence in normal blood not of the more stable varieties in which glucose exists in a simple solution, but of a less stable variety such as that identified by Irvine and his coworkers and styled γ glucose. These experiments were subsequently extended (4) to include the blood of diabetic patients who had been treated with insulin, and from these studies they concluded that in diabetics the decreased amount of blood sugar caused by the injection of insulin contained a greater proportion of normal blood sugar than that of the untreated diabetic.

Another interesting experiment has also been performed by these same workers (5) who have reported that when solutions of glucose and fructose are incubated in vitro at 37° in phosphate buffer solutions together with small amounts of insulin and liver extract, their

rotations were altered in a levo and dextro direction respectively, whereas the copper reducing power remained unaltered.

Recently, however, most of this work has failed to receive confirmation in the hands of other investigators. The experiments of Hewitt and Pryde have been challenged by Stiven and Reid (6), who have repeated their work and have been unable to confirm the former's results. Similarly van Creveld (7) has obtained negative results and also Hume and Denis (8) who report a series of 21 similar experiments in which they noted in 12 experiments no change in the optical activity of glucose which had been brought into contact with the intestinal mucosa of the rabbit, a small upward rotation in 5, and a somewhat greater downward rotation in 4, showing that unmistakable evidences of the existence of polarimetric changes were present in a large percentage of their experiments, but that these changes did not seem to follow any definite trend.

Doubt has also been cast upon the conclusions of Winter and Smith by Hewitt (9) and by Eadie (10) who repeated their experiments upon rabbits. Eadie is also quoted by Macleod (11) as having shown that in extracts of normal blood polarimetric readings are often obtained which are less dextrorotatory than they should be (as judged from their reducing power) and which slowly became greater on standing, but this instead of indicating the existence of y glucose, might have depended on the presence either of glucosides which gradually became hydrolyzed on standing, or of traces of other levorotatory substances which gradually became destroyed. It is also pointed out that the results of Winter and Smith rest upon polarimetric readings which were extremely small in magnitude, and furthermore, that the change in optical activity required a time interval amounting to several days which would not be expected if this were due to a highly reactive type of sugar.

Another investigator who has attacked the same problem and who has in some measure repeated Winter and Smith's work is van Creveld (7). He eventually abandoned the lengthy methods of deproteinization of blood as advocated by Winter and Smith, choosing instead to work with the aqueous humor of the eye, serum ultra-filtrates and artificially produced transudates. With the aqueous humor

he noted that reduction and optical rotation determinations showed close agreement and mutarotation could not be detected. With the serum ultrafiltrates and transudates no changes were noted in optical rotation, but there always was a small difference between optical rotation and reduction in favor of the former.

Visscher (12) has also repeated Winter and Smith's experiments and reports that the supposed differences between normal and diabetic blood which they have noted could be produced by varying the H ion concentration of the blood filtrate. If the filtrate was nearly neutral it resembled normal blood, if strongly acid diabetic blood. He also suspected that optically active substances other than dextrose, which were not eliminated by the deproteinization of the blood, might play an important rôle in the observation. Quite recently Denis and Hume (13) in a careful and broad repetition of Winter and Smith's work have likewise failed to corroborate the latter's work.

Apparently, therefore, the balance of evidence obtained by the more recent investigators, who have been quoted above, seems to indicate that this is a rather sterile method of attack in our efforts to investigate dextrose metabolism. The field has not, however, been exhausted. Clark's original work does not seem to have been repeated and the proof or disproof of his theory is evidently of fundamental importance in our conception of the manner in which glucose may possibly be influenced within the body in preparation for its utilization.

With the thought that the urine might contain enzymes or other factors which are present in the blood of normal and diabetic individuals, it seemed interesting to study its effect upon added sugar by a series of polarimetric observations. This is evidently not quite comparable to a study of the actual difference between the sugar physiologically present in blood and that seen in diabetics. However, a comparison of the sugar in normal urine and that found in the diabetic is difficult because of the rather complex nature of the former and its exceedingly small quantity.

In the course of this work the problem divided itself naturally into a number of different phases; the original primary object of this study was the effect which normal urine might have upon added glucose as compared with diabetic urine upon the glucose naturally present.

Secondarily, a further comparison was drawn between the effect of both normal and diabetic urine upon added glucose. The incidental problem, which proved to be of prime importance was that of a comparative study of the optically active substances in normal and diabetic urine and their tendency to change on standing.

Our experiments are in some measure comparable to those of Hewitt and deSouza (14). These investigators, working on the basis that the sugar of the blood could be best removed through the physiological action of the kidneys, studied the optical and reducing properties of various sugars which were excreted in the urine of experimental animals after their intravenous injection. As a result of their experiments they concluded that, following the intravenous injection of equilibrated solutions of α-glucose, α-fructose and α-galactose into rabbits and dogs, no stereo-chemical changes were noted and the equilibrium of the sugars was unaltered in the excreted urine. They further emphasized the fact that polarimetric estimation of reducing sugar in the urine may give fallacious results unless controlled by other methods.

METHODS

For the estimation of glucose by copper reduction, Benedict's quantitative method was employed (15). In using this method it was found necessary to adhere strictly to certain points of technique in order to secure uniform results and, although the procedure is well known, the exact technique as employed in these experiments is given. It was as follows: Twenty-five cc. of Benedict's copper sulphate solution were put into a small wide mouthed flask, together with 7 gm. of anhydrous Na_2CO_3. This solution was boiled over a low flame for exactly 5 minutes and then 3 cc. of distilled water were added. The solution of which the glucose content was to be determined was then added drop by drop from a 10 cc. burette graduated in twentieths of a cubic centimeter. In the case of urine, when the titration was about two thirds finished a drop of octyl alcohol was added to prevent excessive foaming. As the end point was approached a time interval of 3 seconds was allowed between each drop to promote complete reduction. In the event that less than 4 cc. of glucose solution were required to complete the reduction, the solution

was diluted accordingly. In all instances the determinations were run in duplicate or triplicate. Using this method on various glucose solutions which were generally of a concentration of about 1 per cent, the average error from a series of 10 determinations was calculated to be 0.5 per cent, the maximum being 0.8 per cent. This degree of accuracy compared favorably with that obtained by the Folin-McEllroy method (16) which showed a slightly greater error in our hands. Benedict's method proved also to be more advantageous for these experiments in that it was the simpler of the two.

For the standardization of the reducing method a series of sugar determinations were run upon known solutions of glucose, the value of which had been determined polarimetrically. For the specific rotation of glucose +52.8° was adopted.

The polarimetric determinations were made with a Reichert instrument which was graduated to read in hundredths of a degree. The readings were made in a 189.4 mm. tube using a 100 watt Mazda lamp and an appropriate dichromate solution filter for the light source. Final determinations represented the average of 5 successive readings not varying over 0.03°. This gave results which could be compared with a fair degree of accuracy to the third decimal place.

The urine employed was obtained freshly passed from normal individuals and from patients with apparently uncomplicated diabetes mellitus. Only those specimens of urine were chosen which did not contain acetone or diacetic acid and which failed to show appreciable quantities of albumen by the routine clinical tests. For the added sugar Merck's dextrose was used in all of the experiments.

A freshly prepared 5 per cent solution of glucose was made up for each experiment. This was allowed to boil for ten minutes to obviate mutarotatory phenomena, and was then cooled and made up to its original volume.

From the freshly voided specimens of normal and diabetic urine 25 cc. samples were transferred into 100 cc. volumetric flasks, 20 cc. of the 5 per cent glucose solution were added and the whole diluted to the mark, making a final concentration of 1 per cent glucose. At the same time 25 cc. samples of the same urine specimens were diluted with water to a volume of 100 cc. without the addition of glucose. A 1 per cent solution of glucose in water was also made to serve as

a control for each experiment. The solutions were then stoppered and placed in the water bath at 38° and as soon as possible polariscopic readings were commenced. These were continued on the diluted samples of urine, the diluted urine plus glucose and the control at one half to one hour intervals for a period of five hours. At the beginning and end of this time copper reducing determinations were run on each of the solutions containing glucose. At the end of 5 hours one drop of toluol was added to each specimen and the solution placed on ice. On the following day a single polariscopic reading and copper reducing determination was made.

Owing to the difficulties of obtaining strictly sterile urine the experiments were not carried out under absolute aseptic technique. The glassware containers were sterilized and ordinary precautions to avoid contamination were utilized. In a few instances bacterial growth became apparent in the urine during the initial 5 hours which was invariably evidenced by the fact that the urine became cloudy, and at the same time both polariscopic and copper reduction values began to show a parallel fall. Specimens showing such evidences of contamination were always discarded.

RESULTS

One representative experiment has been charted in graphic form (fig. 1) in order to illustrate the manner in which the results have been recorded and subsequently studied. From this chart it will be noted that the curves at the bottom of the figure designate a series of polariscopic readings upon plain urine; Nos. 1 A and 2 A representing normal specimens which remain levorotatory throughout the course of the experiment and No. 3 A a diabetic specimen which is dextrorotatory for the first 6 hours followed by a sharp drop below the zero mark on the following day. The three curves in the upper half of the figure, Nos. 1, 2 and 3 designate a corresponding series of polariscopic readings made simultaneously upon the same urine specimens to which 1 per cent glucose solution had been added; and a fourth curve, No. 4, represents similar readings upon a control solution of 1 per cent glucose.

Curves 1 and 2 of normal urine-glucose solution start with relatively high polariscopic readings which diminish slightly during the first

6 hours. Curve 3 the diabetic urine-glucose solution starts relatively low and climbs upward during the first six hours to be followed by a sharp drop on the following day. The control solution of 1 per cent glucose is represented by curve No. 4 which roughly maintains a

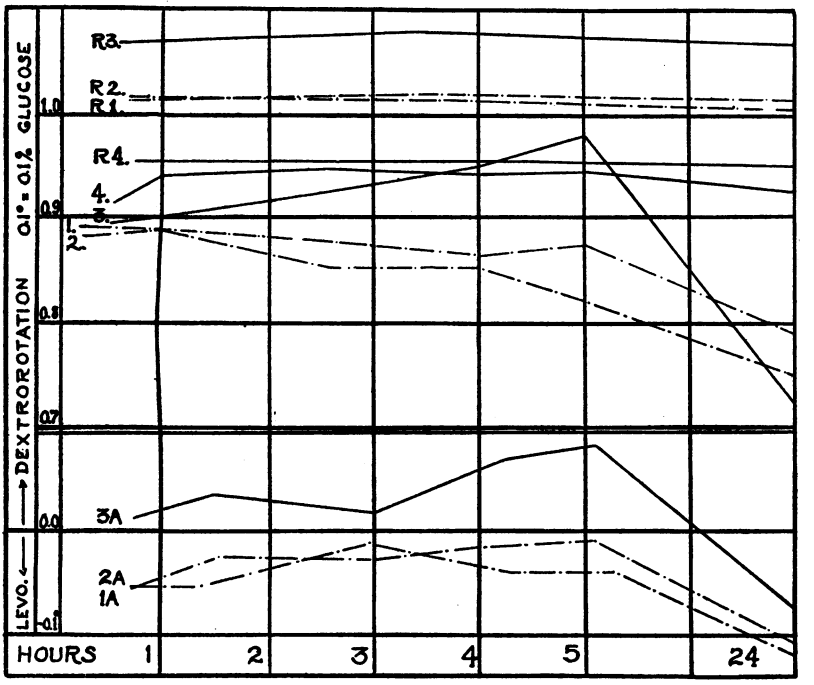

FIG. 1. TYPICAL EXPERIMENT SHOWN IN GRAPHIC FORM

Lines 1 A and 2 A represent polariscopic readings upon normal urine; 3 A diabetic urine. Lines 1 and 2 represent polariscopic readings upon normal urine to which 1% glucose has been added and line 3 diabetic urine similarly treated. Line 4 a control solution of 1% glucose. Lines R 1, 2, 3, and 4, represent corresponding reducing values of the solutions containing glucose.

straight line throughout the experiment. One cannot but notice the distinct influence which curves 1 A, 2 A and 3 A seem to exert upon 1, 2 and 3. They can hardly be said in this instance to show definite parallelism but the major fluctuations noted in the curves 1, 2 and 3 show a counterpart in curves 1 A, 2 A, and 3 A, particularly the latter.

At the top of the figure Curves R 1, R 2 and R 3 designate the series of values obtained from reducing determinations made upon the urine-glucose solutions. It will be noted that the reducing value curves adhere quite closely to a straight line throughout the experiment and that they are considerably higher in the case of the urine-glucose solutions than the values obtained by estimating the glucose content polariscopically. In the case of the control, however, curve R 4 the polariscopic and reducing values approach each other quite closely.

The further results of the series of experiments are shown graphically by composite curves. The changes encountered in the optical activity of normal urine alone are given in figure 2 and in tabular

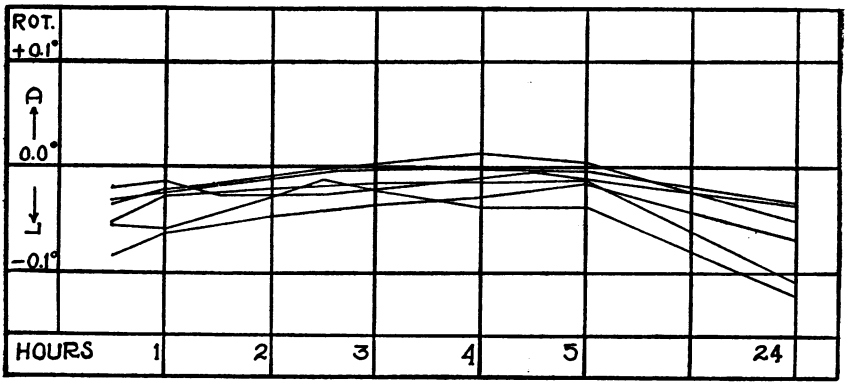

FIG. 2. ASSEMBLED CURVES OF POLARISCOPIC READINGS UPON NORMAL URINES

form by table 1. The results of a series of seven experiments are given in which readings were made at hourly and half hourly intervals for a period of 5 hours followed by a final reading at the end of 24 hours. It will be noted that appreciable changes occur during this period of time and, that in the 7 experiments shown, a fairly uniform trend is followed. In all instances the initial reading of the normal urine samples proved to be levorotatory, varying in degree from −0.020° to −0.085°. On standing at body temperature a gradual diminution in the levorotation invariably occurred so that in the course of 3–4 hours the reading in all instances approached the zero point and in one instance (number 7) it became dextrorotatory. The final reading taken at the end of 24 hours after the specimens had been

TABLE 1

Normal urine

Number	½ hour	1 hour	1½ hours	2 hours	2½ hours	3 hours	3½ hours	4 hours	4½ hours	5 hours	24 hours
	degrees	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*
1	−0.067	−0 050			−0.045			−0.015		−0 006	−0 064
2	−0.085	−0.063				−0.038				−0.028	−0.070
3	−0.053	−0.058			−0.013			−0.039		−0.039	−0.121
4	−0.055	−0.025			−0.028			−0.011		−0.010	−0.111
5	−0.034	−0.028	−0.015		−0.002		−0.002		−0.008		−0.040
6	−0.020	−0.013	−0.028		−0.016		−0.018		0.005		−0.038
7	−0.032	−0.025		0.027	+0.002			+0.012		−0.003	−0.051

kept on ice invariably showed a shift back towards the original reading, and in two instances the final determination was negatively greater than the original.

In the same manner the changes encountered in the optical activity of diabetic urine alone are shown in figure 3 and table 2. The initial readings in this series all proved to be dextrorotatory although the amount naturally varied far more than in the normal samples, being from +0.008° to +0.162°. It will be noted that successive readings in this series showed a rather irregular picture. Fairly wide fluctuations were observed and in two instances a well defined rise was noted at the end of 3 hours followed by a subsequent fall

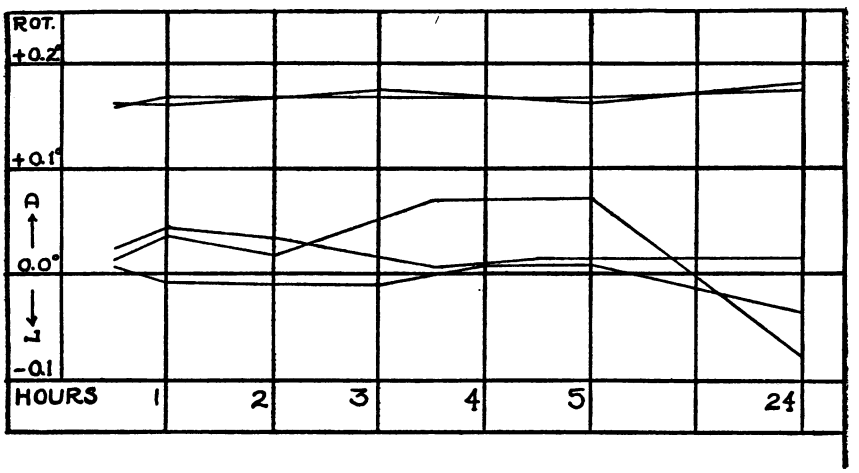

FIG. 3. ASSEMBLED CURVES OF POLARISCOPIC READINGS UPON DIABETIC URINES

at the end of 24 hours well below the zero mark. In general the fluctuations of the other three specimens during the first 5 hours did not show any definite trend, but adhere more or less to a straight line.

In comparing the assembled curves in the case of both normal and diabetic urine one is impressed with the tendency for the readings to shift above and below the zero point. This might be attributed to some optically active substance shifting from a dextrorotatory to a levorotatory character or vice versa. It is, however, more probable that the readings represent the total effect of several optically active substances presumably including small quantities of sugar and of

TABLE 2

Diabetic urine

Number	½ hour	1 hour	1½ hours	2 hours	2½ hours	3 hours	3½ hours	4 hours	4½ hours	5 hours	24 hours
	degrees	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*	*degrees*
1	+0.163	+0.160				+0.175				+0.165	+0.180
2 +(I)*	+0 162	+0 173				+0 167				+0.167	+0.182
3 −	+0.013	+0.035		+0.018				+0.071		+0.075	+0.075
4	+0.024	+0.045	+0.040	+0.034			+0.007		+0.016		+0.017
5	+0.008	−0.007		−0.009		−0.006		+0.011	+0.010		−0.033

* *Note:* Nos. 1 and 2 are samples of the same specimen. To No. 2, 10 units of insulin were added. It will be noted that there are on appreciable changes as a result of the addition of insulin.

glucuronic acid or glucuronates, some of which are dextrorotatory and others levorotatory in character. Changes in any one of these optically active substances would of course influence the total reading. It is clear that in adding glucose to urine one is adding a substance which is optically active to a solution which already contains optically active substances and the polariscopic reading of the resultant solution will represent the sum total of these. A study, therefore, of the changes in the optical rotation of the added glucose might seem to be largely dependent upon the associated changes usually occurring in urine. In order to estimate, therefore, the degree of rotation of the added sugar, it might seem justifiable to read the urine with and without added sugar at stated intervals, and substract the readings of the simple urine from that of the urine to which glucose has been added. The values obtained, however, by this method would be valid upon the assumption, of which we have no assurance, that the usual changes in optical activity noted in simple urine actually takes place, once glucose has been added.

On the basis of our 7 experiments, curves have been drawn to represent, upon the assumption just named, the values of the optical rotation of the sugar added to urine. The ordinates in these curves designate the differences between simultaneous readings of the urine with and without added sugar. The results of normal and diabetic samples are shown in figures 4 and 5 respectively.

In the case of the normal samples of urine a gradual apparent diminution of about 0.05° is observed during the first few hours with as a rule a subsequent slight rise.

With the diabetic urines the curves are irregular but without any consistent rise or fall. An explanation of the minor fluctuations is not attempted but when the curves are viewed critically it does not seem that the optical activity of the added sugar has been appreciably influenced by the urine.

It will be finally noted that in all of these experiments the reducing determinations of the sugar in urine remain constant, but as stated before they show values considerably higher than those obtained by polariscopic determinations.

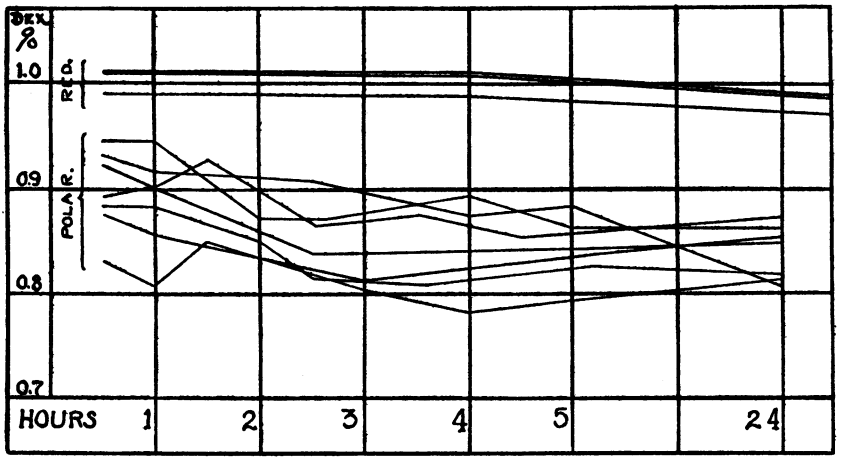

FIG. 4. ASSEMBLED CURVES OF THE INCREMENTS IN POLARISCOPIC AND REDUCING VALUES FROM GLUCOSE ADDED TO NORMAL URINE

The low er curves represent only the computed value of the glucose increment as determined polariscopically; the upper curves represent the combined reducing values of urine and the glucose increment.

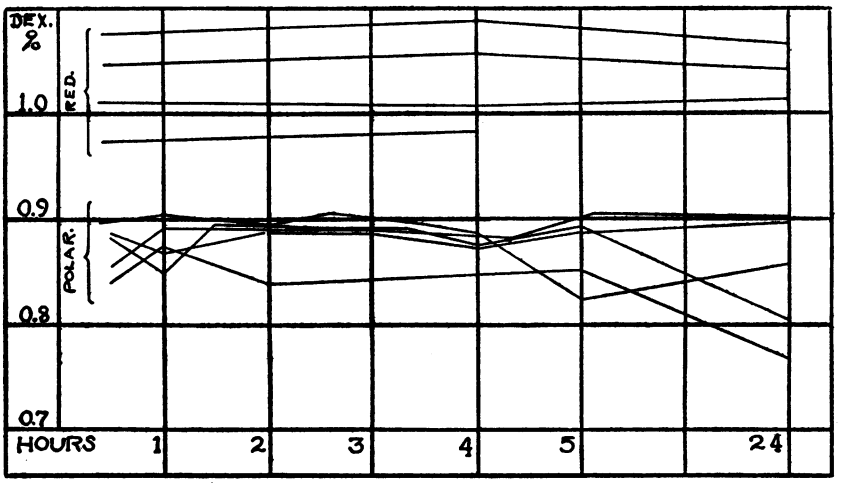

FIG. 5. ASSEMBLED CURVES OF THE INCREMENTS IN POLARISCOPIC AND REDUCING VALUES FROM GLUCOSE ADDED TO DIABETIC URINE

The lower curves represent only the computed value of the glucose increment as determined polariscopically; the upper curves represent the combined reducing values of urine and the glucose increment.

SUMMARY

On standing appreciable changes in the optical activity of dilute samples of normal urine occur. These consist in a diminution of the levorotation noted in fresh urine until at the end of 3 to 5 hours the zero point is approached. Subsequently until the end of 24 hours there is an increase of levorotation and return to initial values.

Similar changes are noted in dilute samples of diabetic urine but the general course of these changes seems to be more irregular than in the normal.

The increment in polariscopic readings produced by the addition of 1 per cent glucose to normal urine diminishes slightly during the first few hours with subsequently, up to 24 hours, no further change.

Polariscopic readings produced by 1 per cent glucose added to diabetic urine shows only such variations over a period of 24 hours as could be accounted for by the changes occurring in the optically active substances already present in the urine.

The difference in behaviour of glucose when added to normal and diabetic urine, is, however, quantitatively too slight to permit deductions as its true significance.

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