CXXVIII. THE RELATION OF GLYCOSURIA TO GLYCAEMIA AND THE DETERMINATION OF THE RENAL THRESHOLD FOR GLUCOSE.

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INTRODUCTION.

GLYCOSURIA, as the most obtrusive finding in diabetes mellitus, has, since its association with this disease was established, aroused considerable interest both as to its production and as to its significance. Prior to the day of Claude Bernard the presence of sugar in the blood was regarded as denoting an abnormal and definitely pathological condition, and glycosuria was considered to be its attendant, and equally pathological, manifestation. His demonstration that glycaemia was of normal occurrence, and that hyperglycaemia was productive of glycosuria, was the first step towards the modern ideas on the subject, and, in addition, it provided the data upon which Bernard [1877] based his conception of the renal threshold as that level of sugar in the blood at which glycosuria appeared. Some years later the discovery by von Mering [1888] of phlorhidzin diabetes showed that glycosuria could exist apart from hyperglycaemia, but it was only with the realisation that there existed a benign clinical type of this disorder that the idea of the presence of a renal factor in all cases of glycosuria became generally accepted.

Apparently Bernard was of the opinion that when the sugar in the blood exceeded a certain level the kidney permitted sugar to "spill" over into the urine. A definite line could therefore be drawn between glycosuria and aglycosuria. This idea was universally accepted until it was attacked by Benedict, Osterberg and Neuwirth [1918]. They maintain that, in the urine of normal individuals, sugar is always present, and that, after carbohydrate meals, an increase in amount may be detected. They have suggested the term glycuressis for this phenomenon, and claim that there is no such thing as a definite threshold. Folin and Berglund [1922] have criticised this work on the ground that the carbohydrates present in normal urine are not of the nature of glucose, but are unusable carbohydrates which the body naturally excretes. Until glucose has been isolated from normal urine this conception of glycuressis must be considered unproven.

The work of Richards [1929] has produced very strong evidence in favour of the filtration-reabsorption theory of kidney function and considered along

with the recent work of Ni and Rehberg [1930] has thrown considerable light upon the nature of the renal threshold. Richards has demonstrated in frogs the presence of glucose in the glomerular filtrate and its absence from the urine. By studying the blood-sugar and the glycosuria in dogs after the intravenous administration of glucose, in conjunction with Rehberg's creatinine filtrate method of estimating the amount of reabsorbed fluid, Ni and Rehberg have shown that as the blood-sugar rises the amount of glucose reabsorbed by the tubules rises also, but that this increase becomes less and less the higher the level attained by the blood-sugar, and that when the difference in concentration between the sugar in the blood and the sugar in the lumen of the tubules exceeds a certain limit, there is no further increase in reabsorption. It appears therefore that for every concentration of blood-sugar a definite amount of glucose per minute is filtered through the glomerulus, and that also for the same value a definite amount of glucose per minute is absorbed from the lumen of the tubules. Thus there will be one value of the blood-sugar at which the tubules just fail to absorb all the glucose filtered, and glycosuria will occur. This value will correspond to the renal threshold.

A peculiar phenomenon in connection with the threshold was first observed by Frank [1913] and has since been confirmed by many other investigators. Frank found that the level of the blood-sugar at which glycosuria appeared was not the same as that at which it disappeared. In his experience the former was usually the higher, but many cases have been reported in which the latter was the higher. Most observers have been inclined to accept this phenomenon as indicating the presence of two different normal thresholds, but Faber and Hansen [1923] in their review of the subject remark, "we cannot really regard the difference as a sign of change in the threshold; it is a phenomenon by itself which must be separated from the level of the threshold." And up to the present there has been no definite evidence to disprove this statement.

Thus it appears that the balance of evidence is in favour of a renal threshold, or thresholds, of a definite level, and that there is some indirect evidence as to the nature of the underlying mechanism.

METHODS PREVIOUSLY ADVOCATED FOR THE DETERMINATION OF THE RENAL THRESHOLD.

When considering the methods which have been recommended for the determination of the renal threshold, one must remember that some of these methods were evolved before much of the now commonplace knowledge of the behaviour of the blood-sugar was discovered, and in assaying any method it is essential to bear the following three facts in mind. First, that the blood-sugar varies considerably throughout the 24 hours according to the food intake; second, that the level of blood-sugar at which glycosuria appears usually differs from the level at which it disappears; and third, that in most,

if not all diabetics, during the night, the blood-sugar does not exhibit a uniform decrease but rises steadily in the early hours of the morning, and then decreases again slowly. This latter phenomenon, noted by Hatlehol [1924], has been confirmed by the writer.

The methods will now be described, and in the consideration of methods (a) and (b) the debt to the critical review of Faber and Hansen [1923] must be acknowledged.

(a) After the night's fast the blood-sugar is estimated, and the urine is tested for the presence or absence of sugar. This procedure is repeated several times during treatment, and from the data thus collected the threshold is adjudged to lie between certain values of the blood-sugar.

Certain workers [Williams and Humphreys, 1919] have based their conclusions upon the presence or absence of sugar in a 24-hour specimen, but in the light of the known variations of the blood-sugar the inaccuracy of such conclusions is obvious. The most accurate modification of the test is that in which the bladder is emptied after a night's fast, urine is passed again a short time later, and the blood-sugar estimated half-way between the two evacuations. From the data thus collected some knowledge of the relationship of the blood-sugar values to the presence or absence of glycosuria may be gathered, but it is obviously impossible by any modification of this test to avoid the discrepancies introduced by the two different thresholds. It is the usual experience with diabetics that as the patient reacts satisfactorily to treatment the value of the morning blood-sugar decreases, and there is a concomitant decrease, and possibly disappearance, of the glycosuria. In a case in which the threshold of appearance is higher than the threshold of disappearance, glycosuria may occur on one occasion and not on another with a blood-sugar of approximately the same value. Failure to take this fact into consideration would account for the common statement that, during treatment, there is a rise in value of the renal threshold.

It appears that this method, and the conclusions drawn from it, have little or no value.

(b) After the oral administration of a standard dose of glucose, the blood-sugar is estimated at frequent intervals, and the urine voided at frequent known intervals is tested for sugar. By noting the highest value of the blood-sugar both on the rise and on the fall of the curve, at which the urine is sugar-free, and similarly the minimum value at which glycosuria occurs, an approximate idea of the two thresholds may be obtained.

This method has been used extensively but is open to criticism on the score of the practical difficulties which prevent its accuracy. The frequency with which the bladder can be emptied is limited, and 10-minute specimens are as much as can be expected from an unpractised person. It will easily be seen that, under such natural restrictions, with a rapidly rising or falling blood-sugar curve, considerable error is inevitable, but that on a slowly falling curve a reasonable degree of accuracy can be attained.

Another criticism, which applies equally to all methods involving qualitative testing of the urine for sugar, is the question of the sensitivity of the reagent. The lower limit of sensitivity of Benedict's reagent is in the region of 0.09 % of glucose. Now, if the threshold is exceeded just before a urination the sugar-containing urine then passed will be diluted by the urine already in the urinary tract, and the resulting concentration of sugar may be too small to be detected by the reagent. In cases of renal glycosuria where the concentration of sugar excreted is very low this is a point of considerable importance.

A method similar in principle, and limitations, is that in which the glycosuric is starved until he is sugar-free, blood-sugar estimations being performed the while. As the slope of such a blood-sugar curve is very gradual, provided one does not meet the nocturnal rise of blood-sugar, one might expect to get a moderately accurate idea of the situation of the threshold of disappearance, but according to Faber and Hansen [1923] this point appears to be lower after starvation than after administration of glucose. Further information is wanted on this point before the factor of experimental error either in this latter method or their control method can be ruled out.

(c) On different days graded doses of glucose are given to the patient, the blood-sugar estimated frequently in each experiment, the resulting curve charted, the urine passed frequently and tested for sugar. Curves of varying heights are obtained. The peaks on the lowest curve with glycosuria and the highest curve without are noted and the threshold is said to lie between these two points. The authors of this method, Faber and Hansen [1923], point out that it is essential to estimate the blood-sugar frequently in order to hit the peak of the curve, and also, that it is equally essential to obtain frequent urine specimens as the concentration of the sugar passed, in those experiments near the border line of glycosuria, is so small that dilution renders impossible its detection by the reagent. They claim that it is quite possible, with practice, so to judge the dose of sugar that the peaks of neighbouring curves may be made to lie very close to each other. The reason behind the evolution of this method is to be found in the statement by the above authors quoted previously, namely, that two thresholds do not exist, there being but one threshold which has the apparent phenomenon of the double threshold superimposed upon it. From the results given later two separate thresholds do appear to exist, and, in that case, their figures will correspond to determinations of the threshold of appearance.

(d) By a modification of Ambard's formula, Chabanier, Lebert and Lobo-Onell [1929] have produced an equation expressing a theoretical relationship between blood- and urine-sugars and the renal threshold. Their results and conclusions are so much at variance with the observations on this subject that they need not be discussed further.

It will be seen that none of the methods above described attains to any high degree of accuracy, and that they are all equally inapplicable in the case of a patient who is never sugar-free. The second method described probably

gives the least distorted results of all, but its limited accuracy has always left doubts as to the validity of conclusions based upon its results.

NEW METHOD.

The theoretical basis.

The question of the renal threshold is so intimately bound up with the problem of the relationship of glycosuria to glycaemia that they cannot be discussed apart. These various relationships are well brought out by the data obtained from a sugar tolerance curve, in which frequent observations of blood-sugar and urine-sugar values are made at definite intervals, and then charted on the same time scale. It is immediately obvious that, whilst there is no apparent correspondence between the glycaemia and the total amount of urinary sugar, there is a definite parallelism between the concentration of sugar in the blood and the concentration of sugar in the urine (Figs. 1 and 2).

Fig. 1.

Fig. 2.

This correspondence is so close that it suggests a proportionate relationship between the two variables. If now the figures for the concentration of glucose in the urine are plotted along the abscissa against points on the ordinate denoting the preceding blood-sugar values at those points in time midway between two consecutive urinations, points are obtained lying along a straight line which cuts the ordinate at some point above zero (Figs. 3, 5 and 7). From

such a graph it is possible to read off the blood-sugar values corresponding to any given concentration of urinary sugar.

Now when the blood-sugar is below and until it attains the renal threshold, no sugar appears in the urine. When the threshold is just reached, sugar is still absent from the urine and immediately it is passed a definite concentration of sugar appears. On such a graph, therefore, the blood-sugar value corresponding to zero on the abscissa is the renal threshold value, and this is the point where the straight line cuts the ordinate.

It will be noted on reference to Figs. 5 and 7 that two lines are obtained. One marked "rise" is drawn through the points obtained whilst the blood-sugar curve is rising, and on the other marked "fall" through those taken during the fall of the curve. This point is well brought out when the data from two consecutive tolerance curves are taken. The two different threshold values obtained correspond respectively to the threshold of appearance and the threshold of disappearance. In Fig. 3 the values charted being taken

Fig. 3. (Linear graph corresponding to Fig. 2.) Note that the values taken before the fall was established fall off the line.

exclusively from a fall curve (Fig. 2), only the threshold of disappearance is shown. In all cases in which two lines are obtained it will be found that these are never continuous; they are separated by an indeterminate zone corresponding to the higher blood-sugar values found round the peak of the tolerance curve, and in this zone various points interpolated from these values fall. Only in those cases in which the peak is flattened into a plateau can a sufficient number of points be obtained to give some idea of the happenings in this area. From the data shown in Fig. 8 it will be seen (Fig. 9) that these points tend to fall on a smooth curve, in this case continuous with the fall line, which, if continued, would make a sharp angle with the rise line. Consideration of these and other figures suggests that the junction between the two lines is effected, not by a single, but by an S-shaped curve, and that this is not necessarily enclosed in its entirety by the two lines.

From the linear graph it is possible to deduce a formula by which the renal threshold may be obtained. Firstly, it will be seen that for equal variations in blood-sugar values there are equivalent variations in the figures for the concentration of urinary sugar. Secondly, that the ratio between the height of the blood-sugar above the renal threshold and the corresponding urinary sugar is constant.

Let B_1, B_2, B_3 = value of the blood-sugar in mg./100 cc.

Let W_1, W_2, W_3 = value of the urinary sugar in mg./100 cc.

Let x = renal threshold.

Then $B - x$ = head of blood-sugar above the renal threshold.

Now
$$\frac{B_1 - x}{W_1} = \frac{B_2 - x}{W_2} = \frac{B_3 - x}{W_3} = K,$$

and when

$$B_1 - B_2 = B_2 - B_3,$$

then

$$W_1 - W_2 = W_2 - W_3.$$

Consider a blood-sugar value which is $(B_1 - B_2)$ mg./100 cc. above x , i.e. $(B_1 - B_2) + x$.

When the blood-sugar falls from B_1 to B_2 the concentration of sugar in the urine falls from W_1 to W_2 , i.e. $(W_1 - W_2)$ mg./100 cc. Therefore the blood-sugar value $(B_1 - B_2) + x$ corresponds to a urinary sugar concentration $W_1 - W_2$.

Therefore

$$\frac{[(B_1 - B_2) + x] - x}{W_1 - W_2} = K = \frac{B_1 - x}{W_1},$$

$$\frac{B_1 - B_2}{W_1 - W_2} = \frac{B_1 - x}{W_1},$$

whence

$$x = \frac{W_1 B_2 - W_2 B_1}{W_1 - W_2}.$$

Thus it is theoretically possible to find the renal threshold value from two consecutive figures expressing the urine-sugar in mg./100 cc., and the two corresponding blood-sugar values.

The technique of the method.

The patient is starved for 18 hours before the test is to be performed. During this time he is allowed water *ad lib.*, and at the 14th hour, after emptying the bladder, he is given 1 pint of water. At the 16th hour the bladder is emptied again, another pint of water taken, and at 17½ hours this is repeated. By this means the mucous membrane of the bladder is washed free from any adherent sugar. At the 18th hour the resting blood-sugar is determined, the bladder emptied, and 50 g. of glucose given by mouth. At 10-minute intervals further blood and urine samples are taken, and after each urination the patient is given 300 cc. of water to drink. Each specimen of urine is tested for sugar and only those specimens saved which give a positive reaction.

The collection of the blood offers no difficulty, but various points must be attended to in the collection of the urine. The large amount of water taken

not only removes the discrepancy referred to later, but provides sufficient fluid for the frequent micturitions necessary. Under its influence it will be found quite easy to obtain 10-minute samples from men, but in women it is essential to pass a catheter. The most convenient way of doing this is by means of a male gum elastic catheter complete with a well-fitting spigot. At the beginning of the test this is passed and secured in position by means of tapes and adhesive plaster. All that it is then necessary to do in order to collect a specimen is to remove the spigot and press on the anterior abdominal wall. When the urine ceases to flow the pressure is maintained and the catheter withdrawn slightly to ensure that there is no retention due to the tip projecting too far into the bladder. The point to be insisted on in both sexes is that the bladder be emptied as completely as possible at each urination.

The question now arises as to the efficiency with which the urinary tract can be evacuated and the error introduced into the glucose concentration figures by that degree of urinary retention which is inevitable. Obviously it is impossible to empty the ureter and the pelvis of the kidney, and the urine contained in them will appear in the next specimen. This part of the tract usually contains about 5 cc. of fluid. The diuresis induced during the investigation is of the order of 100 to 200 cc. per 10 minutes. If the blood-sugar values are rising or falling slowly the concentration of urinary sugar rises or falls by small amounts in consecutive specimens, and the correspondence between the two is practically perfect. If the variations are more marked the corresponding error becomes more pronounced, but this can be overcome to some extent by increasing the diuresis. For this reason it is always more important to have a marked diuresis on the rise, which is rapid, than on the fall of the blood-sugar curve, which is slow. The correspondence demonstrated in the ensuing results shows that there is here no serious source of error.

The first specimen, after the renal threshold has been passed on the rise of the curve, will possibly be diluted by the urine passed immediately previous to this. For this reason this specimen may be misleading and in that case, when the figures relative to it are charted, a discrepancy will be revealed. This specimen should not however be discarded, for, as pointed out in the section discussing the accuracy of the method, sugar may have been present in the specimen previous to this but have been diluted beyond the limits of detection of the qualitative reagent, and in that case the first sample giving a positive reaction will contain its full complement of sugar. On the fall a similar discrepancy may arise, for whilst the specimen passed immediately after the fall below the threshold may contain a sufficient concentration of sugar to give a qualitative reaction, this may still fall short of the maximum concentration possible.

We have seen that when the curve is in the region of the peak, values are obtained which on charting give points falling in the indeterminate zone between the two lines. These points are serious sources of error if included in the calculation of the threshold. Therefore, values should always be taken as

near each threshold as possible. Consecutive specimens after the appearance of sugar on the rise fulfil this condition, but in order to obtain similar specimens on the fall one is driven, if the curve is at all prolonged, to forming an opinion, from clinical data, as to the time when the fall will be well established. If the urine passed before the test is commenced contains sugar, it is wise to assume that the curve will take a considerable time to return to the normal level. All the data will be obtained, and the determination considerably shortened, if the blood-sugar is first depressed by the injection of 15 units of insulin and then raised by the subsequent administration of glucose.

It is hardly necessary to point out that if the blood-sugar curve is falling slowly specimens may be collected at longer intervals than 10 minutes, and quite accurate results obtained.

Theoretically, each threshold may be determined from two urine and two blood samples, but in practice it is more accurate to take three specimens of each, to chart them, draw the line which corresponds most closely to the three points, and by reading off two sets of values from this line apply the equation and obtain the threshold.

To obtain the following data MacLean's method was used for the estimation of the blood-sugar, and for the urinary sugar Benedict's method in the early cases and the polarimeter in the later. There have been many criticisms of the estimation of sugar by the polarimeter. The instrument used was a Hilger M2 with direct vision spectroscope attachment and the light was provided by a mercury vapour lamp. The specific rotation of glucose was taken as $[\alpha]_D + 52.5^\circ$ and the readings were obtained for the mercury green line corrected to α_D by dividing with the factor 1.18. The error involved in the reading amounted to 0.02° . With the diuresis obtained the specimens were clear and it was found that such a urine, containing no glucose, showed no rotation.

Lastly it must be emphasised that to obtain good results the conditions laid down above must be adhered to strictly, as under other conditions discrepancies may occur.

The accuracy of the method.

In discussing the accuracy of this method there are two distinct considerations to be taken into account. One is the accuracy of the methods used in the determination of the necessary data, and the second is the accuracy of the method itself relative to these data.

The accuracy attainable by the various methods used in the determination of the blood-sugar is still under dispute and will not be discussed. As regards the urine-sugar, the majority of urines analysed being of low concentration in sugar, Benedict's method did not give an end-point sufficiently sharp, but owing to the conditions of the experiment accurate readings in practically all the specimens containing their full complement of sugar could be obtained on the polarimeter.

With regard to the second consideration, the whole question turns upon the variation in position of the line allowed by the points in the linear graph. The conditions necessary for the obtaining of good results had been laid down when the experiment, set forth in Figs. 4 and 5, was performed. It will be

Fig. 4.

Fig. 5. Linear graph corresponding to Fig. 4.

seen (Fig. 5) that on the fall the line cuts each point and that no variation is possible. The threshold of disappearance is definitely 181 mg./100 cc. On the rise a variation is allowed and the maximum value of this is 2 mg., that is, the threshold of appearance lies between 221 mg./100 cc. and 223 mg./100 cc. Greater variations do occur, but even on the rapidly rising curve of a case of renal glycosuria these should not exceed 6 or 7 mg. of the total blood-sugar, whilst on a slowly moving curve practically no latitude should be allowed.

One of the advantages of the method is that it is not limited by the sensitivity of qualitative reagents. This is well illustrated by case 6 and the case used for Figs. 4 and 5. Consider, in the former, the rising blood-sugar curve. By the above method the threshold of appearance is 250 mg./100 cc. The 250 mark is crossed at 23 mins., but at 28 mins. a urine giving a negative

Benedict's reaction is passed. The preceding specimen, also with a negative Benedict's reaction, was passed at 17 mins. From the blood-sugar curve and linear graph the following data can be adduced.

At 28 mins. the blood-sugar is 268 mg./100 cc. corresponding to 200 mg./100 cc. urinary sugar.				
„ 27	„	266	„	170
„ 26	„	262	„	140
„ 25	„	258	„	90
„ 24	„	254	„	50
„ 23	„	250	„	0

Fig. 6. Case of renal glycosuria. Figures taken from *Acta Med. Scand. Supp. XXVII*, p. 209 (Malmros).

Fig. 7. Linear graph corresponding to Fig. 6.

The average content of sugar in the urine over the 5 mins. is 130 mg./100 cc., but this is diluted by urine passed in the preceding 6 mins., and, if we assume that the diuresis is approximately the same over these 11 mins., then the average concentration of urinary sugar passed at 28 mins. is 60 mg./100 cc., which is undetectable by Benedict's reagent.

In all cases in which the threshold has been determined by the above method, and in which a rough idea of the threshold could be directly obtained, no discrepancy has been found. .

It may then be concluded that even with the limited accuracy of the means of estimation at present available, the method gives practically perfect results.

RESULTS.

In Table I are given the results in 20 cases. Eleven of these have been arrived at from estimations done by the writer, and nine from figures taken from the literature. It is worthy of comment that in few instances, amongst

Table I.

Own cases				Cases from literature			
No.	Case	Threshold of appearance mg./100 cc.	Threshold of disappearance mg./100 cc.	No.	Reference	Threshold of appearance mg./100 cc.	Threshold of disappearance mg./100 cc.
1	D.W.	—	128	1	Malmros [1928], p. 209	79	20
2	E.F.	—	232	2	„ p. 241	30	103
3	S.D.	—	266	3	„ p. 210	—	70
4	H.W.	206	200	4	„ p. 233	86	104
5	E.P.	200	195	5	Hatlehol [1924], p. 236	110	142
6	G.L.	250	304	6	„ p. 237	234	224
7	I.H.	160	182	7	Bailey [1919], Case 2	73	50
8	H.P.	250	235	8	„ Case 7	232	192
9	H.M.	223	181	9	Goldblatt and Ellis [1931], Case 3, glucose	129	170
10	M.J.	194	175				
11	A.J.	152	144

Fig. 8.

Fig. 9. Linear graph corresponding to Fig. 8. The "rise" and "fall" lines are taken from a previous experiment.

the multitude of figures recorded for glucose tolerance curves, were the corresponding percentages of urinary sugar noted, and in some cases in which they were noted the experiments were unsuitable for calculation by reason of their complicated nature. Perfect results are not to be expected from such figures, as the exact time relationships between the urine and blood samples are not recorded.

It will be noted that in no example are the two thresholds at the same level, although in many instances they lie very close together. This latter

Table II.

Case	Blood		Urine			
	Time in mins.	Sugar mg./100 cc.	Time in mins.	Volume cc.	Sugar %	Total sugar g.
6 Diabetic	0	189	—	—	—	—
	19	236	—	—	—	—
	32	282	28	62	0	0
	49	332	47	78	0.538	0.429
	59	350	57	51	0.870	0.444
	75	374	70	78	1.144	0.891
	88	374	85	117	1.238	1.44
	179	349	175	650	0.850	5.52
	190	337	187	120	0.650	0.78
	202	318	199	76	0.385	0.293
	218	308	215	67	0	0
	228	299	—	—	—	—
10 Diabetic	0	—	0	—	0	0
	12	182	14	—	0	0
	24	200	27	—	0	0
	35	216	39	100	0.105	0.105
	46	237	50	83	0.298	0.248
	57	268	60	73	0.52	0.285
	68	288	71	84	0.73	0.6
	78	310	81	82	0.86	0.71
	87	322	90	82	0.99	0.81
	98	330	100	91	1.06	0.96
	108	306	112	120	0.96	1.15
	119	278	121	115	0.72	0.83
	131	253	133	120	0.56	0.67
	142	243	144	112	0.46	0.51
9 Diabetic	0	103	7	—	0	0
	12	208	18	65	0	0
	22	231	29	35	0.250	0.087
	34	244	35	36	0.515	0.196
	43	260	45	47	0.780	0.364
	52	271	54	63	0.598	0.405
	62	254	65	88	0.427	0.375
	72	237	74	90	0.330	0.298
	82	218	85	113	0.226	0.256
	91	207	95	109	0.097	0.105
	101	191	106	93	0	0
	111	172	115	70	0	0
	120	157	—	—	—	—
11 Renal glycosuric	0	103	—	—	—	—
	14	140	10	24	0	0
	22	177	27	15	0.12	0.028
	40	189	35	14	0.525	0.077
	53	176	49	43	0.25	0.108
	66	160	62	86	0.13	0.111
	82	142	78	170	0.065	0.110
	99	125	—	—	0	0
	119	114	—	—	—	—

apparent correspondence is deceptive, for in every case the two lines giving the thresholds showed distinctly different inclinations.

In Table II the figures from four of the author's experiments are set forth. It will be noticed that the diuresis is not marked in any of them, and we may say at once, that the minimum diuresis required for correspondence varies within wide limits for different individuals.

DISCUSSION.

The primary observation upon which the preceding work is based is that, whilst, under the above conditions, there is a relationship between the concentration of sugar in the urine and the concentration of sugar in the blood, the total amount of sugar in the urine bears no relation to the glycaemia but rather reflects the water output. The important corollary of this observation is that the concentration of sugar in the urine is independent of the diuresis. Now the sugar in the urine is derived from the sugar in the blood, and yet it is impossible to lower the blood-sugar level by creating a diuresis and transferring large amounts of sugar from the blood to the urine. The concentration of sugar in the blood remains steady, the dependent concentration of urinary sugar is unaltered, the total amount of sugar in the urine varies within wide limits. Point is lent to this statement when it is remembered that whilst a patient with a blood-sugar of 300 mg./100 cc. may by diuresis be made to lose 10, 15 or 20 g. of sugar each hour, yet the total amount of sugar in his blood lies between 12 to 15 g. There can only be one conclusion from this phenomenon, and that is that as the body must be supplying sugar to the blood to keep the concentration constant, then the blood-sugar is being maintained at that concentration in spite of all influences to the contrary. Regarded from this point of view the sugar tolerance curve takes on an importance greater than that of a passive expression of the quantity of sugar in the blood awaiting storage, and the high blood-sugar of the diabetic acquires a new significance. Each level of glycaemia must be looked upon as a dynamic rather than a static condition; each as the optimum level for the needs of the body under the particular combination of circumstances then obtaining.

In explanation of the above observations, and the relationships deduced from them, the following theory is offered.

It is a well-known fact that insulin can be demonstrated in the body in tissues other than the pancreas, and that, in a diabetic who has died in coma, insulin may be recovered in surprising quantities from other structures than that organ. It is assumed that in life an insulin factor (I.F.) is present in all living tissues. Under a particular set of circumstances the body requires a definite amount of energy per unit of time, and of this energy each tissue requires its own particular quota. To produce this energy per unit of time a certain amount of carbohydrate must be utilised at a certain rate. Let it be assumed that the utilisation of glucose in the organism for the production of energy involves two consecutive reactions, (a) the formation, by the inter-

action of the glucose and tissue I.F., of an insulin-glucose compound, and (b) the breakdown of this compound with the simultaneous oxidation of the glucose and the production of energy. It is suggested that these reactions conform with the law of mass action and that the concentration of available oxygen required for reaction (b) is constant. In that case the initial velocity of reaction (b) will be proportional to the concentration of the insulin-glucose compound which itself is being formed, according to reaction (a), at a rate proportional to the product of the concentrations of insulin and glucose. Now under resting conditions the demand of the body for production of energy may be regarded as practically constant. If this demand is satisfied by the degradation of the insulin-glucose compound, the concentration in the tissues of the latter must also remain constant. It follows then that the formation of the insulin-glucose compound is proceeding with unvarying velocity, and therefore that the product of the concentrations of I.F. and glucose, which determine this velocity, is a constant quantity. Thus, when the production of energy is constant, if the concentration of insulin be increased, then a fall in the concentration of the blood-sugar is to be anticipated and *vice versa*.

The common observations of (1) the reduction in blood-sugar concentration following injection of insulin, and (2) the increasing blood-sugar concentration which accompanies the progressive restriction of insulin by disease, would follow as immediate corollaries of the above hypothesis. As a further example on this basis may be explained the results which follow administration of glucose.

It is well known that after the oral administration of glucose to a patient at rest there is a rise and fall in blood-sugar values, immediately preceded by a sharp rise and sharp fall in the respiratory quotient and the metabolic rate. The theory outlined above affords an explanation of these phenomena if the additional assumption be made that an increase in concentration of the blood-sugar does not cause an immediate decrease in that of the I.F. In that case the increase in concentration of the blood-sugar, subsequent to ingestion of glucose, will increase the velocity of reaction (a), and the resulting higher concentration of the insulin-glucose compound will bring in its train an increased rate of degradation and hence of energy production. The latter will manifest itself in a raised metabolic rate, the increased degradation in an approximation of the respiratory quotient to unity.

The limit of increase in these related phenomena will be determined by the concentration available in the tissues of I.F. and as this falls the respiratory quotient and metabolic rate will revert to resting values in spite of the persistence of a high blood-sugar. Since the product of the concentrations of insulin and glucose must be maintained at a certain level in order to satisfy the minimal energy requirements of the body, the concentration of the blood-sugar will not be permitted to decrease until the concentration of the I.F. again rises towards normal levels, that is, the delay in return of the

blood-sugar to its normal value will be inversely proportional to the rate of renewal of the I.F. in the tissues. The prolongation of the blood-sugar curve of a diabetic patient whose capacity to produce insulin is deficient receives therefore a logical explanation.

It might be supposed at first sight that the theory proposed above would demand also an increase in metabolic rate as the result of increasing the concentration of insulin-factor by injection of insulin. The absence of an effect of this kind [Himsworth, unpublished] may be ascribed to the extreme readiness with which the relative excess of glucose is removed from the blood and synthesised into glycogen as the result of the inherent, but varying, capacity of most tissues to effect this object, and not attributed to the existence of a mechanism for the reduction of the effective concentration of the insulin-factor by its storage as an inert substance.

We may regard, then, the rise of blood-sugar in the diabetic as comparable with the rise of blood pressure in chronic interstitial nephritis, and both as compensatory phenomena. The examples need not be multiplied, but the theory offers reasonable explanations of phenomena so far removed as the specific dynamic action of carbohydrates, the results obtained in the combined pancreatectomies and hepatectomies of Mann and Magath, and the constancy of the blood-sugar in the face of diuresis.

The secondary problems arising out of the above primary observations centre around the significance of the linear relationship between the concentrations of sugar in the blood and in the urine. The line itself is an expression of the constant ratio which exists between the height of the blood-sugar above the renal threshold and the concentration of sugar in the urine. There is no similar relationship between the latter and the total blood-sugar.

If we consider the relationship in the light of the secretion theory of renal function, then the renal threshold becomes that level of the blood-sugar required to induce in the tubules the secretion of glucose, and from a consideration of the ratio it would appear that secretion proceeds until an equilibrium is established between the concentration of glucose in the lumen fluid and the concentration of the excess of blood-sugar over the renal threshold.

From the point of view of the filtration-reabsorption theory one may say that the failure of reabsorption is proportional to the excess of blood-sugar over the renal threshold. Ni and Rehberg [1930] have shown that the amount of glucose reabsorbed does not rise until a certain level of blood-sugar is reached and then remain constant, but that reabsorption of glucose goes on even when the blood-sugar is far above the normal. It will be noticed that Ni and Rehberg are speaking in terms of amounts, but if their figures are examined some correspondence will be noticed between the concentrations reabsorbed and the blood-sugar values. It appears quite possible then to interpret their results as indicating that the concentration of sugar reabsorbed by the tubules is governed by the blood-sugar. Above the threshold our own

results support this possibility. We know that the urinary sugar is derived from the blood-sugar and because its concentration is proportional to the excess of the latter above the threshold, it is logical to suppose that any removal of glucose from the filtered fluid in its passage through the tubules occurs also in a concentration proportional to the head of blood-sugar above the threshold. The ratio of excess of blood-sugar to urinary sugar concentration is constant above the threshold, and, if the above argument is correct, then the ratio of excess of blood-sugar over the threshold to concentration of reabsorbed fluid is also constant, and as sugar is escaping and appearing in the urine this can only mean that above the threshold the maximum "re-absorption ratio" has been brought into play. Now the excess of sugar is but a fraction of the whole blood-sugar, and it is unreasonable to imagine that the effect of this excess upon the lumen fluid is different from that exercised by the remainder. Thus it would appear that below the threshold the blood-sugar still governs the concentration of the glucose reabsorbed from the glomerular filtrate. The expression $\frac{B-x}{W} = K$ is mathematically untenable in this latter situation and certainly, if the tubules are working at the maximum reabsorption ratio, they are absorbing sugar at a greater concentration than they need to avoid glycosuria. Theoretically this would leave an excess of water in the tubules and if one considers that many volumes of blood must be filtered to provide one volume of urine then the excess would be considerable, and one would expect a diuresis occurring before the threshold was passed to diminish progressively as sugar appeared. But, in experiments in which the intake of water per unit of time is kept constant, this is the reverse of experience. Apparently then the maximum reabsorption ratio does not come into play until the renal threshold is reached, and in that case the renal threshold may be defined as that value of the blood-sugar required to induce the maximum ratio of reabsorption between the concentration of the blood-sugar and the concentration of sugar in the reabsorbed fluid.

The greater the change in concentration of urinary sugar per unit change in concentration of the blood-sugar the more nearly horizontal the line in the graph, and the smaller the relative change the more nearly vertical the line. The inclination of the line or the value of the ratio might thus be regarded as an indication of the permeability of the kidney to glucose. This does not appear to be characteristic of any group of cases and on the basis of it no differentiation of glycosuria into types can be made. The ratio, however, is constant either on the rise or on the fall in any particular experiment, and as it determines the renal threshold, it establishes conclusively the existence of two separate thresholds, a threshold of appearance and a threshold of disappearance. On the mechanism of their differentiation, however, it throws no light.

Reference must now be made to an interesting discrepancy which occurred during the course of the work.

An investigation was carried out in the usual manner, save that no water was given to the patient after the administration of the glucose solution. The

results are charted in Figs. 10 and 11. The blood- and urinary-sugar curves taken on the rise do not run parallel (Fig. 10) and it will be seen that sugar was

Fig. 10.

Fig. 11. Linear graph corresponding to Fig. 10.

excreted in concentrations which increased in unexpected proportion to the blood-sugar. After the first specimen the diuresis fell off markedly. In Fig. 11 are shown the lines obtained on charting the two sets of values. The figures obtained on the rise fall on a curve whilst those obtained on the fall lie on the usual straight line. In all the experiments previous to this, determinations had been made on the fall, no attention paid to diuresis, and perfect results obtained. Also in figures taken from the literature with slowly rising blood-sugar curves no discrepancy had been observed. It appears then that with a rapidly rising blood-sugar curve, to obtain correspondence with the urinary sugar, a diuresis is essential.

The interpretation of this observation appears to be as follows. Fluid is filtered off at the glomerulus at a definite level of the blood-sugar, and if owing to diuresis it passes rapidly along the lumen, then when it reaches the tubules it is subjected to the influence of blood of approximately the same concentration as its own precursor and is concentrated proportionately. If, however, it passes slowly down the tubules, whilst the sugar content of the blood is rising rapidly, then it is exposed in the tubules to a blood-sugar value much higher than that at which it was filtered and is concentrated excessively. The result will be a failure of proportionality between urine and blood-sugar figures, and with a progressive fall in diuresis there will be a progressive rise

in the disproportion. On the fall the same argument would explain the occurrence of a more dilute urine than was expected in association with a given blood-sugar, but the slower decrease of the blood-sugar on the fall does not predispose to widely varying sugar contents in the glomerular and peritubule bloods, and thus the discrepancy will tend not to occur.

Poulsso[n] [1930] has shown that if the tubules are paralysed by phlorhidzin sugar behaves as a non-threshold substance, that is, it is excreted *in toto* and the whole mechanism of proportionate excretion previously described presumably breaks down. Thus the tubules appear to be the seat of the proportionate concentration mechanism and in the light of the above experiment it appears justifiable to conclude that the controlling factor of this mechanism is the sugar content of the blood in the peritubule capillaries.

SUMMARY.

1. A review of the literature and methods previously used in the determination of the renal threshold for glucose is given.
2. A new method is described for determining the renal threshold accurately.
3. The results of calculations made from figures taken from the literature and from those obtained in the present investigation are recorded.
4. It is demonstrated that the concentration of urinary sugar is independent of the diuresis, and that it is proportional to the excess of the blood-sugar concentration over the renal threshold.
5. The nature of the renal threshold is discussed in the light of these results and a theory is advanced to explain the lack of dependence of the urinary sugar concentration on the diuresis.
6. Evidence is produced that the concentration of the sugar reabsorbed is determined by the sugar content of the blood in the peritubule capillaries.

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