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OXIDATION OF GLUCOSE AS FUNCTION OF ITS SUPPLY

By M. WIERZUCHOWSKI

From the Institute of Physiology of the John Casimir University, Lwów, Poland

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THE laws of the total utilization of glucose introduced by vein at constant speed in normal resting dogs over a large range of the rates of supply have been described previously [Wierzuchowski, 1936*a*]. The infusion rates covered were from 1 to 9 g./kg./hr. and the blood-sugar concentrations produced by them reached the value of 3800 mg./100 c.c., which appears to be the lethal concentration [Wierzuchowski, 1936 *b*]. With the rate of supply amounting to some 5 g. glucose/kg./hr., the highest limit of resting assimilation was encountered, and this was no longer exceeded when the rate of glucose supply and of glucose concentration in the internal medium was raised far above the critical limit. In the present work we intend to show whether there exists also a limiting rate of glucose oxidation when glucose supply and its concentration in the body is raised in a resting normal dog. Therefore the determinations of the respiratory exchange were performed on some of the dogs used in the earlier work mentioned above [Wierzuchowski, 1936*a*].

METHODS AND THEIR VALIDITY

The methods of glucose infusion, blood sampling, blood and urine analyses, and of the administration of the diet, were described in the author's former work [1936*a*]. Only the methods concerning the respiratory exchange will be given here.

Two mongrel adult bitches, Ordo. and Andro., of regular bodily shape, were trained for about 1 year before the experiments were started. On the day of experiment, early in the morning, and always exactly at the same time, an enema of definite volume of tepid water was administered

to the dog to wash out fæces, which were almost ready to be expelled after the last meal given between 1 and 2 p.m. on the preceding day. Two hours later, after a short walk, she was laid down on a comfortable cushioned table in the room where she had formerly been trained. The temperature of the room was 26° C., with a variation of ± 0·3°. A catheter was introduced into the bladder, the urine was washed out, and the collection of the preliminary N sample started. Under novocain anæsthesia two small skin incisions were made for isolation of the veins, one for the cannula and one for blood sampling.

After 2 hr. rest the first basal determination was made. An open circuit system was used. A rubber mask, constructed especially for each individual, was put on the dog's mouth, which had been shaved long before the test. The mask had a large rubber cushion perfectly fitting the dog's mouth and leaving as little dead space as possible. The tightness of the Sadd flutter valves, as well as that of all the connexions, and of the large well-balanced spirometer for the collection of the expiratory air, was checked carefully for each experiment. As the inspiratory, outside garden air was used, conducted by tubing, which was protected against wind. For each determination the mask was put on separately, its tightness on the dog's mouth being tested each time with due care. This is the point of technique on which the correctness of the results largely depends. It requires much experience and patience both on the part of the experimenter and on the part of the animal. Even when the basal conditions are most carefully observed, the R.Q. depends largely: (1) on the manner in which the mask is put on the dog's mouth, (2) on the length of time it is left on the dog's mouth, and (3) on the frequency of its application during the day.

The further steps were performed according to the indications of Boothby & Sandiford [1920] with all the precautions recommended by these authors. The determination proper lasted usually 10 min. During the experiment the dog was slightly covered, lying always in the same position in order to make her heat-radiating surface uniform [Bohnenkamp, 1932]. Two to four basal determinations were performed, during which the number of respirations was measured several times. For each determination body and outside temperature and barometer were read. When the preliminary values were established, glucose infusion was started, and precisely at this moment a collection of the expiratory air was begun again. During a 6 hr. glucose infusion the respiratory exchange was determined at the beginning of each hour. It was not found desirable to make more frequent determinations, which would have resulted in irritating the

skin and immediately influencing unfavourably the R.Q.'s. After the infusion was concluded the determinations were continued until the specific dynamic action of glucose was over, i.e. usually during the 7 hours which followed the end of the infusion, this term being prolonged only when necessary.

During the experiments the dogs avoided every movement except that caused by respiration. After the experiment they remained normal and ate the ample meal given them. Thirty-six respiratory experiments were performed.

Air analyses were made at least in duplicate on Haldane burettes, checked daily on outdoor air (accepted limits: 0·03–0·04 p.c. CO_2, 20·92–20·94 p.c. O_2). 97 p.c. of the analyses were accurate within ±0·01 p.c. of the sample volume, i.e. within ±0·5 p.c. of the total value of the increase or decrease of the gas concentration found in experiment.

Calculations were performed according to the rules of indirect calorimetry [Lusk, 1931*a*], with the help of planimetric interpolation. Planimetric calculation, in comparison with the ordinary one, gave an error not higher than ±0·4 p.c., as may be judged from the following:

Value examined	Absolute error	Percentage of the whole value
Heat	±0·08 cal./hr.	±0·33
R.Q.	±0·0027	±0·38

In these calculations the values for protein metabolized were found from the urine N, determined by macro-Kjeldhal procedure. Three portions of the urine N were collected (Table I): (1) the preliminary sample

TABLE I. Nitrogen elimination in urine by the dog Andro. in connexion with intravenous glucose supply at various rates. Average of fourteen experiments

Glucose infusion rate g./kg./hr.	N in urine: Preliminary g./hr.		During 6 hr. of infusion g./hr.		During 7 hr. after infusion g./hr.	
Saline infusion	0·132		0·494		0·090	
1	0·089		0·167		0·150	
2	0·155		0·173		0·093	
3	0·057		0·188		0·068	
4	0·168		0·305		0·078	
5	0·160		0·267		0·034	
6	0·141	0·134	0·226	0·280	0·074	0·067
7	0·068		0·249		0·058	
8	0·134		0·352		0·093	

(before infusion), (2) a specimen during infusion (accumulative 6 hours' sample), and (3) the final post-injection portion. The only portion which seems to correspond to the metabolized protein is the preliminary one.

The two other fractions are a result of undetermined interplay between at least two factors:

(1) *Washing out* of N by the increased flow of urine is shown by the fact that roughly there is some proportionality between the rate of glucose infusion and the increase of urine N in the infusion period over the preliminary value (Table I). With the increasing rate of glucose supply the volume of fluid introduced also increases, glucose solution being administered always at the same concentration.

(2) The second factor is the *N sparing action* of glucose, recognizable by the fact that N elimination with saline was much greater than with any rate of glucose infusion (Table I). For the higher rates of infusion the average N elimination was 0·280 g. N/hr. in comparison with the preliminary value, which was 0·134 g./hr. The increase was twofold. In saline experiment the N rose from the basal value of 0·132 to 0·494 g./hr. —a fourfold increase.

Therefore the preliminary portion of urine N was used exclusively for the indirect calorimetry calculations. But in this way the N-sparing action of glucose infused was neglected, so that the error thus caused should be estimated. This was done in a series of experiments performed for some other purpose (Table II). The average N-sparing action under

TABLE II. Nitrogen-sparing action in two dogs during continuous intravenous injection of glucose at 6 g. rate during 10 hr.

Dog	Wt. of dog kg.	Total N spared g.	Glucose assimilated g.	N spared per g. of glucose assimilated g.	N spared per kg./hr. g.	N spared corresponds to c.c. O_2 per kg./hr. c.c.	N spared corresponds to g. glucose per kg./hr. g.
Colomb.	12·40	1·222	315·33	0·00388	0·00657	39·0	0·0523
Penel.	12·08	0·416	318·19	0·00131	0·00229	13·6	0·0182
Average	—	—	—	0·00260	0·00443	26·3	0·0353

the influence of a high dosage of intravenous glucose amounts to 0·00443 g. N/kg./hr., which equals 26·3 c.c. O_2. Such an amount of O_2 may have been ascribed erroneously to the protein combustion per kg./hr. It corresponds to 0·035 g. of glucose oxidation. If the largest value of glucose oxidized per kg./hr. is about 0·7 g., the minus error thus made is about 5 p.c. The correct value should be perhaps 0·735 g./kg./hr. This error is, however, too small to invalidate our results.

There are also some other errors introduced by non-consideration of the N-sparing action: the non-protein R.Q. is slightly raised; the total heat is influenced only negligibly. At the peak of the N-sparing action of glucose protein supplies, on an average, only about 3 p.c. of the total heat

production. This value corresponds closely to the quantity obtained by Wierzuchowski & Ling [1925] on a hog fattened by carbohydrate diet.

Some additional checks on our technique will be shown in the following sections (fractionated ventilation, fasting non-protein R.Q., etc.).

Results

(1) *Average ventilation rate and fractionated ventilation during full glucose action*

The average ventilation rate, followed throughout the experiment, shows an increase, at the beginning, of glucose infusion, which, during the progress of infusion, attains a degree characteristic for each infusion rate,

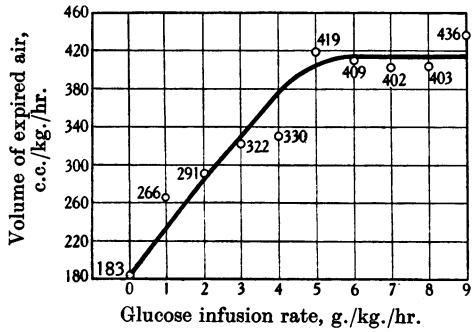

Fig. 1. Mean ventilation rate in two dogs during the fourth to sixth hour of glucose infusion at constant rate of 1–9 g./kg./hr.

whereupon, after the exogenous glucose supply is over, it returns more or less slowly to the basal level. The most significant part of this curve is then the peak value in comparison with the initial one (183 c.c./kg./min.). Up to the infusion rate of 5 g./kg./hr. there are in general definite quantitative relations between the glucose supply and the ventilation (Fig. 1). At this point, however, the maximum ventilation is attained, 419 c.c./kg./min., and a further rise of glucose supply even up to the rate of 9 g./kg./hr. does not produce any significant increase of the ventilation.

As a test for the validity of the R.Q.'s the ventilation rate during the 10 min. of the determination was fractionated, the spirometer tape being read each minute (Table III). The temperature of the spirometer, however, was not read for each minute separately. When we compare the average ventilation for the first 5 min. with the mean value for the second 5 min., there is always a somewhat larger ventilation rate during the second 5 min. (Table III). In the basal determination this increase

TABLE III. During the 10 min. respiratory test the approximate ventilation rate was read each minute. Only the average values for the third to sixth hour of glucose infusion at various rates are given in litres of expired air per minute. Dog Ordo., 10·5 kg. body weight. Average of eleven experiments

Glucose infusion rate	Minutes										Average values		Average non-protein
g./kg./hr.	1	2	3	4	5	6	7	8	9	10	First 5 min.	Second 5 min.	R.Q.
0	2·08	2·12	2·07	2·13	1·99	2·16	2·17	2·17	2·16	2·09	2·08	2·15	0·718
1	2·44	2·44	2·43	2·43	2·49	2·49	2·58	2·58	2·55	2·55	2·45	2·55	1·002
2	2·78	2·78	2·67	2·67	2·74	2·74	2·77	2·77	2·88	2·88	2·73	2·81	0·977
3	3·22	3·22	3·01	3·01	3·13	3·13	3·15	3·15	3·21	3·21	3·12	3·17	0·999
4	3·43	3·04	3·03	3·15	3·08	3·07	3·42	3·28	3·27	3·33	3·15	3·27	1·020
5	3·78	3·60	3·60	3·48	3·58	3·57	3·51	3·37	3·70	3·64	3·41	3·57	1·039
6	4·39	4·06	4·23	3·84	4·19	4·16	4·08	4·48	4·15	3·98	4·14	4·17	1·053
7	4·12	4·23	4·12	4·10	4·38	4·14	4·23	4·19	4·17	4·32	4·19	4·21	1·019
8	4·12	4·14	4·03	4·22	4·00	4·20	4·33	4·41	4·31	4·70	4·10	4·39	1·026
9	4·10	4·28	4·39	4·45	4·03	4·41	4·34	4·24	4·61	4·83	4·25	4·49	1·048

amounts to 3·3 p.c. of the value obtained during the first 5 min., and during glucose infusion up to 7 g./kg./hr. on average 2·6 p.c. With the two highest rates of supply the increase is somewhat larger: 7·1 and 5·6 p.c. Whereas the slight increases could be ascribed to the slow heating up of the air in the spirometer not corrected for temperature, the somewhat larger increases at the two highest rates show, perhaps, that the sensory stimulus of the mask is less tolerated.

If there is, however, any slight washing out of CO_2 in such determinations, it is not noticeable, since our basal R.Q.'s are as close to the fat level as obtainable in such experiments and the R.Q.'s at the advanced stage of glucose action between 4 and 9 g. rate of infusion are consistently alike. Moreover, if there were a certain volume of CO_2 eliminated over unity it would not invalidate our results, for the R.Q.'s in most parts of all the present experiments are over unity in any case.

(2) *Undifferentiated respiratory exchange*

It lends our conclusions a certain directness which cannot be underestimated that most of the facts described later on, and based on elaborate values prepared according to the rules of indirect calorimetry, may be deduced already from the coarse undifferentiated volumes of O_2 consumption (Fig. 2), CO_2 elimination (Fig. 3) and the R.Q. Concerning these, no such doubts can be raised as may exist in the case of the figures obtained by means of indirect calorimetry.

(*a*) *O_2 consumption.* Under the influence of the increasing glucose supply the O_2 consumption rises over basal only to a certain limit (Fig. 2). This limit of increase amounts to 0·22 l. O_2/kg. body weight per hour and

is not surpassed when the glucose supply is increased from 6 to 9g./kg./hr. The curves for the corresponding rates intermingle on Fig. 2. During the second hour of infusion the values show a peak, after which some of them drop slightly. Most of the values reach the initial level within 7 hr. after infusion, with the exception of values for 9 g./kg./hr., when there is some retention of glucose (Fig. 2). On returning to the basal level some of the curves drop somewhat below it.

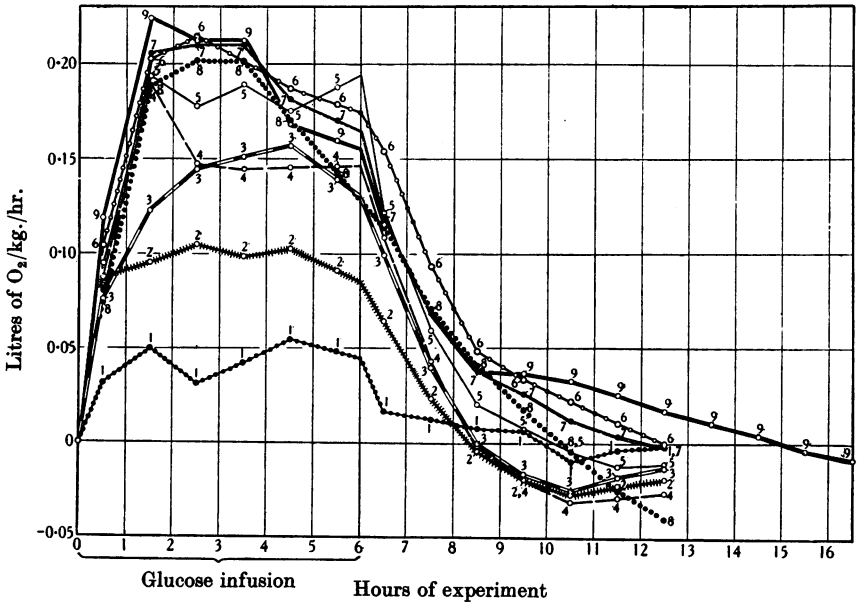

Fig. 2. Increase of O_2 absorption in two dogs as result of continuous infusion of glucose at a rate of 1–9 g./kg./hr. Values represent increases over the basal level. Numbers denote injection rate.

(*b*) *CO_2 elimination.* The values for the increase of CO_2 elimination are more regular than those for O_2 consumption. The higher the infusion rate, the greater is the increase of CO_2 elimination (Fig. 3). This connexion ends at 6 g. rate. Between the rates of 6 and 9 g./kg./hr. there is no more increase of CO_2 elimination. The highest increment thus obtained amounts to about 0·33 l. CO_2/kg./hr. It is almost 50 p.c. higher than the highest increase of O_2 consumption in Fig. 2. This is because the R.Q. is changed from the fat to carbohydrate level, which requires about 0·10 l. of additional CO_2/kg./hr. Because the rise *in toto* is higher than that of O_2 consumption, it also lasts longer and therefore does not reach the basal line within the observation period.

(*c*) *Respiratory quotient.* The undifferentiated R.Q. is only slightly and regularly lower than the differentiated one, and shows all the same features as non-protein R.Q., which will be analysed in the following sections.

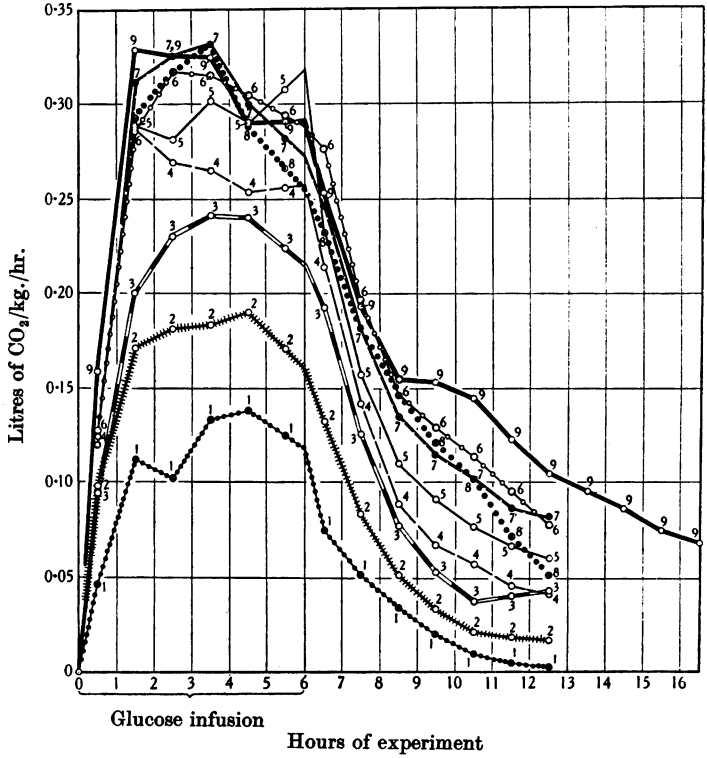

Fig. 3. Increase of CO_2 elimination over the basal level, considered as zero, in two dogs, in connexion with continuous infusion of glucose (1–9 g./kg./hr.). Numbers at each dot signify the rate of glucose supply.

(3) *First moments of glucose inflow at various rates*

It seems to be important from the standpoint of the theory of the specific dynamic action of carbohydrates what happens first with the respiratory exchange as the consequence of glucose infusion. To this end two series of experiments were performed: one during the first 10 min., the other one during the first 20 min. of glucose supply at various rates.

During the *first* 10 *min.* (Table IV) no distinct change in the R.Q. appears during the influence of glucose. In some cases there is a slight rise, in some others an insignificant drop. On the average the R.Q. amounts to 0·712 before infusion, and to 0·707 during the first 10 min. of

infusion. In spite of that, there is a slight but proportionate rise of O_2 absorption when the inflow of glucose is increasing. At first, at 1 g. rate of supply no rise occurs; it is visible at 2 g. rate, increases at higher rates, and attains, with the highest rates of infusion, a 10 p.c. increase. As the

TABLE IV. Undifferentiated respiratory exchange during the first 10 min. of glucose infusion at a rate of 1–9 g./kg./hr. Dog Ordo. Average of seventeen experiments

Glucose infusion rate g./kg./hr.	Before infusion			First 10 min. of infusion		
	O_2 consumption l./hr.	CO_2 elimination l./hr.	R.Q.	O_2 consumption l./hr.	CO_2 elimination l./hr.	R.Q.
1	3·513	2·551	0·726	3·509	2·441	0·696
2	3·532	2·557	0·724	3·794	2·672	0·704
3	3·895	2·793	0·717	4·343	3·145	0·724
4	4·258	3·013	0·708	4·380	3·060	0·700
5	3·995	2·814	0·705	4·308	3·077	0·715
6	4·058	2·962	0·730	4·259	2·908	0·683
7	4·207	3·012	0·718	4·112	2·936	0·717
8	4·270	2·936	0·687	4·602	3·277	0·712
9	4·250	2·953	0·693	4·707	3·360	0·714
Average	3·999	2·844	0·712	4·224	2·986	0·707
Increase	—	—	—	0·225	0·142	0·631

R.Q. remains approximately without change during the first 10 min. of supply, the CO_2 elimination evidently increases parallel with the O_2 absorption. The average increase of O_2 over basal amounts to 5·6 p.c. and of CO_2 to 5 p.c. If we subtract the basal values of both gases from the values obtained during the first 10 min. of infusion we get an R.Q. of the excess which amounts to 0·631 (Table IV). It indicates that fat has probably been oxidized to cover the expense of the increase of O_2 absorption during the first 10 min. of glucose supply. Similar phenomena have been observed with glucose taken by mouth [Bornstein & Holm, 1928; Deuel, 1927; Carpenter & Fox, 1930; Dann & Chambers, 1930, and others; cf. Lusk, 1931 *b*]. Here an analogous phenomenon is found during intravenous supply. It lasts, however, only a short time.

When the period of initial observation is *increased to* 20 *min.* (Table V) the average increase of O_2 consumption for all the rates of glucose administration reaches 12·5 p.c. and that of CO_2 output to 20·7 p.c. The average R.Q. grows from 0·728 to 0·781. The R.Q. of the excess metabolism is consequently 1·21, indicating a great change in the metabolic mixture. All these phenomena justify the conclusion that glucose oxidation appears in 20 min. experiments. As there is none during the first 10 min., it sets in apparently during the second 10 min. of intravenous glucose supply, no matter what rate of supply has been employed.

TABLE V. Respiratory exchange during the first 20 min. of the intravenous glucose supply at a constant rate of 1–8 g./kg./hr. Dog Andro. Average of fourteen experiments

Glucose infusion rate g./kg./hr.	Before infusion			After 20 min. of infusion		
	O_2 absorbed l./hr.	CO_2 eliminated l./hr.	R.Q.	O_2 absorbed l./hr.	CO_2 eliminated l./hr.	R.Q.
1	6·249	4·560	0·731	6·436	4·781	0·743
2	6·292	4·659	0·740	8·392	6·449	0·769
3	5·748	4·231	0·736	6·451	4·989	0·773
4	6·808	4·977	0·731	7·041	5·755	0·816
5	6·891	5·097	0·739	7·364	5·581	0·757
6	6·605	4·790	0·725	7·955	6·363	0·801
7	6·559	4·675	0·713	7·160	5·484	0·767
8	6·474	4·578	0·707	7·262	5·953	0·819
Average	6·453	4·696	0·728	7·258	5·669	0·781
Increase	—	—	—	0·805	0·973	1·209

(4) *Glucose oxidation during the first 3 hr. of glucose infusion*

The average non-protein R.Q. of the two dogs was 0·709. From this pure fat level the R.Q. rises in most cases during the first 3 hr. of infusion (Table VI) and attains its maximum value for the given rate in the fourth

TABLE VI. Non-protein R.Q. and glucose oxidized during the first 3 hr. of continuous intravenous injection of glucose at various rates. Average of twenty-nine experiments on two dogs

Glucose infusion rate g./kg./hr.	Non-protein R.Q.			Glucose oxidized, g./kg./hr.		
	1 hr.	2 hr.	3 hr.	1 hr.	2 hr.	3 hr.
1	0·792	0·934	0·960	0·143	0·386	0·402
2	0·822	0·994	0·993	0·205	0·480	0·495
3	0·829	0·981	0·997	0·215	0·529	0·596
4	0·868	1·021	1·052	0·319	0·702	0·615
5	0·848	1·013	1·025	0·270	0·684	0·660
6	0·846	0·989	1·026	0·276	0·674	0·714
7	0·862	1·019	1·035	0·297	0·715	0·735
8	0·861	0·991	1·012	0·302	0·698	0·730
9	0·862	1·021	1·015	0·326	(0·760)	0·745

to sixth hour of infusion (Fig. 4). The values of the R.Q. for the first hour increase with the rate of glucose supply up to 4 g. rate, when they reach 0·86. With the higher rates there is no more rise of the R.Q. In the second hour it reaches the unity, but with the higher rates of infusion only. In the third hour the unity is exceeded at higher rates of supply. In this way, commencing with the 4 g. rate upwards, the curve of the R.Q. is approximately alike for all injection rates.

Correspondingly, glucose oxidized during the first infusion hour increases with the rate of supply, but even at the highest rates does not go much over 0·3 g./kg./hr. and this only from the 4 g. rate up. In the second

hour the highest oxidation rate attained is 0·7 g./kg./hr. If it sometimes exceeds in the second hour the value obtained in the third hour this should be attributed to the slight shivering which sometimes appeared at the beginning of the second hour of infusion. The values in the third hour approximate closely the definite equilibrated values at the later stages of infusion, and exceed 0·7 g./kg./hr. Except one unusually high figure in the second hour at 9 g. rate, the curves of glucose oxidized for the three highest injection rates are almost similar. It is clear that with the dynamic change of oxidation from the basal fat level to the maximum resting carbohydrate level there is a certain maximum rate which cannot be exceeded by the increase of constant glucose supply.

The period of the first hour of glucose infusion falls on the so-called first phase of glucose assimilation [Wierzuchowski, 1936*a*]. There is certainly in this hour, besides a depressed glycogen formation, a slower rate of glucose oxidation, as manifested by the absence of rise of the R.Q. in the first 10 min. of glucose infusion and the values of glucose oxidation encountered in the first injection hour.

(5) *Maximum level of the oxidative glucose removal during the fourth to sixth hour of infusion*

During the last 3 hr. of glucose infusion at various rates the transformation phenomena were stabilized at a certain level; hence the average metabolic values at this period are significant for the corresponding infusion rates.

(*a*) *Non-protein respiratory quotient and the fat formation.* At this phase of the experiment there is only a slight rise of the non-protein R.Q. with the rising rate of glucose supply. The average R.Q. for these three infusion hours amounts to 1·001 at 1 g. rate, reaches 1·053 at 5 g. rate, and then remains at this level, oscillating between 1·022 and 1·054 during the action of the highest concentrations of glucose obtained in the blood (Fig. 4).

In connexion with R.Q. some facts about lactic acid should be noted here. With the increasing rate of glucose inflow, the lactic-acid concentration in the blood rises over basal value until the 7 g. rate (Fig. 4), when the maximum level is reached. When glucose is being pumped in at the highest speeds, from this rate up, it does not increase any further [Wierzuchowski & Chmielewski, 1935*a*, *b*]. This seems to indicate that this form of glucose transformation does not increase any further when a certain limit of glucose concentration in the body is overstepped. This happens at the level of about 1300 mg. of blood sugar/100 c.c. Non-protein R.Q. seems not to depend at this moment to any larger extent on

blood lactic-acid concentration. For instance, at 4 g. rate there is an R.Q. of 1·042, and in the blood there is an increase of 18·5 mg. of lactic acid over basal; at 9 g. rate the R.Q. is about the same, 1·044, and in the blood there is an increase of lactic acid equal to 30·8 mg./100 c.c., i.e. 66 p.c. more than at 4 g. rate (Fig. 4).

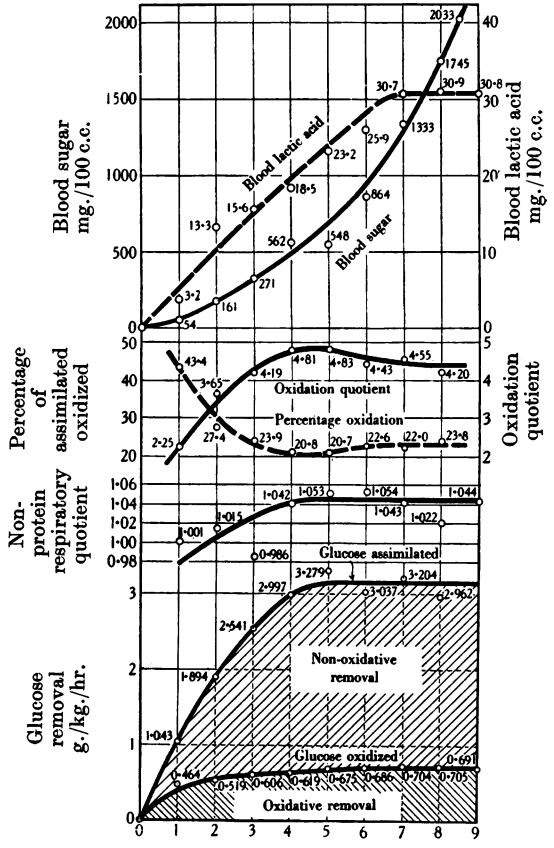

Fig. 4. Average metabolic relationships during the fourth, fifth and sixth hour of continuous glucose infusion at various rates. Oxidation in two dogs, assimilation in four dogs (see footnote on p. 13).

The steadiness of the R.Q. at the higher rates of supply and its relative independence of lactic acid concentration suggest its validity as a measure of fat formation from glucose, so far as one may judge in the light of Krogh & Lindhard's work [1920], the intensity of this transformation only by

the R.Q. rise *above* unity. If estimated in this way by Lusk's method [1931*a*] the process seems to be fairly slight (Table VIII). It must be concluded, that after the workshops of the body oxidizing and polymerizing glucose have been overcharged with labour, there is no indication whatsoever that the oxido-reduction processes may transform the superadded glucose into fat. Just about the moment when the glucose oxidation ceases to increase, in spite of the violently rising glucose concentration in the body fluids, the fat formation does not show any further progress either (Table VIII).

(*b*) *Glucose oxidation.* At 1 g. rate of glucose supply 0·46 g. of glucose are oxidized per kg./hr. (Fig. 4). At 2 g. rate 0·52 g. are burnt and the value increases with the rate of supply, until with the 6 g. rate of injection, the highest capacity to oxidize glucose is reached. In this way, by increasing the intravenous glucose inflow from 1 to 9 g./kg./hr., i.e. 900 p.c., only a rise of glucose oxidation from 0·46 to 0·71 g./kg./hr., i.e. of 66 p.c., may be obtained.

(*c*) *Oxidation quotient,* $\frac{\text{glucose assimilated}}{\text{glucose oxidized}}$, is low at the lowest rates of glucose supply and amounts to 2·25 at 1 g. rate (Fig. 4) and to 3·65 at 2 g. rate. At 3 g. rate it is 4·19 and with the higher rates it reaches 4·8 or a somewhat lower figure. Similarly, the percentage of assimilated oxidized glucose changes (Fig. 4) from 43 p.c. at 1 g. rate of infusion, to 21 p.c. at 4 and 5 g. rate. The depression of the oxidation quotient and the corresponding rise of the percentage oxidized between the rates of 6 and 8 g./kg./hr. is due to the decline of the values of assimilation, calculated according to a special formula.[1] The formula is not quite exact for the highest rates of infusion.

(*d*) *Glucose oxidation and blood-sugar concentration.* It is clear from Fig. 4 that the limit of both oxidation and assimilation is attained at the blood-sugar concentration of some 1000 mg./100 c.c. This is the level which may be called the diabetic threshold level, or the limit of the maximal tolerance for glucose. Further increase of blood sugar does not influence further the oxidation of glucose, or assimilation, or fat formation. The blood sugar rises then very sharply and reaches, at 9 g. rate, the average value of 2023 mg./100 c.c., i.e. a twofold increase as compared with the level of 1000 mg.

(*e*) *Incremental values.* With each gram of the increase in glucose supply per kg. per hour there occurs a change in the metabolic values

[1] To get the values of the assimilation rate at this most important phase of the experiment, the average data for four dogs (two in addition to the couple aforementioned) were borrowed from Table V (formula 9) of a former paper [Wierzuchowski, 1936*a*]. Being more general they serve the purpose better.

showing how the gram of glucose supplied in excess of the 1 g. lower rate of injection has been utilized. This change may be followed on Fig. 5.

A 1 g. increase upon the rate of supply has a different influence on the blood sugar at different levels of inflow. Whereas between zero and 1 g. rate the blood sugar increases by 52 mg./100 c.c. only, this increase between 6 and 7 g./kg./hr. and between 7 and 8 g./kg./hr. amounts to 458 and 479 mg./100 c.c. respectively, i.e. is nine times greater than between zero and 1 g. rate.

The increments of glucose assimilation and oxidation for each 1 g. increase of glucose supply in the same period follow a different curve (Fig. 5). Whereas the increments of assimilation decrease fairly regularly almost as a straight line, the increments of oxidation, very large at 1 g. rate of supply, decrease rapidly, when the rate of supply increases, to an insignificant amount at higher rates of infusion. By subtracting the increments of oxidation from the increments of assimilation the non-oxidative increments are obtained (Fig. 5). With the exception of the value for 1 g. rate they are very large in comparison with the increments of oxidation.

From the data on Fig. 4 we may conclude that there is a shift in the manner in which glucose is utilized when the rate of glucose administration is changed: whereas at the lowest rate of infusion (1g./kg./hr.) two-fifths of the glucose molecules which are assimilated choose the oxidative way of transformation, at higher rates only one-fifth undergo this process, the rest being subjected to non-oxidative removal. Fig. 5 shows in detail how this shift occurs. The relatively large oxidation at 1 g. rate is exceptional. At the higher injection rates oxidative increments become very small in comparison with the non-oxidative ones. The latter are often ten times larger, as may be inferred from the relationship

$$\frac{\text{increase of glucose assimilation}}{\text{increase of glucose oxidation}}$$

called *incremental oxidation quotient*:

Gram increment of the rate of glucose supply, g./kg./hr.	0–1	1–2	2–3	3–4	4–5	5–6
Incremental oxidation quotient	2·3	12·0	9·1	12·5	4·1	0

(6) *Decrease of glucose oxidation after the infusion is over*

(*a*) *Respiratory quotient.* With 1–2 g. rate (Table VII) the fall of the R.Q. begins immediately after glucose infusion has been stopped. At 4 g. rate it is not until in the third hour that a significant decline of the R.Q. may be seen. With these lower infusion rates the higher the speed of

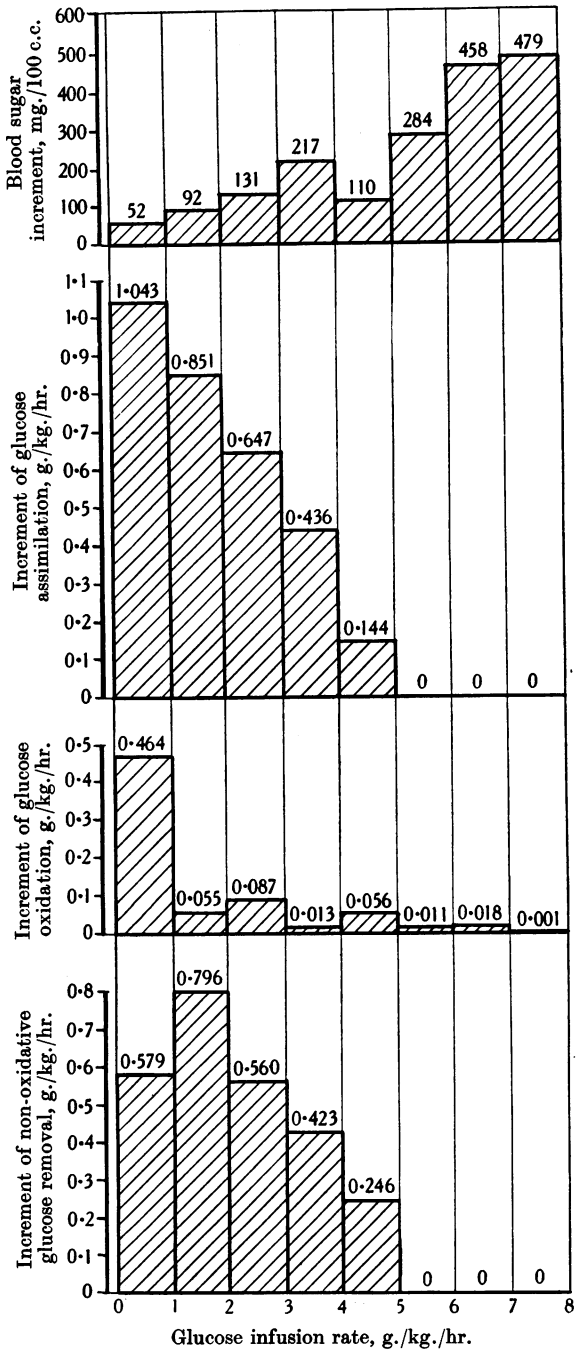

Fig. 5. Increments of blood sugar, of glucose oxidation and assimilation (original values find in Fig. 4) per 1 g./kg./hr. increase of glucose supply. Fourth to sixth hour of glucose infusion. Oxidation in two dogs, assimilation in four dogs.

TABLE VII. Average non-protein R.Q. and glucose oxidized in the hours following glucose infusion at a rate of 1–9 g./kg./hr. in two dogs

Glucose infusion rate g./kg./hr.	Value	Non-protein R.Q.'s (upper values) and glucose oxidized, g./kg./hr. (lower value)							
		1 hr.	2 hr.	3 hr.	4 hr.	5 hr.	6 hr.	7 hr.	8 hr.
1	R.Q.	0·922	0·866	0·826	0·812	0·792	0·759	0·741	—
	g.	0·321	0·228	0·161	0·134	0·106	0·064	0·041	—
2	R.Q.	0·972	0·942	0·921	0·905	0·888	0·863	0·846	—
	g.	0·439	0·347	0·287	0·252	0·223	0·196	0·177	0·188
3	R.Q.	1·015	0·996	0·970	0·940	0·915	0·903	0·899	—
	g.	0·556	0·469	0·379	0·318	0·276	0·265	0·263	—
4	R.Q.	1·033	1·023	1·005	0·990	0·975	0·942	0·916	—
	g.	0·607	0·479	0·388	0·375	0·352	0·331	0·298	—
5	R.Q.	1·046	1·021	1·001	0·985	0·974	0·963	0·938	—
	g.	0·593	0·498	0·433	0·399	0·372	0·360	0·324	—
6	R.Q.	1·069	1·036	1·022	1·010	1·006	0·987	0·966	—
	g.	0·639	0·558	0·496	0·469	0·443	0·407	0·373	—
7	R.Q.	1·054	1·029	0·994	0·977	0·982	0·961	0·961	—
	g.	0·612	0·533	0·455	0·425	0·391	0·379	0·377	0·286
8	R.Q.	1·025	1·004	0·990	0·984	0·985	0·963	0·942	—
	g.	0·624	0·543	0·470	0·442	0·406	0·373	0·334	0·344
9*	R.Q.	1·072	1·048	1·032	1·032	1·019	0·982	0·954	—
	g.	0·614	0·551	0·508	0·508	0·502	0·454	0·403	0·382

* One dog only.

glucose supply, the slower the decrease of the R.Q. Finally, at the 5 g. rate a condition is reached when further increase of glucose supply does not influence further the fall of the R.Q. Therefore the course of the R.Q. curve is approximately equal for the 5–8 g. rates.

(*b*) *Glucose oxidized.* After the glucose supply ceases the values begin to drop immediately. Until the 6 g. rate the curves bear direct relationship to the speed of glucose entrance. Between 6 and 8 g. rate the curves are all alike. They no longer attain the value of 0·7 g./kg./hr. This shows that there is a limit of oxidation also during the disappearance of the metabolic phenomena connected with glucose supply, in spite of the fact that there is no oversaturation with glucose. The possible explanation is as follows:

It is probable that the fuel burnt at this period is not free glucose, but glycogen stored to a certain limited extent, equal for all the higher rates of glucose supply. Therefore with all these rates (6–8 g.) there is an equal starting amount of glycogen in the tissues at the moment when glucose administration from outside has been cut off. The result is an equal rate of glycogen oxidation.

For 9 g. rate the R.Q. curve and the curve of glucose oxidation decreases at a slower rate than between the 6 and 8 g. rate, because there is a large glucose retention in the body as a consequence of a kidney lesion, conditioned by the strain of high diuresis [Wierzuchowski, 1936*b*].

(7) *Balances of glucose during* 12 *hr. observation period*

(*a*) *Assimilation.* The 12 hr. period of observation (during first 6 hr. glucose infusion at constant rate) covers in the present experiments the totality of assimilation. The corresponding values in Table VIII were

TABLE VIII. 12 hr. balance of the transformations of glucose injected constantly by vein at various rates in 1 kg. dog (6 hr. of infusion + 6 hr. of after-infusion period). Mean values of two dogs

Glucose infused g./kg./12 hr.	Glucose assimilated (retained) g./kg./12 hr.	Glucose oxidized g./kg./12 hr.	Fat formation: Glucose turned into fat g./kg./12 hr.	Fat formation: Fat formed g./kg./12 hr.	Lactic acid eliminated in urine g./kg./12 hr.	Glycogen formed. Hexosephosphoric esters (glycocholia?) g./kg./12 hr.	Oxidation quotient	Percentage of glucose assimilated, oxidized
6	6·00	3·34	0·06	0·02	0·0006	2·60	1·80	55·6
12	11·46	4·48	0·19	0·07	0·0026	6·79	2·57	39·1
18	15·52	5·42	0·12	0·04	0·0060	9·97	2·88	35·0
24	18·90	5·83	0·23	0·27	0·0126	12·83	3·25	30·9
30	21·15	6·29	0·64	0·24	0·0235	14·20	3·40	29·7
36	23·10	6·73	0·75	0·28	0·0338	15·59	3·38	29·2
42	23·82	6·65	0·81	0·30	0·0363	16·32	3·19	27·9
48	23·93*	6·71	0·62	0·22	0·0566	16·54	3·66	27·3
	(24·58)					(17·19)	(3·68)	(27·3)
54	27·43†	7·04†	0·79	0·29	0·0731	19·53†	3·90†	25·7

* One experiment, with exceptionally higher values, excluded. In brackets, the average value for the given infusion rate without this exclusion.

† Kidney lesion. Values obtained in one dog only. Much longer period of assimilation than with all preceding rates.

obtained by subtraction of glucose excreted from the values of glucose injected. Practically the highest limit of utilization appears at 6 g. rate (if one exceptionally high value is excluded, see note in Table VIII). It sometimes occurs that the limit of assimilation is not reached within the experimental period, and with the infusion rates which, on other occasions, showed the highest limit of utilization in the same dog. This limiting value amounts from 23 to 24 g. of glucose per 1 kg. dog. The values for 9 g. rate, although perfectly valid, appear in somewhat abnormal conditions (see note in Table VIII) and should not be compared with values obtained at lower infusion rates.

(*b*) *Glucose oxidation.* The longer the period of observation is considered beyond the 4 hours of the greatest intensity of oxidation (during infusion),

the lower the average quantity of glucose oxidized, since the periods of lower intensity of glucose oxidation are included. The highest quantity of glucose oxidized in Table VIII (except for 9 g. rate) amounts to 6·7 g. at 6 g. rate, i.e. 0·56 g./kg./hr., a lower value than in the three peak hours. Although the average value for 12 hr. is naturally lower than the value for three peak hours, the highest limit of oxidation appears at the same infusion rate as during the three peak hours. It illustrates the phenomenon, described in the former sections, that between the 6 and 8 g. rate of supply a certain limiting rate of glucose oxidation appears *not only during the peak hours*, but also *at the rise* to the highest limiting intensity and *at its decrease* to the initial level. The oxidative phenomena in connexion with the glucose injected never cease within the 12 hr. of observation. They continue even when the blood sugar has fallen to the initial level, i.e. when the removal of glucose from the blood is over. It is suggested that the material burnt, after this moment is past, is glycogen formed from the injected glucose. Between 1 g. rate of supply and 8 g. rate there is an eightfold increase of the stream of glucose molecules, but the amount of glucose oxidized is only doubled (from 3·35 to 6·71 g./kg./12 hr.).

In the oxidation quotient (Table VIII) a shift is seen from the value of 1·80 at 1 g. rate to the value of 3·40 at 5 g. rate. The former indicates that at the end of the 12 hr. period 56 p.c. are oxidized, and the latter, that only 27 p.c. are combusted. There is a similar shift, as already observed during the three peak hours, glucose oxidation being equally active as glycogen formation at the lowest rate of infusion, and at the higher rates the latter form of glucose utilization being the prevailing one.

(*c*) *Fat formation.* If judged by the Lusk method [1931*a*] of calculation, fat formed rises with the rising glucose administration from 0·02 g. at 1 g. rate to 0·28 g. at 6 g. rate, which is practically the limit of the fat-forming power of the body under these conditions (Table VIII). For this purpose 0·06–0·8 g. of glucose must be transformed.

(*d*) *Lactic acid formation.* In the 12 hr. observation period a limit of lactic acid excretion cannot be found (Table VIII), because in the post-infusion period no limit can be obtained, whereas it exists in the period during the infusion. Therefore lactic acid elimination in urine, as a form of glucose escape from the body, increases in 12 hr. balances in direct relationship with glucose supply. Even the highest values are, however, insignificant from the standpoint of glucose balance.

(*e*) *Glycogen formation.* When we subtract from the total glucose assimilation the values for glucose oxidation, glucose transformed into fat and glucose escaped in the form of urine lactic acid, a remainder is left

which undergoes the change into glycogen or hexose phosphoric esters, and perhaps escapes into the bowels with bile [Aszódi, 1934, and others], being afterwards reabsorbed. This remaining fraction of glucose reaches its limit at 6 g. rate of infusion, and amounts to some 16g. /kg./12 hr. (Table VIII). This value is within the range of the amounts of glycogen found by Butsch [1934] in dogs during long continuous glucose infusion when the limit of glycogen-forming capacity of the body was probably reached.

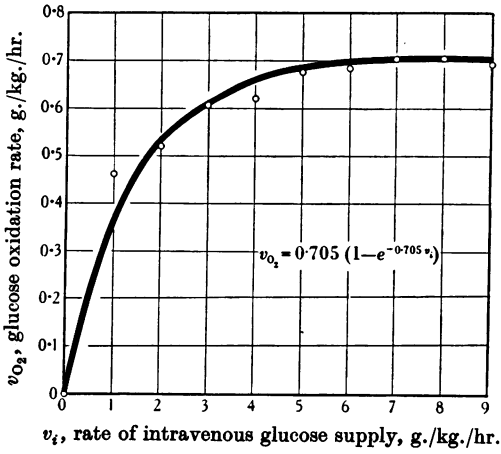

Fig. 6. Glucose oxidation as function of supply during the fourth to sixth hour of infusion.

(8) *Law of glucose oxidation with regard to glucose supply*

If the average values of glucose oxidized during the fourth to the sixth hour of infusion are plotted as ordinates against the rates of glucose supply as abscissæ, an exponential curve of the following shape may be drawn through them (Fig. 6):

$$v_{O_2} = 0{\cdot}705(1-e^{-0{\cdot}705 v_i}),$$

in which v_{O_2} is the velocity of glucose oxidation when this hexose is infused at the speed v_i, both values being expressed in g./kg./hr. Only the amount for 1 g. rate of supply gives a larger deviation, the remaining one being within $\pm 2{\cdot}1$ p.c.

Discussion

Glucose oxidation by the dog and by Saccharomyces. Before the *Saccharomyces* reach the highest limit of glucose oxidation in sugar media at different concentrations they have an optimum of glucose oxidation

between the concentration of 0·2 and 0·5 p.c. [Kluyver & Hoogerheide, 1933]. At 1 p.c. the value falls to a somewhat lower, definite constant level, which does not change any further when glucose concentration is raised to 10 p.c. (Fig. 7). No such optimum is found in the dog. This optimum in yeast has its counterpart in the case of the dog, the chief rise of glucose oxidation being here accomplished exactly at the same blood-sugar concentration as the optimum in yeast. Further increase of sugar concentration in the circulating internal medium of the dog adds only some 16 p.c. more to that value (Fig. 7). The highest level of glucose oxidation in the dog appears at 1 p.c. concentration and persists at higher

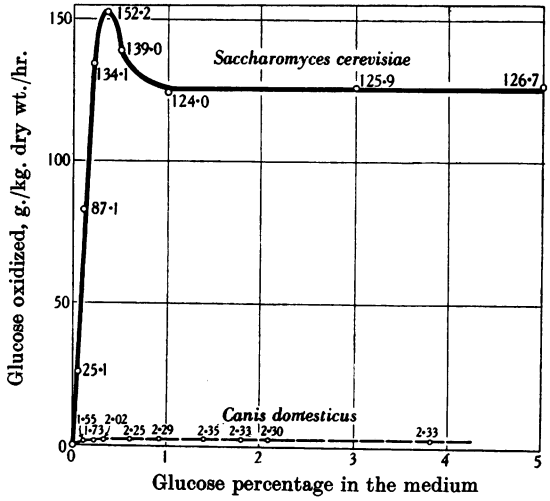

Fig. 7. Glucose oxidation as function of glucose concentration: (*a*) in the external medium in *Saccharomyces*, and (*b*) in the internal medium of the dog.

concentrations. Per kg. dry weight per hour the oxidative consumption of glucose by yeast (152 g./kg./hr.) is thirty-six times greater than by the dog (2·35 g./kg./hr.). If calculated per square metre the values show a discrepancy in another direction.

Establishment of a special form of diabetic disorder. When glucose concentration in the liquids of the body at rest is raised by means of different rates of constant, intravenous glucose influx, three stages of the capacity to oxidize and, in general, to transform glucose may be differentiated, characterized by the various rates of glucose supply:

(1) At 1 *g. rate* of glucose infusion, which approaches the maximal *physiological* rate of glucose absorption from the intestine, the aglycosuric oxidation and assimilation is observed, the oxidation playing a very large

part in the assimilative phenomena, which is characteristic for this lowest rate used.

(2) Between 2 *and* 6 *g. rate* of glucose infusion, when the renal threshold is overstepped, there is a special limit of oxidation and of assimilation for each infusion rate. A definite percentage of glucose assimilated is oxidized, higher at lower rates and lower at higher rates of supply, the increases under each new addition in the rate of inflow being very slight over the value found at 1 g. rate of supply. Therefore the more unphysiological the glucose supply and the higher the blood-sugar concentration, the larger the percentage of glucose stored as glycogen and hexose phosphoric esters.

(3) Finally, between 6 *and* 8 *g. rate* of supply, when the highest limit of utilization is reached, the following phenomena take place: (*a*) the ventilation rate per minute shows no further increase when the supply is raised further, (*b*) also the O_2 intake and (*c*) the CO_2 output does not increase and, consequently, (*d*) glucose oxidation shows a steady level, uninfluenced at the highest point of glucose action by the rising glucose supply. Also the mode of increase of glucose oxidation from the initial value and of decrease to the basal level seems to be limited, so that the limitation of glucose combustion appears during the total observation period applied. The highest limiting rate of glucose oxidation is 0·7 g./kg./hr. and of glycogen formation 2·4 g./kg./hr. The latter is then 3·5 times larger than the former. As the condition resembles a diabetic disturbance, it has been proposed to establish this metabolic form as a separate entity and to call it *overflow diabetes* [Wierzuchowski, 1936*a*].

Different laws apply to experiments made upon exercising animals and to still higher rates of supply (9 g./kg./hr. and upwards), the last ones causing in a short time a serious damage to the kidneys.

Criteria of the overflow diabetes. The following facts disclose this condition as a diabetic state. They are all based on the effect of an additional dose of glucose, introduced intravenously during this abnormality:

(1) The additional dose of glucose increases the blood sugar to a much higher point than the same dose under normal conditions (Fig. 5). At these high concentrations the sugar-absorbing power of the organs *in situ* reached a limit and did not grow under the influence of the supplementary dose [Wierzuchowski *et al.* 1935].

(2) Therefore it is almost completely excreted in the urine (97 p.c.) [Wierzuchowski, 1936*a*, 1937*a*].

(3) Under the influence of this supplementary dose the blood lactic-

acid concentration does not increase any further, nor does the quantity of lactic acid excreted in the urine.

(4) There is no increase of glucose oxidation or of fat formation under the influence of the additional dose (present work).

(5) Neither is there any rise of heat production under its influence [Wierzuchowski, 1937*b*].

(6) The glucose quota attributed to the glycogen and phosphate esters' formation likewise does not increase either.

In this way the list of the arguments in favour of a limit in glucose-transforming power of the body at the highest rates of glucose supply seems to be fairly complete, and includes the chief known metabolic forms of glucose.

Suitability of this diabetic disturbance for experimental studies. This condition has this advantage that it may be provoked in a normal animal without extirpation of the pancreas and without intoxication with phlorizin. After the experiment the animal remains normal, provided the kidneys are not affected [Wierzuchowski, 1936*b*]. Under the influence of subcutaneous glucose, kidney lesions were seen in rabbit by Bouckaert *et al.* [1936]. The chief organs affected by the long continuous glucose infusion are pancreas and hypophysis [Jacobs & Colwell, 1936]. This may throw some light on the mechanism of overflow diabetes.

Certainly none of the existing forms of experimental diabetic derangements offers a model subject for the study of the new formation of glucose in the body, each one of them having its serious drawbacks. Being of completely different origin from the other forms of diabetic abnormalities, the overflow diabetes offers specific opportunity for the studies of glyconeogenesis; for it is to be expected, that if sugar is eventually formed from some substances under these conditions, it will probably not be metabolized, but almost quantitatively excreted. The diabetic form described, being a conservative one for the animal, may be used for long series of experiments.[1] The diabetogenic rate of glucose supply lies between 6 and 8 g./kg./hr. The possibility of glucose formation from amino acids in overflow diabetes is now being tested [Wierzuchowski *et al.* 1935].

[1] It is obvious, in these perhaps more than in any other diabetic studies, that the experiments should be taken in a discriminate way, as they are accomplished on one and the same individual within months and even years, and it must be considered that during such relatively long periods of a dog's life the capacity of the organism to dispose of glucose may change not only quantitatively but also qualitatively. The limit of this capacity is different in various individuals, and may change in one and the same individual in connexion with all the possible changes in the bodily state. The experimenter should make the conditions as constant as possible and select for the experiment the most appropriate moments with regard to constancy. The exact description of the methods will be given soon.

A few general remarks. The most satisfactory correlations recently found between O_2 intake and sugar assimilation were those of Cori & Cori [Cori, 1931] on mice, of Carpenter & Fox [1930] on man and of Cruickshank & Startup [1933*a*, *b*; 1934] on isolated heart. They showed that oxidation of glucose is only one form of its utilization, the rest of the glucose intake being utilized in some other way; moreover, they substantiated the value of the respiratory quotient, as measure of oxidation processes, on which the present work is based. During its progress it was not forgotten that Yater *et al.* [1933] recently called the R.Q. an hypothesis.

The present work supports the view that the lowest rates of glucose supply chiefly favour the oxidation, and that the higher ones favour the storage processes and lactic-acid formation. When the limit of the capacity of the cells is attained all the metabolic forms of glucose transformation cease to develop, because it would perhaps disturb the fixity of the internal medium if they increased without limit. If such a limit does appear in a normal animal, it certainly may appear in a diabetic one. In this way the principle of glucose non-utilization is extended from the normal organism to the diabetic one. The appearance of the limit, on too great demands being made upon the cells, agrees with the ideas of Voit [1881], Lusk [1931*b*] and Barcroft [1934] (principle of maximal activity and of the fixity of internal medium).

Summary

Glucose was infused in two dogs during 6 hr. at a constant rate from 1 to 9 g./kg./hr. and the respiratory exchange determined. Complete indirect calorimetry calculations were correlated with the disappearance of the infused glucose (assimilation) and with lactic-acid formation.

Glucose oxidation begins in the second 10 min. after the infusion has been started. At the beginning of glucose infusion the values of oxidized glucose rise from the pure fat level to certain characteristic stabilized amounts. After the infusion is finished they drop to the basal level. The following conclusions refer to the stabilized values between the fourth and sixth hour of glucose infusion.

As the chief increase of glucose oxidation occurs at 1 g. rate of supply, it may be concluded that only blood-sugar increases met with within the limits of the ordinary life and occurring at this rate of glucose supply favour oxidation. The higher infusion rates increase especially the storage of the material infused in the usual forms, glucose oxidation being only

slightly increased upon each new addition to the rate of supply. At the lowest rate of infusion used (1 g./kg./hr.) two-fifths of glucose assimilated are oxidized and three-fifths are transformed in another manner. At the higher rates only one-fifth of the assimilated material is oxidized and four-fifths are otherwise disposed of.

With the rising glucose supply the values of ventilation, of O_2 intake, CO_2 elimination and of the respiratory quotient increase, until at a certain rate of supply (at 6 g. rate) the values attain the limit and do not increase any further in spite of the increase in the rate of infusion. This concerns all the more important known forms of assimilative glucose removal; i.e. glucose oxidation, fat deposition, glycogen, hexose phosphoric esters and lactic-acid formation. In this way the values of glucose oxidation in the later period of infusion, considered as function of glucose supply, follow an exponential curve of definite shape.

For these highest rates (6–8 g./kg./hr.) also the curves of the increase of oxidation (first to third hour of infusion) to the definite level at each rate of infusion are likewise almost equal to each other, and the same happens with the curves of the decrease of oxidation in the post-infusion period.

All the values obtained by the respiratory exchange were associated with corresponding blood-sugar levels. The limit of the capacity to oxidize glucose is reached at the level of blood sugar amounting to about 1000 mg./100 c.c. Above this level a condition is obtained in a normal animal (dog), which approaches one of diabetes, as it fulfils a number of criteria, enumerated above, which could be postulated for such a diabetic state.

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