CXX. THE GLUCOSE METABOLISM OF KIDNEY TISSUE *IN VITRO*. I.

By JAMES TUTIN IRVING (*Beit Memorial Research Fellow*).

From the Biochemical Laboratory, Oxford.

(Received July 7th, 1927.)

RECENT work on the relationship between glycolysis and respiration in normal tissues by Warburg and Meyerhof and their associates has shown that the changes previously found to take place in muscle, occur in most body cells. The experiments to be described below have been undertaken partly to determine more fully the part glucose takes in these changes, and also to see if the theory of surface activity previously adumbrated by me [1926] for blood corpuscles applies to other body cells. So far only the kidney has been investigated.

EXPERIMENTAL.

In all the experiments undertaken, fresh rabbit's kidneys were employed. They were perfused with warm saline till free from blood and the cortex was then cut off with small scissors and finely chopped with a razor. Weighed amounts of this were then placed in isotonic phosphate buffer at a p_H of about 8.0. The present writer has found that the best proportions of kidney tissue to phosphate buffer are one part of the former to four of the latter, and this proportion has been used throughout. Enough glucose (Kahlbaum) was added to bring the final concentration up to from 0.10 % to 0.20 %.

Sugar estimations were carried out on the supernatant fluid by the method of Hagedorn and Jensen. In the absence of glucose the reducing power did not change, or rose very slightly, while the distribution of glucose between buffer and kidney appeared to reach an equilibrium almost immediately. It thus appeared justifiable to attribute changes in reducing power to alterations in glucose content.

The tubes containing the suspension were completely immersed in a water-bath at 38° and were continually shaken by a mechanical device so that the contents were thoroughly mixed during the entire time of the experiment.

In several of the experiments, hydroxyquinoline sulphate was used in a concentration of 1 in 1500 as an antiseptic. Control experiments, in which the kidney tissue was removed from a suspension after incubation for 4 hours at 38°, and the phosphate-glucose solution reincubated, showed that the glucose content did not change subsequently. In addition, infection was not at all probable during the short period the experiments lasted, which seldom exceeded 4 hours, and in the majority of cases was no longer than two.

FACTORS GOVERNING THE RATE OF GLUCOSE UTILISATION.

Immediately incubation was begun, the glucose content of the phosphate buffer was found to fall at a rate which was fairly constant for each specimen, but varied somewhat from kidney to kidney, the amount used % per hour being between 0.015 and 0.040 g. Exp. 1 in Table I and Fig. 1, curve 1, show results typical of many obtained. The value of k as calculated for a mono-molecular reaction rises as the reaction proceeds, which would suggest that the rate was linear. The power of utilising glucose was found to be lost or greatly weakened if the kidney was kept for 24 hours at 0°.

In certain experiments, however, discrepant results were obtained which suggested that other factors might be playing a part. A series of experiments was undertaken in which the suspensions were incubated *in vacuo* in Thunberg tubes. The change in reducing power was estimated during this period, and the suspension was then placed in an aerobic tube and reincubated, and changes in the reducing power noted. Typical results of these experiments are shown in Table I, Exps. 2, 3 and 4, and Fig. 1, curve 2.

Table I.

Exp. no.	Conditions	Hours	Glucose %	Glucose utilised %	k
1	Aerobic	0	0.179	—	—
	"	1	0.154	0.025	0.0645
	"	2	0.129	0.050	0.0715
	"	3	0.110	0.069	0.0707
	"	4	0.092	0.087	0.0725
2	Anaerobic	0	0.199	—	—
	"	2	0.202	0	—
	Aerobic	0	0.202	—	—
	"	1	0.173	0.029	—
	"	2	0.155	0.047	—
	"	3	0.148	0.054	—
3	Anaerobic	0	0.226	—	—
	"	2	0.217	0.009	—
	Aerobic	0	0.219	—	—
	"	1	0.199	0.029	—
	"	2	0.186	0.042	—
4	Anaerobic	0	0.195	—	—
	"	1.5	0.195	0	—
	Aerobic	0	0.191	—	—
	"	1	0.170	0.021	—
	"	2	0.161	0.030	—

These figures show that the presence or absence of oxygen plays an important part in the utilisation of glucose, and that this can be completely stopped *in vacuo*, while, during the subsequent aerobiosis, glucose is again utilised. For these results to be consistently obtained, it is essential that the kidney be first thoroughly freed from blood, as otherwise enough oxygen is held by the haemoglobin remaining to promote some glucose utilisation.

The utilisation of glucose was also found to be markedly inhibited by cyanide. Experiments were undertaken using KCN in a final concentration

of $M/500$ and $M/250$. Before being used, the cyanide solution was adjusted to the same p_H as the phosphate buffer. Control experiments with KCN and kidney tissue in the absence of glucose showed that the reducing power did not change, and it was also shown that KCN did not interfere with the sugar estimations.

Fig. 1. The rate of utilisation of glucose.

Curve 1. Aerobic incubation.

Curve 2. Anaerobic and aerobic incubation. At the arrow, the suspension was transferred from an anaerobic to an aerobic tube.

Typical results are shown in Table II.

Table II.

Exp. no.	Hours	Control tube		Experimental tube		Concentration of KCN
		Glucose %	Glucose utilised %	Glucose %	Glucose utilised %	
1	0	0.157	—	0.163	—	$M/500$
	1	0.195	0.062	0.163	0	
	2	0.059	0.098	0.161	0.002	
	0	—	—	0.125	—	$M/250$
	1	—	—	0.129	0	
	2	—	—	0.131	0	
2	0	0.139	—	0.139	—	$M/250$
	1	0.106	0.033	0.132	0.007	
	2	0.081	0.058	0.125	0.014	
3	0	0.181	—	0.163	—	$M/500$
	1	0.145	0.036	0.159	0.004	
	2	0.119	0.062	0.173	0	
4	0	0.138	—	0.129	—	$M/250$
	1	0.104	0.034	0.129	0	
	2	0.079	0.059	0.129	0	
	0	—	—	0.139	—	$M/250^*$
	1	—	—	0.129	0	
	2	—	—	0.129	0.010	

* 0.04 mg. Fe added (as used by Harrison [1924]).

In Exp. 4 iron was added as ferrous ammonium sulphate. This experiment, which is typical of several, shows that added iron does not influence the

inhibition by cyanide. It has also been found that KCN inhibits the glucose utilisation by unperfused kidneys to the same extent as by perfused, which would tend to show that iron is not acting catalytically.

The possibility cannot be disregarded that during these experiments in which no glucose appears to be utilised, an equal amount of other reducing bodies may be formed. Even if this were so, it would still show that glucose is differently utilised aerobically and anaerobically. The constancy of the figures obtained at the beginning and end of anaerobiosis appears to make it more probable, however, that glucose is not being utilised during this period.

Levene and Meyer [1913] showed that chopped kidney tissue *in vitro* was capable of turning glucose into lactic acid, an observation which has also been recorded by Warburg, Posener and Negelein [1924]. The latter writers found, however, that the rate of glycolysis was greater anaerobically than aerobically, a fact which does not at first sight accord with the findings recorded above, but it is possible that different phases of carbohydrate utilisation have been studied in the two cases.

Fig. 2. The influence of cyanide.

Curve 1. Normal glucose utilisation.

Curve 2. Glucose utilisation in the presence of KCN *M*/250.

A few preliminary experiments have shown that the disappearing glucose may be converted into a polysaccharide¹. In addition, it has been found that the contents of the reaction tubes are more acid at the end of the experiments than at the beginning, irrespective of whether glucose has been broken down

¹ Note added August 3rd. More recent experiments have shown that it is improbable that glucose is changed to polysaccharides by kidney tissue *in vitro*. J. T. I.

or not. This fact would make it appear more probable that glucose was taking part in the synthetic phase of the glycogen $\rightleftharpoons$ lactic acid cycle as found by Meyerhof, Lohmann and Meier [1925], particularly as Meyerhof [1926, 2] has shown that glucose is probably not an intermediate in the production of lactic acid from glycogen in muscle. Further work is being done to determine the fate of the glucose which is utilised.

The method of utilisation of glucose in the kidney appears, however, to differ fundamentally from that in the blood corpuscle, since the latter is not inhibited by cyanide [Irving, 1926].

THE SITE OF GLUCOSE UTILISATION.

A series of experiments was undertaken with a view to finding the nature of the agency which activated glucose. Attempts were first made to isolate a "glycolytic" enzyme by extraction, employing a method similar to that used by Meyerhof [1926, 1].

The extracts were usually made by grinding the chopped cortex with isotonic phosphate buffer (p_H 7.8) at -3° . Both the whole mass and the supernatant fluid after centrifuging off the macerated tissue were quite inactive when incubated at 38° . In addition, at the end of the ordinary aerobic experiments described in the previous section, the phosphate buffer, after removal of the kidney tissue, was not found to utilise glucose, so that the enzyme did not appear to be able to diffuse out of the cells. Finally, kidney tissue which had been previously frozen solid for a short period, and then thawed, was found to have completely lost its activity, an observation which Laquer [1922] has also made for muscle.

Alders, Chiari and Laszlo [1927] have recently stated that a glycolytic enzyme can be prepared from several tissues by incubating them in glucose-Ringer solution for 1 hour, and then filtering through a Chamberland candle. The filtrate possesses the power of degrading glucose. The present writer has not succeeded as yet in confirming these experiments, but the method of analysis employed by these authors appears to include other substances besides glucose.

It would thus appear that if an enzyme were responsible for the changes observed, it would be much less stable than that described by Meyerhof [1926, 1]. Further experiments were carried out in order to see if an active surface was involved. Several homologous alcohols, methyl, ethyl, propyl, and *isopropyl* were employed. The minimum inhibiting concentrations were investigated in each case, and were found to be very sharp, save in the case of *isopropyl* alcohol, which for some unknown reason did not give such good results as the others. These concentrations were:

Methyl alcohol...	...	...	9.9 %
Ethyl alcohol ...	...	...	7.8
Propyl alcohol ...	...	...	2.9
<i>iso</i> Propyl alcohol	...	...	1.5

The ratio between these amounts is consonant with a surface action theory. Typical results are shown in Table III.

Table III.

Alcohol %	mg. glucose % utilised in 2 hours		
	Propyl	Ethyl	Methyl
1.5	39	—	—
2.0	9	—	—
2.4	7	—	—
2.9	0	—	—
3.4	0	—	—
3.8	—	14	—
5.7	—	—	23
6.5	—	10	—
7.1	—	18	—
7.4	—	7	22
7.8	—	0	—
8.3	—	0	—
9.1	—	—	9
9.9	—	—	0
10.7	—	—	0

In addition, reversibility of the inhibiting action was demonstrated during the early stages of the experiments. Typical results are shown in Table IV.

Table IV.

In each experiment sample no. 1 was placed in contact with alcohol for the stated period, the kidney tissue was washed in a large excess of saline and then incubated in glucose-buffer solution. Sample no. 2 was incubated in glucose-buffer with the same concentration of alcohol as a control.

Exp. no.	Hours	Sample no. 1		Sample no. 2		Conditions
		Glucose %	Glucose utilised %	Glucose %	Glucose utilised %	
1	0	0.157	—	0.134	—	Propyl alcohol
	2	0.110	0.047	0.136	0	2.9 %. 10 mins.
2	0	0.141	—	0.110	—	Ethyl alcohol
	2	0.090	0.051	0.120	0	7.8 %. 5 mins.
3	0	0.163	—	0.141	—	Methyl alcohol
	2	0.127	0.036	0.141	0	9.9 %. 10 mins.

These experiments thus show that an active surface is probably one of the main factors in the utilisation of glucose by the kidney. Whether the surface acts in the manner pictured by Quastel [1926], or whether the responsible enzyme is adsorbed on some cellular interface, it is at present impossible to say. From the facts adduced above, however, it would appear that, as with the blood corpuscle, the integrity of the cell is essential for the utilisation of glucose.

SUMMARY.

1. Kidney tissue *in vitro* utilises glucose aerobically at a rate which is probably linear. *In vacuo*, or in the presence of KCN, the utilisation of glucose is markedly inhibited, or stopped completely.

2. The integrity of the kidney cell is essential for the utilisation of glucose. It is probable that an active surface is involved.

The writer is indebted to Prof. R. A. Peters for his continued advice and interest in this work.

REFERENCES.

- Alders, Chiari and Laszlo (1927). *Biochem. Z.* **180**, 46.
Harrison (1924). *Biochem. J.* **18**, 1009.
Irving (1926). *Biochem. J.* **20**, 613.
Laquer (1922). *Z. physiol. Chem.* **122**, 26.
Levene and Meyer (1913). *J. Biol. Chem.* **15**, 65.
Meyerhof (1926, 1). *Biochem. Z.* **178**, 395.
—— (1926, 2). *Biochem. Z.* **178**, 462.
Meyerhof, Lohmann and Meier (1925). *Biochem. Z.* **157**, 459.
Quastel (1926). *Biochem. J.* **20**, 166.
Warburg, Posener and Negelein (1924). *Biochem. Z.* **152**, 309.